Vol. 12, no. 02
December 2021

Print- ISSN: 1391-586X
E- ISSN: 2602-9030

JSc

Journal of Science
Eastern University, Sri Lanka

Published by Faculty of Science

JSc

Journal of Science
Eastern University, Sri Lanka

Editor-in-Chief

Dr. T. Ealasukanthan

Editorial Board

Snr. Prof. P. Vinobaba
Prof. S. Thirukkanesh
Prof. A. G. Johnpillai
Prof. F. C. Ragel
Prof. P. Peratheepan
Prof. S. Sithambaresan
Dr. T. Mathiventhan
Mr. S. Sotheeswaran
Mr. K. A. N. K. Karunarathna

Disclaimer: The materials in this publication have been supplied by authors, and the views expressed remain the responsibility of the named authors. The statements and opinions stated in this publication do not necessarily represent the views of the Faculty of Science. No responsibility is accepted by the Faculty of Science for the accuracy of information contained in the text and illustrations.

Print-ISSN: 1391-586X
E-ISSN: 2602-9030
Web: www.jsc.sljol.info/

© 2021 by Faculty of Science, Eastern University, Sri Lanka
All rights reserved

Address: The Editor-in-Chief, Journal of Science, Faculty of Science, Eastern University, Sri Lanka.

Journal of Science - Eastern University, Sri Lanka

Vol. 12, no. 02 **December 2021**

CONTENTS

RESEARCH ARTICLES

Actuarial Analysis of Claim Per Payment Event: Theoretically Developing Estimation Link Between Quantum Mechanics and Insurance Modeling **1**
Ogungbenle, M. G.

An Alternative Method for Constructing Hadamard Matrices **26**
A. P. Batuwita, N. T. S. G. Gamachchige, P. G. R. S. Ranasinghe, A. A. I. Perera

Phytochemicals and Antioxidant Properties of the Leaves of Wild Guava Varieties Grown in Sri Lanka **33**
K. Shanthirasekaram, V. P. Bulugahapitiya, H. Manawadu, C. S. Gangabadage

An Alternative Approach for The Anti-Magic Labelling of a Wheel Graph and a Pendant Graph **47**
K. M. P. G. S. C Kapuhennayake , A. A. I. Perera

Anti-Magic Like Labelling of Marigold Graph **61**
K. M. P. G. S. C Kapuhennayake , A. A. I. Perera

A Monthly Evaluation of Microbiological and Chemical Quality of Bottled Drinking Water **67**
Herath, A. L., Abayasekara, C. L.*

Assessment of Microbiological and Chemical Quality of Spring-water in Riverston of Knuckles Mountain Range in Sri Lanka **79**
Herath, A. L.

Print-ISSN: 1391-586X
E-ISSN: 2602-9030
Web: www.jsc.sljol.info/

Published by Faculty of Science, Eastern University, Sri Lanka

RESEARCH ARTICLE

ACTUARIAL ANALYSIS OF CLAIM PER PAYMENT EVENT: THEORETICALLY DEVELOPING ESTIMATION LINK BETWEEN QUANTUM MECHANICS AND INSURANCE MODELING

Ogungbenle, M. G.

Department of Actuarial Science, Faculty of Management Sciences, University of Jos, Nigeria

ABSTRACT

In general, business, underwriting firms usually write policy terms by imposing additional deductible conditions in order to mitigate the risk of loss and discourage frivolous claims by modifying the indemnity payable by the underwriter where loss occurs. Under deductible conditions, underwriting firms will only be liable in a loss event where it becomes apparent that the loss has exceeded the deductible defined in the policy terms. The maximum accumulated number of losses retained by the insured applying deductible policy modifications is usually set as part of the terms and conditions of the policy documents. This paper develops an analytical framework for evaluating the effect of structural properties of dirac-delta on insurance risk variables with deductible clauses. The objective is to obtain models for the excess of loss random variable in a payment event. In order to achieve this and create analytically sound theoretical platform of investigating payment distribution functions, the quantum structure of dirac-delta is first examined in respect of probability density function. The import of adopting the dirac-delta function in this paper lies in its elegance to permit alternative technique to obtain analytically useful models for insurance severity beneficial to both the insured and insurer with particular reference to rate relativity deductible clause. We then obtained insurance excess of loss severity and variance for an arbitrary policy under deductible coverage conditions. As part of our contributions, theorems in respect of loss were stated and proved for underwriters to see reasons for their applications and use in policy underwriting decisions.

Keywords: *Cost per loss, deductible, loss elimination ratio, risk, severity*

DOI. *http://doi.org/10.4038/jsc.v12i2.32*

1. INTRODUCTION TO SINGULARITY FUNCTIONS

In practice, underwriting firms are bound by set targets for claim out-go by using the claims values already recorded and then use it in projecting the frequency of claims in

*Corresponding author: *moyosiolorun@gmail.com* *https://orcid.org/0000-0001-5700-7738*

respect of the uncertain periods. The potential insurance risk associated with allocation of insurance funds necessitates the need for deep actuarial estimation techniques. Insurance funds are usually invested in many debt and market instruments with the goal of generating real returns on investment so as to meet claim obligations criteria and solvency requirements. In [1] we see that the insurance expected loss associated with both claim per payment and claim per loss which form the basis of this paper is responsible to a large extent for a meaningful fraction of the aggregate liability of the underwriting firms and consequently, the loss is associated with claims demand and uncertainties linked to it through which the insurance schemes provoke the underwriter to forfeiture. Usually, underwriters are frequently engaged in the administration of issues relating to the expected claim liability estimation and in order to deal with these issues, underwriting firms will have to apply actuarial techniques of estimation to obtain critical information over the uncertainties on the liabilities in order to ensure decisive actions relating to the expected claim, payout targets and future insurance pricing. Insurance firms are now groping to cope with the current problems of underwriting risk phenomena, regulatory risk trajectories, solvency requirements risk and demand for insurance policies, market share syndrome, risk management, investment risk and claim payment strategies.

It is imperative to note from the foregoing that the issue of satisfying claim payment terms and conditions has become a hydra-headed problem to the underwriters even though the scheme holders want a financial succor in the event of contingencies as defined in the policy terms happen. We infer from [1-2] that the estimation of claim per payment of the insurance policies allow the risk manager to take drastic actions on claim payment devoid of significant error that could evolve out of the problems already enumerated hitherto and consequently it is objective of this paper to apply expectation and singularity potential theory as working tools to model insurance claims in order to estimate the expected claim per payment liabilities in non-life insurance contracts. This is done by developing a link between singularity and actuarial modeling and fix an actuarial model which provides financial arrangements to cover the expenses resulting from the actuarial treatments of insurance losses. The occurrence of a loss event is a necessary condition for advising a claim.

In other to model an insurance loss, the event surrounding the loss must be well defined such that it does not have too much exposure to risk. Correspondence in the analysis of loss coverage usually assumes a rigorous dimension which could conceal the ease of the underlying idea but the dirac-delta function serves to exemplify much of the difficult expression which is the key tool used to deal with actuarial principles involved and to represent magnitude of insurance loss. In this paper, we will apply the dirac-delta function to obtain the expected cost per payment claim severity under deductible conditions and the variance of the cost per payment loss event under the deductible coverage modifications. Note that the effect of the insured's characteristics on payment amounts functionally depends on the cost sharing arrangements implied by the deductible

clauses such that preference for a small deductible could reflect an anti-selective tendency on the part of the scheme-holders.

In [1-2], it is asserted that dirac-delta function exists naturally in the solution of financial problems but it is often applicable in actuarial risk theory and statistical physics to obtain the distribution of an ideal point mass as a function equal to zero everywhere except for zero and whose integral in the entire real line becomes unity. The behavior of the dirac-delta function is such that it is not a real valued function but only a notation $\delta(z)$ which for apparently defined reasons is considered as if it were a function. The dirac-delta function becomes useful in dealing with a defined notation when addressing quantities associated with some kind of infinity and precisely it is deeply related to an eigen function corresponding to an eigenvalue in the continuum that is non-normalizable. The dirac-delta function is treated as the extension of the Kronecker delta function in the event of the continuous variables. Geometrically, the dirac-delta describes the trajectory of a curve whose width approaches nullity and narrow trough roughly approaching infinity while maintaining the area under the curve finite. From its behaviour, it is a real function of z which becomes zero everywhere except on the inside of a small interval of length $\in$ about the centre s_0 but it is extremely bigger in this interval such that its integral over the same interval approaches unity. Following inferences by authors in [1-2], the dirac-delta function plays fundamental roles in actuarial risk theory under the appropriate limit especially in the evaluation of improper integrals involved in probability. In view of observations by authors in [3-4], important applications of dirac-delta were demonstrated in mathematical statistics and probability theory under univariate and multivariate framework. The unit impulse function otherwise called dirac-delta $\delta(z)$ as observed by authors in [1-2;4-5] and which finds applications recently in actuarial risk is a distribution function rather than a true function and it is only defined within an integral on the extended real line. The function is designated generalized real function but does not actually qualify for the characteristics of a real function.

However, in the Schwartz theory of distributions, the function is applied in the evaluation of an integral kernel of some distribution. Following observations by authors in [1;6-8], it is observed that $\delta(z)$ has singularity at a point on the real line where the integral over the extended real line of the product of a function and dirac-delta produces the functional value of the function at that point. Following the definitions by authors in [1;3;9], dirac-delta permits wider spectrum of applications to describe the singularity characteristics of probability distributions used in statistical mechanics especially in quantum theory.

The dirac-delta function is defined in the work of the authors in [1;3;8-11] as follows

$$\delta_{\eta}(z-\omega)=\delta(z-\omega)=\begin{cases}\infty,\text{if},z=\omega\\ 0,\text{if},z\neq\omega\end{cases} \text{ and } \int_{-\infty}^{\infty}\delta(z-\omega)\,dz=\int_{0}^{\infty}\delta(z-\omega)\,dz=1, \qquad (1)$$

the integral is defined over the extended real line. If $\omega = 0$, we have

$$\delta(z-0) = \begin{cases} \infty, \text{if}, z = 0 \\ 0, \text{if}, z \neq 0 \end{cases} \text{ and } \int_{-\infty}^{\infty} \delta(z-0)\,\mathrm{dx} = 1, \qquad \delta(z-0) f(z) = \delta(z-0) f(0). \tag{2}$$

Furthermore, a particular situation where the product is often defined is that of an integrable real valued function with a dirac-delta structure as long as the real valued function has been well defined at the points of singularities of the dirac-delta. Considering the behavior of the delta function, this is equal to multiplying it by a real number, the value of the integrable real function at the singular point of the delta function.

$$\delta(z-\omega) f(z) = \delta(z-\omega) f(\omega) \tag{3}$$

Borrowing from continuous time finance, we can apply complex representation to define dirac-delta function

$$\lim_{A\to\infty}\left[\int_{-A}^{A} \mathrm{e}^{ihz}\,\mathrm{dh}\right] = \lim_{A\to\infty}\left[\frac{\mathrm{e}^{ihz} - \mathrm{e}^{-ihz}}{iz}\right] = 2\pi \lim_{A\to\infty}\left[\frac{\sin Az}{\pi z}\right] \tag{4}$$

As A becomes large,

$$\frac{\sin Az}{\pi z} \to \delta(z) \tag{5}$$

$$\lim_{A\to\infty}\left[\int_{-A}^{A} \mathrm{e}^{ihz}\,\mathrm{dh}\right] = 2\pi\delta(z) \Rightarrow \delta(z) = \frac{1}{2\pi}\lim_{A\to\infty}\left[\int_{-A}^{A} \mathrm{e}^{ihz}\,\mathrm{dh}\right] \tag{6}$$

As z becomes very large without bounds, we have

$$\int_{-\infty}^{\infty} \frac{\sin Az}{\pi z}\,\mathrm{dz} \to 1 \text{ and } \int_{-\infty}^{\infty}\left(\frac{\sin Az}{\pi z}\right)\mathrm{f}(z)\,\mathrm{dz} \to f(0) \tag{7}$$

But when as z approaches zero becoming smaller we have

$$\frac{\sin Az}{\pi z} = \frac{Az}{\pi z} = \frac{A}{\pi} \tag{8}$$

In this section, we use second order differential equation to explain how direct-delta function evolves, as most problems in actuarial risk literature encounter derivation of models for general insurance and casualty. It is on this basis that we use singularity functions to investigate the behavior of actuarial density functions to enable us obtain models applicable in general insurance business.

Recall in the work of the author in [1] that, the linear equation, $b_1 z'' + \mathrm{a}_2 z' + \mathrm{a}_3 z = \mathrm{f}(\mathrm{s})$, is deeply rooted in many areas of actuarial discipline, especially in financial engineering where it has been used to analyze the term structure and varying time parameters of interest

rates by setting the forcing function $\mathrm{f}(\mathrm{s})=0$ and further assuming that the homogeneous differential equation $b_1 z''+b_2 z'+b_3 z=0$ has equal real roots with constant co-efficient $b_i, \mathrm{i}=1,2,3$. Following definitions by authors in [1;5;8;9;11], one of the most simplest but striking application of integral transform occurs in the treatment of linear differential equations with jump discontinuities or discontinuous forcing functions especially in the analysis of circuit problems and mechanical vibrations.

Recall the definitions by the author in [1] that in the second order differential equation above, $\mathrm{f}(\mathrm{s})$ is a measure of forcing term and the total area under the curve

$$\int_{-\infty}^{\infty} \mathrm{f}(\mathrm{s})\mathrm{ds} = \lim_{\mathrm{a}\to\infty} \int_{-\mathrm{a}}^{\mathrm{a}} \mathrm{f}(\mathrm{s})\mathrm{ds} \tag{9}$$

is the impulsive force.
We define the function

$$\delta_\eta(s-\mathrm{s}_0) = \begin{cases} \dfrac{1}{2\eta}, \text{if}, \mathrm{s}_0-\eta < s < \mathrm{s}_0+\eta \\ 0, \text{if, otherwise} \end{cases} \text{ and } \int_{-\infty}^{\infty} \delta_\eta(s-\mathrm{s}_0)\mathrm{ds}=1 \tag{10}$$

where $\eta>0$

$$\int_{\mathrm{s}_0-\eta}^{\mathrm{s}_0+\eta} \delta_\eta(s-\mathrm{s}_0)\mathrm{f}(\mathrm{s})\mathrm{ds} = \int_{\mathrm{s}_0-\eta}^{\mathrm{s}_0+\eta} \frac{1}{2\eta}\mathrm{f}(\mathrm{s})\mathrm{ds} = (\mathrm{s}_0+\eta-\mathrm{s}_0+\eta)\frac{1}{2\eta}\mathrm{f}(\bar{\mathrm{s}}) \tag{11}$$

$$\int_{\mathrm{s}_0-\eta}^{\mathrm{s}_0+\eta} \frac{1}{2\eta}\mathrm{f}(\mathrm{s})\mathrm{ds} = \frac{2\eta}{2\eta}\mathrm{f}(\bar{\mathrm{s}}) = \mathrm{f}(\bar{\mathrm{s}}), \text{ using the mean value theorem} \tag{12}$$

$$\eta\to 0, \delta_\eta(s-\mathrm{s}_0)\to\delta(s-\mathrm{s}_0), \bar{\mathrm{s}}\to\mathrm{s}_0 \tag{12a}$$

$$\lim_{\eta\to 0}\left[\int_{-\infty}^{\infty}\delta_\eta(s-\mathrm{s}_0)\mathrm{f}(\mathrm{s})\mathrm{ds}\right] = \lim_{\eta\to 0}\mathrm{f}(\bar{\mathrm{s}}) = \int_{-\infty}^{\infty}\lim_{x\to\infty}\left[\delta_\eta(s-\mathrm{s}_0)\mathrm{f}(\mathrm{s})\right]\mathrm{ds} \tag{13}$$

Since $f(s)$ is well behaved having a unique value at every point in its domain

$$\int_{-\infty}^{\infty}\delta(s-\mathrm{s}_0)\mathrm{f}(\mathrm{s})\mathrm{ds} = \mathrm{f}(\mathrm{s}_0) \tag{14}$$

and setting $\mathrm{s}_0=0$, then from equation (15) the dirac-delta function becomes valid in an interval when a rule that integrates its product with another continuous function is assigned hence

$$\int_{-\infty}^{\infty}\delta(s-0)\mathrm{f}(\mathrm{s})\mathrm{ds} = \mathrm{f}(0) \tag{15}$$

Furthermore, following the definition of the author in [12],

$$\delta(\beta-\alpha) = \int \delta(s-\alpha)\delta(\beta-s)\,ds \tag{16}$$

The integral value 1 and the limiting value 0 both define the value of dirac-delta function δ which has a value 1 when $s=0$ and 0 if otherwise. *if*, $\int_{-\infty}^{\infty}\delta(s-s_0)f(s)ds \approx f(\bar{s})$, $\delta(s-s_0)$ is the kernel of the integral transform describing the dimensions of a rectangular paralleliped of length 2η and height $\frac{1}{2\eta}$ and centered at s_0 so that the area of the paralleliped will be 1. $\delta(s-s_0)$ isolates the real value of $f(s)$ at some prescribed point s_0 by the normalizing property of dirac-delta function,

$$\delta(s)=\delta(-s) \text{ and } \delta(s-\bar{s})=\delta(\bar{s}-s) \tag{17}$$

$$\int_{-\infty}^{\infty}\delta(s-\bar{s})ds=\int_{-\infty}^{\infty}\delta(s-\bar{s})ds=1\Rightarrow\int_{-\infty}^{\infty}\delta(t)dt=1, \text{ when } t=(s-\bar{s}) \tag{18}$$

Following the work of the author in [11], we let $f(z)$ be a function on which shift operator is defined as

$$E_{\Delta}f(z)=f(z+\Delta)\Rightarrow E_{-\Delta}f(z)=f(z-\Delta) \tag{19}$$

Define the function $G(\xi)=\int_{-\infty}^{\infty}f(\xi)\xi(z)dz$ where $\xi(z)$ is taken from the space of test function. If we assume that the functional $G[\xi]$ corresponds to $f(z)$, then $E_{\Delta}G[\xi]$ corresponds to $E_{\Delta}f(z)$, therefore

$$E_{\Delta}G[\xi]=\int_{-\infty}^{\infty}f(z+\Delta)\xi(z)dz=\int_{-\infty}^{\infty}f(z)\xi(z-\Delta)dz \tag{20}$$

where $E_{\Delta}G[\xi]=G[E_{-\Delta}\xi]$, if we invoke this definition on the dirac-delta function, then we have,

$$E_{\Delta}\delta(\xi)=\int_{-\infty}^{\infty}\delta(z+\Delta)\xi(z)dz=\delta[\xi(z-\Delta)]=\xi(-\Delta) \tag{21}$$

2.0 APPLICATION OF DIRAC-DELTA TO PROBABILITY DENSITY FUNCTIONS

In this section the goal is to test dirac-delta function on arbitrary random risk, to enable us apply it on excess of loss random variable. In view of the work of the authors in [13-14], the function $f(z)$ defines the final pay-off to a unit linked insurance which is maturing at time z. Consider a case where the origin has been shifted from zero to another point s_0, we have

$$z_1 < s_0 < z_2, \quad \int_{z_1}^{z_2} \delta(z - s_0) f(z) dz = \int_{z_1}^{z_2} \delta(z - s_0) f(s_0) dz = f(s_0) \int_{z_1}^{z_2} \delta(z - s_0) dz \tag{22}$$

$$\int_{z_1}^{z_2} \delta(z - s_0) f(z) dz = f(s_0) \times 1 = \partial(\delta(z)) = f(s_0), \tag{23}$$

$\partial(.)$ is the Laplace transform of $\delta(z)$.

The point $z = s_0$ is a contribution of the integral in equation (22), that is the first term of the Taylor's series expansion of $f(z)$ at the point $z = s_0$ and which vanishes at all other functional values. Again, substituting $s_0 = 0$, we have

$$\int_{z_1}^{z_2} \delta(z - 0) f(z) dz = f(0) \times 1 = f(0) \times 1 = f(0) \tag{24}$$

In view of the work of the author in [2], if $H(z)$ is the unit step function,
then

$$\delta(z - k) = \frac{dH(z - k)}{dz} \tag{25}$$

Now $F_Z(z)$ is the distribution function of a random risk Z with the property
that

$$\frac{dF_Z(z)}{dz} = f_Z(z) \tag{26}$$

Define $F_Z(z) = \sum_{z_i \in \Omega_Z} P(z_i) H(z - z_i)$, where Ω_Z is the support of Z. By the above
property,

$$\frac{dF_Z(z)}{dz} = \frac{d}{dz}\left[\sum_{z_i \in \Omega_Z} P(z_i) H(z - z_i)\right] = \left[\sum_{z_i \in \Omega_Z} P(z_i) \frac{d}{dz}\left[H(z - z_i)\right]\right] \tag{27}$$

so that the probability density function is obtained as

$$\frac{dF_Z(z)}{dz} = f_Z(z) = \sum_{z_i \in \Omega_Z} P(z_i) \delta(z - z_i) \tag{28}$$

where $\Omega_Z = \{z_i\}\ i = 1, 2, 3, \ldots$ and $P(z_i)$ are the mass points. Since the finite moments of the density function exists, then the average value of the random risk Z can be computed using the moments.

$$\langle Z \rangle = \int_{-\infty}^{\infty} z f_Z(z) dz = \int_{-\infty}^{\infty} \left[\sum_{z_i \in \Omega_Z} P(z_i) \delta(z - z_i)\right] z dz \tag{29}$$

$$\langle Z\rangle = \int_{-\infty}^{\infty}\left[\sum_{z_i\in\Omega_Z} P(z_i)\delta(z-z_i)\right]zdz = \left[\sum_{z_i\in\Omega_Z} P(z_i)\int_{-\infty}^{\infty} z\delta(z-z_i)dz\right], \quad (30)$$

since $f_Z(z) = z$.

$$\langle Z\rangle = \int_{-\infty}^{\infty}\left[\sum_{z\in\Omega_Z} P(z_i)\delta(z-z_i)\right]zdz = \left[\sum_{z_i\in\Omega_Z} z_i P(z_i)\right]. \quad (31)$$

$$\langle Z^2\rangle = \int_{-\infty}^{\infty} z^2 f_Z(z)dz = \int_{-\infty}^{\infty}\left[\sum_{z_i\in\Omega_Z} P(z_i)\delta(z-z_i)\right]z^2dz. \quad (32)$$

$$\langle Z^2\rangle = \left[\sum_{z_i\in\Omega_Z} P(z_i)\int_{-\infty}^{\infty} z^2\delta(z-z_i)dz\right] = \sum_{z_i\in\Omega_Z} z_i^2 P(z_i). \quad (33)$$

$$Var(Z) = \langle Z^2\rangle - \langle Z\rangle\langle Z\rangle = \left[\sum_{z_i\in\Omega_Z} z_i^2 P(z_i)\right] - \left[\sum_{z_i\in\Omega_Z} z_i P(z_i)\right]^2. \quad (34)$$

Hence the first two moments and variance are well defined. Furthermore, we can use the following approximation.

$$\int_{-\infty}^{\infty}\delta(z-z_1) f_Z(z)dz = \lim_{z_2\to z_1}\left[\frac{\int_{z_1}^{z_2}\delta(z) f_Z(z)dz}{z_2 - z_1}\right] = \lim_{z_2\to z_1}\left[\frac{f_Z\left(\frac{z_2+z_1}{2}\right)(z_2-z_1)}{z_2-z_1}\right] \quad (35)$$

$$\int_{-\infty}^{\infty}\delta(z-z_1) f_Z(z)dz = \lim_{z_2\to z_1}\left[f_Z\left(\frac{z_2+z_1}{2}\right)\right] = f_Z(z_1) \quad (36)$$

and dirac-delta function is a useful technique in this kind of estimation.

2.1 Underwriting control measures

In view of the work of the authors in [13-20], we observe that as a result of the consequences of the implicit cost such as moral hazard, underwriters have come up with loss control techniques to off-set these hidden costs from insurance contracts and discourage frivolous claims.

Let $a(s)$ define the value of a non-life insurance portfolio at a time s. The amount of loss in the interval

$$(s, s+\delta s) \text{ is given by } a(s) - a(s+\delta s) = l(s, s+\delta s); \quad (37)$$

$$\frac{a(s)-a(s+\delta s)}{\delta s} = \frac{l(s,s+\delta s)}{\delta s} \Rightarrow a'(s) = \frac{-l(s,s+\delta s)}{\delta s}. \quad (38)$$

Thus, if at times $\tau < s < t$, then

$$a(s) = a\left(\frac{t-s}{t-\tau}(\tau) + \frac{s-\tau}{t-\tau}(t)\right) \geq \frac{t-s}{t-\tau}a(\tau) + \frac{s-\tau}{t-\tau}a(t) \tag{39}$$

$$(t-s)a(\tau) + (s-\tau)a(t) \leq a(s)(t-\tau) \tag{40}$$

$$\frac{a(s)-a(\tau)}{s-\tau} \geq \frac{a(t)-a(\tau)}{t-\tau} \geq \frac{a(t)-a(s)}{t-s} \Rightarrow a'(\tau) > a'(s) \tag{41}$$

implying the differentiability of $a(s)$. If the unit time is being discretized as

$$s_k = k\delta s,\ k \in \{0\} \bigcup Z^+ \tag{42}$$

then

$$l(s_k, s_{k+1}) = a(s_k) - a(s_{k+1}) = a(k\delta s) - a((k+1)\delta s) \tag{43}$$

2.2 Theorem 1: Suppose $a(s)$ is a portfolio of non-life insurance policies and assume the scheme-holder incurs losses at a rate $a'(s)$ such that $a'(s) = -\alpha a(s)$ where α is constant. Let $\alpha\delta s$ define the probability that a policy holder incurs losses in an infinitesimal time of δs and define $\theta(s)$ to be the probability that the scheme holder does not incur losses at time s, then for any $\xi \in (s, s+\delta s)$, $h'(s) = -\alpha h(\xi)$ where h is a real probability density function

Proof: By definition $\theta(s+\delta s)$ is the probability that the scheme holder does not incur losses at time $s+\delta s$ given that he has not incurred losses at time s and consequently

$$\theta(s+\delta s) = (1-\alpha\delta s)\theta(s) \tag{44}$$

which implies

$$\theta(s+\delta s) = \theta(s) - \alpha\delta s\theta(s) \tag{45}$$

$$\frac{\theta(s+\delta s)-\theta(s)}{\delta s} = -\alpha\theta(s) \tag{46}$$

$$\theta'(s) = -\alpha\theta(s) \tag{47}$$

this defines an exponential solution pattern as observed in the conclusion by the author in [21] and this informs why we apply exponential distribution in our analysis. Then by definition, it is clear that

$$\theta'(s) = -h(s),\ \theta(s) = \frac{h(s)}{\alpha} \tag{47a}$$

If $h(s)$ is the probability density function of incurring losses at time s, then

$$\frac{d\theta(s)}{ds} = -h(s) \tag{48}$$

$$1-\theta(s+\delta s)-(1-\theta(s))=-\int_{s}^{s+\delta s}\frac{d\theta(\zeta)}{d\zeta}d\zeta=-\int_{s}^{s+\delta s}\theta'(\zeta)d\zeta=\int_{s}^{s+\delta s}h(\zeta)d\zeta=\theta(s)-\theta(s+\delta s) \quad (49)$$

$$\alpha\int_{s}^{s+\delta s}h(\zeta)d\zeta=(h(s)-h(s+\delta s)) \quad (49a)$$

By the mean value theorem,

$$\int_{s}^{s+\delta s}h(\zeta)d\zeta=(\delta s)h(\xi);\ \xi\in(s,s+\delta s) \quad (50)$$

$$\alpha(\delta s)h(\xi)=(h(s)-h(s+\delta s)) \quad (50a)$$

$$h'(s)=-\alpha h(\xi) \quad (50b)$$

The distribution of the random loss in (37) to the underwriting firm is enumerated below Z assumes the loss incurred in a loss event when there is no deductible but does not functionally depend on deductible and Z_L defines the amount incurred in a loss event under deductible while Z_p is the cost per payment in a payment event under deductible modifications. The loss event describes the condition of a loss but payment event is a condition where an underwriter incurs a fraction of a loss or wholly liable to pay everything. In practice, insurance data are only available on incurred payments. Following the work of the authors in [19-20; 22-25] when $Z<c$, the underwriter will repudiate claim advised except on ex-gratia basis to boost its goodwill and information content on losses will not be available thereby creating problems in insurance analysis. However, under deductible terms and conditions, the excess of loss random variable is only captured to the extent that the information content on Z_L is truncated.

As observed in the definitions by authors in [13;24;26-30], deductibles describe intermediate insurance transfer technique between total loss transfer and self-insurance to an underwriter. It has been applied to arouse interest of a few medium-sized employers but the rationale behind the attractiveness does not usually fall in line with the aims of insurance regulators and issues are usually raised by workers' union generally which need to be resolved. An insured with a per loss deductible c will repudiate claims whenever the claim of size Z falls short of or equal to the deductible c. However, when the claim value rises above the value c, the underwriter will pay the excess $(Z-c)$ The amount of loss covered by the underwriter and paid out as claim size in the loss event is defined in [1-2] by

$$Z_{\mathrm{L}}=\begin{cases}0 & \mathrm{Z}\le \mathrm{c}\\ Z-c & \mathrm{Z}>\mathrm{c}\end{cases} \quad (51)$$

$$Z_{\mathrm{L}}, 0 \le Z_{\mathrm{L}} \le z \tag{52}$$

$$Z_{\mathrm{L}} = \left(Z - \mathrm{c}\right)_{+} \text{ where } Z_{+} = \begin{cases} 0 & \mathrm{Z} \le 0 \\ Z & \mathrm{Z}>0 \end{cases} \tag{53}$$

$$Z_{+} = \begin{cases} Z & for \quad \mathrm{Z} \le c \\ c & for \quad \mathrm{Z} > c \end{cases} \tag{54}$$

This is the amount retained by the insured

$$\left\langle Z_{\mathrm{L}} \right\rangle = \left\langle \left(Z - \mathrm{c}\right)_{+} \right\rangle = \int_{0}^{\infty} \Pr\left(Z > \mathrm{c} + z\right) \mathrm{dz} \tag{55}$$

If $$\Pr\left(Z_{\mathrm{L}} = 0\right) = \mathrm{F}_{Z}\left(\mathrm{c}\right) \tag{56}$$

Then Z_{L} has a probability mass point at zero of $\mathrm{F}_{Z}\left(c\right)$ and hence

$$\mathrm{f}_{Z_{\mathrm{L}}}\left(Z\right) = \mathrm{f}_{Z}\left(z + \mathrm{c}\right) \text{ for } \mathrm{z} > 0 \tag{57}$$

The expected value function allows us to assess which losses from the risks, the insurance firm will bear in quantitative terms The random variable $\left(Z - c\right)_{+}$ describes the amount by which Z is greater than the threshold ceiling c,

$$\left(Z - c\right)_{+} = \int_{c}^{\infty} S_{Z}\left(\chi\right) d\chi \tag{58}$$

where $\left(\chi\right)_{+} = \max\left(\chi, 0\right)$. Furthermore,

$$\lim_{c \to -\infty} \left[\left(Z - c\right)_{+} + c\right] = \int_{c}^{\infty} S_{Z}\left(\chi\right) d\chi = \lim_{c \to -\infty} E\left(\max\left(Z, c\right)\right) = \left\langle Z \right\rangle \tag{59}$$

$$\left\langle Z \right\rangle = \int_{0}^{\infty} z f_{Z}\left(z\right) dz, \; f_{Z}\left(z\right) = \frac{\Pr\left(z < Z < z + \delta z\right)}{\delta z} \tag{60}$$

where $\left\langle . \right\rangle$ is the average value function.

We assume the existence of a positive differentiable non decreasing function of finite integral

$$\int_{0}^{\infty} \varphi\left(z\right) dF\left(z\right) < \infty \tag{61}$$

$$\left\langle \varphi\left(z\right) \right\rangle = \int_{0}^{\infty} \varphi\left(z\right) dF_{Z}\left(z\right) = -\int_{0}^{\infty} \varphi\left(z\right) d\left(\int_{0}^{\infty} f_{Z}\left(z\right) dz - F_{Z}\left(z\right)\right) \tag{62}$$

$$\left\langle \varphi\left(z\right) \right\rangle = -\varphi\left(\infty\right)\left[1 - F_{Z}\left(\infty\right)\right] + \varphi\left(0\right)\left[1 - F_{Z}\left(0\right)\right] + \int_{0}^{\infty} \left(1 - F_{Z}\left(\infty\right)\right) d\varphi\left(z\right) \tag{63}$$

$$\varphi\left(0\right) = 0, \varphi\left(0\right)\left[1 - F_{Z}\left(0\right)\right] = 0, and, \varphi\left(\infty\right)\left[1 - F_{Z}\left(\infty\right)\right] = 0, F_{Z}\left(\infty\right) = 1 \tag{64}$$

$$\langle \varphi(z) \rangle = \int_0^{\infty} (1 - F_Z(\infty)) d\varphi(z) \tag{65}$$

Suppose $p(z)$ is the payment function, then

$$\langle p(z) \rangle = \int_0^{\infty} (1 - F(z)) dp(z), p(0) = 0 \tag{66}$$

If $\langle p(z) \rangle = \Omega$, then the mean loss implies that

$$\Omega = \int_0^{\infty} (1 - p(z)) dp(z) \tag{67}$$

By definition,

$$\int_c^{\infty} (1 - F_Z(z)) dz = \int_c^{\infty} S_Z(z) dz = \Omega \tag{68}$$

$$F_{Z_L}(z) = F_Z(z + c), z > 0 \tag{69}$$

$$S_{Z_L}(z) = S_Z(z + c) \tag{70}$$

$$\langle Z_L \rangle = \frac{\langle \max(0, Z - c) \rangle}{\langle z \rangle} = \frac{\langle \min(Z, c) \rangle}{\langle z \rangle} = \int_{-\infty}^{\infty} (z) f_{Z_L}(z) dz =$$

$$\int_0^{\infty} (z) f_{Z_L}(z) dz = \int_c^{\infty} (z - c) f_Z(z) dz \tag{71}$$

$$\langle Z_L \rangle = \int_c^{\infty} (z - c) dF_Z(z) = (z - c) F_Z(z) |_c^{\infty} - \int_c^{\infty} F_Z(z) dz = 1 - \int_c^{\infty} F_Z(z) dz \tag{72}$$

3.0 MATERIAL AND METHODS

The dirac-delta technique has been pesented as a novel advanced method to model insurance severity in order to usher in fresh light into the puzzling investigations in actuarial literature that parametric models tend to equally solve when deductible clauses are built into insurance contracts. The conditional excess of loss random variable remains valid provided there is a payment.

$$Z_p = (Z - c) | Z > c \tag{73}$$

$$Z_p = Z_L | Z_L > 0 \tag{74}$$

$$\langle Z_L \rangle = E(Z_L | Z_L = 0) P(Z_L = 0) + E(Z_L | Z_L > 0) P(Z_L > 0)$$

$$\langle Z_L \rangle = 0 + E(Z_L | Z_L > 0) P(Z_L > 0) = \langle Z_P \rangle P(Z_L > 0) \tag{75}$$

$$F_{Z_L}(z) = F_Z(z+c) \Rightarrow F_{Z_L}(0) = F_Z(c) \tag{75}$$

$$S_{Z_L}(z) = S_Z(z+c) \Rightarrow S_{Z_L}(0) = S_Z(c) \tag{76}$$

$$\langle Z_L \rangle = \langle Z_P \rangle S_{Z_L}(0) = \langle Z_P \rangle S_Z(c) \tag{77}$$

$$\langle Z_P \rangle = \frac{\langle Z_L \rangle}{S_Z(c)} = \frac{\langle \max(0, Z-c) \rangle}{\langle z \rangle S_Z(c)} = \frac{\langle \min(Z,c) \rangle}{\langle z \rangle S_Z(c)} \tag{78}$$

$$f_{Z_p}(z) = \frac{F_Z'(z+c)}{(1-F_Z(c))} = \frac{f_z(z+c)}{(1-F_Z(c))}, S_{Z_p}(z) = \frac{S_Z(z+c)}{(1-F_Z(c))} \tag{79}$$

$$\langle Z_P \rangle = \int_0^\infty (z)\, dF_{z_p}(z) = \int_0^\infty (z) \frac{dF_Z(z+c)}{(1-F_Z(c))} = \int_c^\infty (z-c) \frac{dF_Z(z)}{(1-F_Z(c))} = \frac{\langle z_L \rangle}{(1-F_Z(c))} \tag{80}$$

$$\langle Z_P \rangle = \frac{1}{(1-F_Z(c))} \int_c^\infty (Z-c) \sum_{j=1}^m P_j \delta(Z - z_j^*) dz = \frac{1}{(1-F_Z(c))} \sum_{j=1}^m P_j \int_c^\infty (Z-c) \delta(Z - z_j^*) dz$$

$$\langle Z_P \rangle = \frac{\sum_{j=1}^m P_j (z_j^* - c)}{(1-F_Z(c))} = \frac{\sum_{j=1}^m P_j z_j^*}{(1-F_Z(c))} - \frac{c \sum_{j=1}^m P_j}{(1-F_Z(c))} \tag{81}$$

$$\langle Z_P \rangle = \frac{\sum_{j=1}^m P_j (z_j^* - c)}{(1-F_Z(c))} = \frac{\sum_{j=1}^m P_j z_j^*}{(1-F_Z(c))} - \frac{c}{(1-F_Z(c))}, \sum_{j=1}^m P_j = 1 \tag{82}$$

$$\langle Z_P \rangle = \frac{\sum_{j=1}^m P_j (z_j^* - c)}{\left(1 - \int_{-\infty}^c f_Z(t)\, dt\right)} = \frac{\sum_{j=1}^m P_j z_j^*}{\left(1 - \int_{-\infty}^c f_Z(t)\, dt\right)} - \frac{c}{\left(1 - \int_{-\infty}^c f_Z(t)\, dt\right)} \tag{83}$$

$$\langle Z_P \rangle = \frac{\sum_{j=1}^m P_j (z_j^* - c)}{\left(1 - \int_0^c f_Z(t)\, dt\right)} = \frac{\sum_{j=1}^m P_j z_j^*}{\left(1 - \int_0^c f_Z(t)\, dt\right)} - \frac{c}{\left(1 - \int_0^c f_Z(t)\, dt\right)} \tag{84}$$

Based on the use of the density function in equation (84), we state and prove the following theorem.

3.1 Theorem 2:

$$\text{Let } Z = \begin{cases} 0 & \textit{with probility } p \\ B & \textit{with probability } (1-p) \end{cases} \tag{85}$$

then p can be estimated as

$$\text{(i) } p = \left(\frac{F_Z(t) - F_B(t)}{S_B(t)} \right); \tag{86}$$

$$\text{(ii) } q = \frac{f_Z(t)}{f_B(t)} . \tag{87}$$

Proof

Let $$I_B = \begin{cases} 0 \quad having \quad probability \quad p \\ 1 \quad having \quad probability \quad (1-p) \end{cases} \tag{88}$$

be the indicator function of the random loss B.

Hence $Z = BI_B$. Suppose $B > 0$ is the loss event, but if $I_B = 0$, then $Z = 0$.
For any real $t > 0$,

$$\int_{-\infty}^{\infty} f_Z(t)dt = \Pr(t \geq 0) = 1 - \Pr(t < 0) = 1 \tag{86}$$

$$\int_{-\infty}^{t} f_Z(s)ds = \Pr(Z \leq s) = \Pr(I_B = 0)\Pr(Z \leq s \mid I_B = 0) + \Pr(I_B = 1)\Pr(Z \leq s \mid I_B = 1) \tag{87}$$

$$\int_{-\infty}^{t} f_Z(s)dt = \Pr(I_B = 0)\Pr(BI_B \leq s \mid I_B = 0) + \Pr(I_B = 1)\Pr(BI_B \leq s \mid I_B = 1) \tag{88}$$

$$\int_{-\infty}^{t} f_Z(s)ds = (p)\Pr(s \geq 0) + (1-p)\Pr(B \leq s) = p + (1-p)F_B(s) \tag{89}$$

$$\int_{0}^{t} f_Z(s)ds = (p)\Pr(s \geq 0) + (1-p)\Pr(B \leq s) \tag{90}$$

$$F_Z(s) = p \times 1 + (1-p)F_B(s) = p \times (1 - F_B(s)) + F_B(s) = p \times (S_B(s)) + F_B(s) \tag{91}$$

$$\left(\frac{F_Z(s) - F_B(s)}{S_B(s)} \right) = p \tag{92}$$

From equation (91),

$$F_Z'(s) = (1-p)F_B'(s) \Rightarrow f_Z(s) = (1-p)f_B(s) = qf_B(s) \tag{93}$$

$$q = \frac{f_Z(s)}{f_B(s)} \tag{94}$$

3.2 Theorem 3: As a consequence of the application of expected value function, we also state and prove the following. If $Z > 0$ is a random risk and let $\eta(Z)$ be a differentiable function of Z such that $\lim_{Z \to 0} \eta(Z) = 0$, then

$$E\left(\eta\left(Z\right)\right)=\int_{-\infty}^{\infty}\eta'\left(Z\right)\Pr\left(Z>s\right)ds=\int_{0}^{\infty}\eta'\left(Z\right)\Pr\left(Z>s\right)ds \tag{95}$$

Proof:

Let $$I_{Z>s}=\begin{cases}1 & for \quad Z>s\\ 0 & elswhere\end{cases} \tag{96}$$

Then by definition,

$$E\left(I_{Z>s}\right)=\Pr\left(Z>s\right) \tag{97}$$

$$\int_{-\infty}^{\infty}\eta'\left(s\right)I_{Z>s}ds=\int_{0}^{Z}\frac{d\eta\left(s\right)}{ds}ds=\int_{0}^{Z}d\eta\left(s\right)ds= \tag{100}$$
$$\eta\left(Z\right)-\eta\left(0\right)=\eta\left(Z\right)-0=\eta\left(Z\right)$$

$$E\left(\int_{-\infty}^{\infty}\eta'\left(s\right)I_{Z>s}ds\right)=\int_{0}^{Z}E\left(\eta'\left(s\right)I_{Z>s}\right)ds=\int_{0}^{Z}\left(\eta'\left(s\right)\right)E\left(I_{Z>s}\right)ds=E\left(\eta\left(Z\right)\right) \tag{101}$$

$$E\left(\int_{-\infty}^{\infty}\eta'\left(s\right)I_{Z>s}ds\right)=\int_{0}^{Z}\left(\eta'\left(s\right)\right)\Pr\left(Z>s\right)ds \tag{102}$$

$$E\left(\int_{0}^{\infty}\eta'\left(s\right)I_{Z>s}ds\right)=\int_{0}^{Z}\left(\eta'\left(s\right)\right)\Pr\left(Z>s\right)ds=E\left(\eta\left(Z\right)\right) \tag{103}$$

The distribution of loss above shows the probability of a defined magnitude coupled with the probability of a particular loss exceeding or falling under a certain loss. The distribution of loss can then be employed to calculate both the expected loss excess of the deductible amount and the expected proportion of total losses at a defined ceiling. The severity model represents the actuarial technique of achieving the expected size of claims which an insurance firm may likely experience in a given period and the cost of average claim. As reported in the work of the author in [25], we see that in the severity technique, past data profile is used to model the estimated average size of claims and the average cost per claim. A high frequency of claims may indicate that the underwriting firm expects a large number of claims. Insurance experts apply advanced actuarial models to determine the probability that insurance firm will pay out a claim and summarize insurance data set which will be subsequently needed and properly interpreted for underwriting decision process. Appraising actuarial model to compute rate differentials may not be immediately apparent since policy holder behavior may influence the frequency the number of events and the severity of the events. Thus, the magnitude by which loss is eliminated is the reduction in the loss incurred.

$$LE\left(z\right)=\left\langle z\right\rangle-\left\langle z_{L}\right\rangle. \tag{104}$$

The loss elimination ratio:

$$LER=\frac{\left\langle z\right\rangle-\left\langle z_{L}\right\rangle}{\left\langle z\right\rangle}=\frac{\left\langle z\right\rangle-\left\langle \max\left(0,Z-c\right)\right\rangle}{\left\langle z\right\rangle}=\frac{\left\langle \min\left(Z,c\right)\right\rangle}{\left\langle z\right\rangle} \tag{105}$$

computes the ratio of the reduction in the expected mean loss of an underwriter writing a contract due to defined deductible conditions c to the expected loss of same underwriter writing a full coverage

$$\left\langle \min(\theta, c) \right\rangle = \int_0^c \left(1 - F_\theta(z)\right) dz \tag{106}$$

3.3 Data analysis

In general insurance practice, *data on deductibles* is usually unavailable because they are claims which are only borne by individual scheme holder and moreover because of the confidentiality of insurance data base. Instead of the raw deductible data, we obtained rate relativity on deductible through a non-life insurance *agent* operating in property insurance market at Lagos. In order to present logical arguments, we solve the following standard empirical problem by considering an insured risk Y with unit sum insured of insurance cover with specified deductibles C under an *assumption* of exponential distributions $0.1 \le C \le 1, Z \sim EXP(\alpha), \alpha = 1$. For ease of computation, we consider the exponential distribution.

3.4 Exponential distribution

$$S_Z(C) = e^{-\alpha C}, g_Z(C) = \alpha e^{-\alpha C}, H_Z(y) = \frac{g_Z(C)}{S_Z(C)} \tag{107}$$

$$\left\langle Z_L \right\rangle = \left\langle (z - 0.15)_+ \right\rangle = \int_{0.15}^{\infty} e^{-z} dZ = e^{-0.15} = 0.86071, \tag{108}$$

$$S_Z(0.15) = e^{-0.15} = 0.86071 \Rightarrow \frac{\left\langle Z_L \right\rangle}{S_Z(C)} = \left\langle Z_P \right\rangle_{\exp} = \int_0^{\infty} e^{-z} dz = 1 \tag{109}$$

Now, $\left\langle Z_p \right\rangle = \int_0^{\infty} g_Z(z) dz = 1$, hence, we can see that $\left\langle Z_L \right\rangle < \left\langle Z_p \right\rangle$ that is the cost per loss amount is less than the cost per payment amount. The loss eliminated (*LE*) and loss elimination ratio (*LER*) are as given below

$$LE(z) = \frac{1}{\alpha} - \left\langle Z_L \right\rangle = 1 - \left\langle Z_L \right\rangle, LER(z) = \frac{\left\langle Z \right\rangle - \left\langle Z_L \right\rangle}{\left\langle Z \right\rangle} \tag{110}$$

$$LR(z) = \frac{1}{\alpha} - E(Z_L) = 1 - 0.86071 = 0.13929$$

TABLE 1: Relativity of Deductible and Loss Elimination Ratio for Exponentially Distributed Claim

Deductible C	**Cost Per Loss** Z_L	**Loss Ratio (LR)**	**Loss Elimination ratio(LER)**	**Change in LER**
0.1	0.904837	0.095163	0.095163	-
0.15	0.860708	0.139292	0.139292	0.0441
0.2	0.818731	0.181269	0.181269	**0.042**
0.25	0.778801	0.221199	0.221199	0.0399
0.3	0.740818	0.259182	0.259182	0.038
0.35	0.704688	0.295312	0.295312	0.0361
0.4	0.67032	0.32968	0.32968	0.0344
0.45	0.637628	0.362372	0.362372	0.0327
0.5	0.606531	0.393469	0.393469	0.0311
0.55	0.57695	0.42305	0.42305	0.0296
0.6	0.548812	0.451188	0.451188	0.0281
0.65	0.522046	0.477954	0.477954	0.0268
0.7	0.496585	0.503415	0.503415	0.0255
0.75	0.472367	0.527633	0.527633	0.0242
0.8	0.449329	0.550671	0.550671	0.023
0.85	0.427415	0.572585	0.572585	0.0219
0.9	0.40657	0.59343	0.59343	0.0208
0.95	0.386741	0.613259	0.613259	0.0198
1	0.367879	0.632121	0.632121	0.0189

From the table 1 above, we observe that as the deductible increases, the loss eliminated also increases and consequently, the ratio of the loss eliminated seems directly proportional to the deductibles but from the last column and below the indicator level of **4.2%** , it seems high deductibles would not offer a reasonable fraction of the eliminated loss due to the underwriter. Consequently, column 4 represents the ratio of a reduction in the expected loss for an underwriting firm which writes a scheme with a deductible clause or with a policy limit imposed on the expected loss where the firm provides full insurance cover.

The considerations for deductible are quite different depending on policy terms & conditions and on the risk preferences of the scheme holder. The underwriter may consider to apply the above hypothetical table of deductible relativity and use it as a guide to confirm if the deductible proposed by the scheme holder could offer a reasonable level of losses eliminated to the underwriter. However, the insurance firm should be cautious as high deductible is not financially ethical for the insured because they would bear a higher percentage of the losses arising from the insured peril. However, high deductibles may be imposed because of underwriting cost saving

conditions, loss control motivations and the burden of falling residuary insurance market. Irrespective of the operating deductible, the goal is to make the scheme holder risk-conscious since he would pay the proportion of the total loss. However, provided that large number of losses is lower than the deductible, the administrative costs incurred by the underwriter to offset liabilities and maintain solvency will drop hence the premium payable by the policy holder will decline compared to full coverage conditions.

From the computations above, we see that a policy limit at $C = 0.150$ for instance shows a loss elimination ratio of 0.139292 meaning that roughly 13.92% of losses incurred will be eliminated by introducing a modification of 0.15. We observe further that the cost per loss values in column 2 are strictly less than unity which is the value of cost per payment in a payment event, implying that cost per loss will always be strictly less than the cost per payment, hence $\langle Z_L \rangle < \langle Z_p \rangle = 1$. Theoretically, it is expected that the net present value of cash in-flows to the underwriting firm will exceed the underwriting income in the event that the investment income cash flow is assumed. This is because premium charged is paid to the underwriter at the time when scheme is incepted, but claims are assumed to be paid in the long run and it is therefore reasonable to assume that the underwriter would have earned return on invested premium.

4. RESULTS AND DISCUSSION

Using the earlier definition of $\langle Z_p \rangle$, we find that

$$\langle Z^2{}_{\mathrm{p}} \rangle = \frac{1}{\left(1 - F_Z(c)\right)} \int_{-\infty}^{\infty} (z - \mathrm{c})^2 \, dF_{Z_{\mathrm{L}}} \mathrm{dz} \tag{111}$$

Because density is only defined on the real line, we integrate from zero to infinity

$$\langle Z^2{}_{\mathrm{p}} \rangle = \int_0^{\infty} (z - \mathrm{c})^2 \, \mathrm{f}_{Z_{\mathrm{L}}}(z) \mathrm{dz} \tag{112}$$

But by the definition of deductible, we integrate from c to infinity

$$\langle Z^2{}_{\mathrm{p}} \rangle = \frac{1}{\left(1 - F_Z(c)\right)} \int_{\mathrm{c}}^{\infty} (z - \mathrm{c})^2 \sum_{\mathrm{j}=1}^{\mathrm{m}} \mathrm{P_j} \delta\left(z - \mathrm{z_j}^*\right) \mathrm{dz} \tag{113}$$

$$\langle Z^2{}_{\mathrm{p}} \rangle = \frac{1}{\left(1 - F_Z(c)\right)} \sum_{\mathrm{j}=1}^{\mathrm{m}} \mathrm{P_j} \int_{\mathrm{c}}^{\infty} (z - \mathrm{c})^2 \, \delta\left(z - \mathrm{z_j}^*\right) \mathrm{dz} \tag{114}$$

$$\langle Z^2{}_{\mathrm{p}} \rangle = \frac{1}{\left(1 - F_Z(c)\right)} \sum_{\mathrm{j}=1}^{\mathrm{m}} \mathrm{P_j} \left(\mathrm{z_j}^* - \mathrm{c}\right)^2 = \frac{\sum_{\mathrm{j}=1}^{\mathrm{m}} \mathrm{P_j} \left(\mathrm{z_j}^*\right)^2}{\left(1 - F_Z(c)\right)} - \frac{2\mathrm{c} \sum_{\mathrm{j}=1}^{\mathrm{m}} \mathrm{P_j z_j}^*}{\left(1 - F_Z(c)\right)} + \frac{\sum_{\mathrm{j}=1}^{\mathrm{m}} \mathrm{P_j c}^2}{\left(1 - F_Z(c)\right)} \tag{115}$$

$$\left\langle Z^2{}_p \right\rangle = \frac{\sum_{j=1}^{m} P_j \left(z_j^*\right)^2}{\left(1-F_Z(c)\right)} - \frac{2c\sum_{j=1}^{m} P_j z_j^*}{\left(1-F_Z(c)\right)} + \frac{c^2}{\left(1-F_Z(c)\right)} \tag{116}$$

$$\mathrm{Var}\left(Z_p\right) = \frac{\sum_{j=1}^{m} P_j \left(z_j^*\right)^2}{\left(1-F_Z(c)\right)} - \frac{2c\sum_{j=1}^{m} P_j z_j^*}{\left(1-F_Z(c)\right)} + \frac{c^2}{\left(1-F_Z(c)\right)} - \left(\frac{\sum_{j=1}^{m} P_j z_j^*}{\left(1-F_Z(c)\right)} - \frac{c}{\left(1-F_Z(c)\right)} \right)^2 \tag{117}$$

$$\mathrm{Var}\left(Z_p\right) = \frac{\sum_{j=1}^{m} P_j \left(z_j^*\right)^2}{\left(1-F_Z(c)\right)} - \frac{2c\sum_{j=1}^{m} P_j z_j^*}{\left(1-F_Z(c)\right)} + \frac{c^2}{\left(1-F_Z(c)\right)} - \left(\left(\frac{\sum_{j=1}^{m} P_j z_j^*}{\left(1-F_Z(c)\right)} \right)^2 + \left(\frac{c}{\left(1-F_Z(c)\right)} \right)^2 - 2\frac{\sum_{j=1}^{m} P_j z_j^*}{\left(1-F_Z(c)\right)} \left(\frac{c}{\left(1-F_Z(c)\right)} \right) \right) \tag{118}$$

$$\mathrm{Var}\left(Z_p\right) = \frac{\sum_{j=1}^{m} P_j \left(z_j^*\right)^2}{\left(1-F_Z(c)\right)} - \frac{2c\sum_{j=1}^{m} P_j z_j^*}{\left(1-F_Z(c)\right)} + \frac{c^2}{\left(1-F_Z(c)\right)} - \frac{\left(\sum_{j=1}^{m} P_j z_j^*\right)^2}{\left(1-F_Z(c)\right)^2} - \frac{c^2}{\left(1-F_Z(c)\right)^2} + \frac{2c\sum_{j=1}^{m} P_j z_j^*}{\left(1-F_Z(c)\right)^2} \tag{119}$$

$$\mathrm{Var}\left(Z_p\right) = \frac{\sum_{j=1}^{m} P_j \left(z_j^*\right)^2}{\left(1-F_Z(c)\right)} - \frac{2c\sum_{j=1}^{m} P_j z_j^*}{\left(1-F_Z(c)\right)} + \frac{c^2}{\left(1-F_Z(c)\right)} - \frac{\left(\sum_{j=1}^{m} P_j z_j^*\right)^2}{\left(1-F_Z(c)\right)^2} - \frac{c^2}{\left(1-F_Z(c)\right)^2} + \frac{2c\sum_{j=1}^{m} P_j z_j^*}{\left(1-F_Z(c)\right)^2} \tag{120}$$

$$\mathrm{Var}\left(Z_p\right) = \frac{\left(1-F_Z(c)\right)\sum_{j=1}^{m} P_j \left(z_j^*\right)^2}{\left(1-F_Z(c)\right)^2} - \frac{2c\left(1-F_Z(c)\right)\sum_{j=1}^{m} P_j z_j^*}{\left(1-F_Z(c)\right)^2} + \frac{c^2\left(1-F_Z(c)\right)}{\left(1-F_Z(c)\right)^2} - \frac{\left(\sum_{j=1}^{m} P_j z_j^*\right)^2}{\left(1-F_Z(c)\right)^2} - \frac{c^2}{\left(1-F_Z(c)\right)^2} + \frac{2c\sum_{j=1}^{m} P_j z_j^*}{\left(1-F_Z(c)\right)^2} \tag{120a}$$

$$\mathrm{Var}\left(Z_{\mathrm{p}}\right)=\frac{\left(1-F_Z\left(c\right)\right)\sum_{\mathrm{j}=1}^{\mathrm{m}}\mathrm{P}_{\mathrm{j}}\left(z_{\mathrm{j}}^{*}\right)^2}{\left(1-F_Z\left(c\right)\right)^2}-\frac{\left(\sum_{\mathrm{j}=1}^{\mathrm{m}}\mathrm{P}_{\mathrm{j}}z_{\mathrm{j}}^{*}\right)^2}{\left(1-F_Z\left(c\right)\right)^2}-\frac{2\mathrm{c}\sum_{\mathrm{j}=1}^{\mathrm{m}}\mathrm{P}_{\mathrm{j}}z_{\mathrm{j}}^{*}}{\left(1-F_Z\left(c\right)\right)^2}+\frac{2c\left(F_Z\left(c\right)\right)\sum_{\mathrm{j}=1}^{\mathrm{m}}\mathrm{P}_{\mathrm{j}}z_{\mathrm{j}}^{*}}{\left(1-F_Z\left(c\right)\right)^2}$$
$$+\frac{\mathrm{c}^2}{\left(1-F_Z\left(c\right)\right)^2}-\frac{\mathrm{c}^2\left(F_Z\left(c\right)\right)}{\left(1-F_Z\left(c\right)\right)^2}-\frac{\mathrm{c}^2}{\left(1-F_Z\left(c\right)\right)^2}+\frac{2c\sum_{\mathrm{j}=1}^{\mathrm{m}}\mathrm{P}_{\mathrm{j}}z_{\mathrm{j}}^{*}}{\left(1-F_Z\left(c\right)\right)^2} \tag{121}$$

$$\mathrm{Var}\left(Z_{\mathrm{p}}\right)=\frac{\left(1-F_Z\left(c\right)\right)\sum_{\mathrm{j}=1}^{\mathrm{m}}\mathrm{P}_{\mathrm{j}}\left(z_{\mathrm{j}}^{*}\right)^2}{\left(1-F_Z\left(c\right)\right)^2}-\frac{\left(\sum_{\mathrm{j}=1}^{\mathrm{m}}\mathrm{P}_{\mathrm{j}}z_{\mathrm{j}}^{*}\right)^2}{\left(1-F_Z\left(c\right)\right)^2}+\frac{2c\left(F_Z\left(c\right)\right)\sum_{\mathrm{j}=1}^{\mathrm{m}}\mathrm{P}_{\mathrm{j}}z_{\mathrm{j}}^{*}}{\left(1-F_Z\left(c\right)\right)^2}-\frac{\mathrm{c}^2\left(F_Z\left(c\right)\right)}{\left(1-F_Z\left(c\right)\right)^2} \tag{121a}$$

$$\mathrm{Var}\left(Z_{\mathrm{p}}\right)=\frac{\left(1-F_Z\left(c\right)\right)\sum_{\mathrm{j}=1}^{\mathrm{m}}\mathrm{P}_{\mathrm{j}}\left(z_{\mathrm{j}}^{*}\right)^2}{\left(S_Z\left(c\right)\right)^2}-\frac{\left(\sum_{\mathrm{j}=1}^{\mathrm{m}}\mathrm{P}_{\mathrm{j}}z_{\mathrm{j}}^{*}\right)^2}{\left(S_Z\left(c\right)\right)^2}+\frac{2c\left(F_Z\left(c\right)\right)\sum_{\mathrm{j}=1}^{\mathrm{m}}\mathrm{P}_{\mathrm{j}}z_{\mathrm{j}}^{*}}{\left(S_Z\left(c\right)\right)^2}-\frac{\mathrm{c}^2\left(F_Z\left(c\right)\right)}{\left(S_Z\left(c\right)\right)^2} \tag{122}$$

This variance of the random claim size under the deductible policy contract accounts for the fluctuations of risk indicators and defines the degree of variations of outcome produced from the model. The variance of cost per payment may likely fall within many standard deviations of its severity so that small variance will lead to prectitable probability outcome especially when computing the probability that an insurance firm will make aggregate loss or profit over all its insured schemes. Thus the magnitude by which loss is eliminated (*LE*) is the reduction in the loss incurred

$$LE\left(z\right)=\left\langle z\right\rangle-\left\langle z_L\right\rangle \tag{123}$$

Based on the definitions in equations (58), (65), (66) and (71), if S is the sum insured; $k=\left(1-c\right)$, the coverage level for $0\le k\le 1$; Z the risk insured; then the premium of insurance contract under the defined deductible level chosen by the policy holder could be obtained based on a convex actuarial premium rating function $p\left(k\right)$ as follows.

4.1 Theorem 4: Let h be the premium equation which can be written linearly as a function of k

$$h\left(k\right)-p\left(k_0\right)=p'\left(k_0\right)\left(k-k_0\right)=m\left(k-k_0\right) \tag{124}$$

where

$$m = \left.\frac{dp(k)}{dk}\right|_{k=k_0} \text{ is the gradient of } h \tag{125}$$

then

$$p(k_0) \le p(k_1) + \frac{(k_0 - k_1)}{(k_2 - k_0)}\left(p(k_2) - p(k_0)\right) \tag{126}$$

Proof: From our arguments in equations (37), we can write the premium equation linearly as

$$p(k) - h(k) = p(k) - \left(p(k_0) + m(k - k_0)\right) = p(k) - p(k_0) - m(k - k_0) \tag{127}$$

$$p(k) - h(k) = p(k) - \left(p(k_0) + m(k - k_0)\right) = \left(p'(\theta) - p'(k_0)\right)(k - k_0) \tag{128}$$

for $y_0 < \theta < y$.

If p is convex, then $p' > 0$, therefore it implies that either both $p'(\theta) - p'(k_0) > 0$ and $(k - k_0) > 0$ or both $p'(\theta) - m < 0$ and $(k - k_0) < 0$, hence in either case

$$p(k) \ge h(k) \Rightarrow p(k) - h(k) \ge 0 \tag{129}$$

consequently,

$$p(k) - h(k) = p(k) - \left(p(k_0) + m(k - k_0)\right) \ge 0 \tag{130}$$

again, either

$$\frac{p(k) - p(k_0)}{k - k_0} \le m; k < k_0 \tag{131}$$

or

$$\frac{p(k) - p(k_0)}{k - k_0} \ge m; k > k_0 \tag{132}$$

and consequently, if we have $k_1 < k_0 < k_2$, then

$$\frac{p(k_0) - p(k_1)}{k_0 - k_1} \le \frac{p(k_2) - p(k_0)}{k_2 - k_0} \Rightarrow p(k_0) \le p(k_1) + \frac{(k_0 - k_1)}{(k_2 - k_0)}\left(p(k_2) - p(k_0)\right) \tag{133}$$

Suppose $p(k)$ is an increasing and convex function such that $p'(k) > 0$ and $p''(k) > 0$, then the total premium for the chosen coverage level k can then be $S \times k \times p(k)$. Under the distribution of underlying risk, the premium function above can be defined in the form

$$p(k) = \frac{1}{Sk}\int_0^{S(1-C)} \max(0, Sk - Z)\, dF_Z(z) \tag{134}$$

$$p(k) = \frac{1}{Sk}\int_0^{S(1-C)} \max(0, Sk - Z)\, f_Z(z) dz \tag{135}$$

where $f_Z(z)$ is the probability density function of the insured risk.

We note that

$$\frac{1}{SK}\int_0^{S(1-C)} \max(0, Sk - Z)\, f_Z(z)dz < \int_{-\infty}^{\infty} f_Z(z)dz = 1 \tag{136}$$

$$\int_0^{S(1-C)} \max(0, Sk - Z)\, f_Z(z)dz < Sk \text{ , for every } k > 0 \tag{137}$$

and such that the distribution function of the coverage level is

$$F_Z(Sk) = \int_0^{S+C-1} f_Z(z)\,dz \tag{138}$$

CONCLUSION

The technique of computing mean loss subject to contract modifications was investigated where we obtained and compared models for computing amount paid in a loss event and in a payment event. Insurance contracts are modified to achieve typical payment functions such as deductible the effect of which is appraised in this paper. In describing a platform of applying generalized functions to study the behavior of risk functions, the dirac-delta technique has been applied to formulate insurance model regarding claim severities and the variance function. The dirac-delta function has then been successfully applied with the objective of drawing attention to some grey area applications of this function in general business insurance. Part of the motivation for using dirac-delta functions, lies in its elegance to permit alternative technique to obtain analytically useful models for insurance severity. In this paper, the severity of an insurance contract under direct delta function with particular rate relativity deductible clause is obtained but this could lead to a critical issue if the rate relativity deductible is not known or missing. Dirac-delta function approach is technically much more convenient in terms of computational superiority and soundness. After appraising the dynamics governing the severity, we are able to build a loss model by applying the dirac-delta function.

The paper presents a dirac-delta-deductible method in the analysis of severity coverages. Correspondence in the analysis of severity coverage usually assumes a rigorous dimension which could conceal the ease of the underlying idea but the dirac-delta function serves to exemplify much of the difficult expression which is the key tool used to deal with actuarial principles involved and to represent magnitude of insurance loss. In this paper, we have applied the dirac-delta function to obtain: **(i)**The expected cost per payment claim severity under deductible policy of general insurance using first moment as reported in (84) **(ii)**The second moment of cost per payment under deductible policy contract of general insurance as reported in (116) **(iii)**The variance of the cost per payment loss event under the deductible coverage modifications obtained and reported in equation (122).

REFERENCES

[1] Ogungbenle, M.G., Adeyele J. S., and Mesioye A.E. (2020.). Modeling moments of insurance claim size under dirac-delta function. *Daffodil International University Journal of Science and Technology*, ***15***(1), 43-51.

[2] Ogungbenle, M.G. (2021a). A theoretical Cauchy-Bunyakovsky-Schwartz inequality estimation of upper bound for the expected claim per loss payment in non-life insurance business. *Journal of New Frontiers in Mathematics and Statistics*, ***2***(2), 1-19.

[3] Mohammed H.R. (2011). Dirac's function and its application in solving some problems in mathematics. *Journal of Al-Qadisiya for Computer Science and Mathematics* ***3***, 1-15.

[4] DeLyra J. L. (2018). Complex analysis of real functions I – complex-analytic structure and integrable real functions. *Transnational Journal of Mathematical Analysis and Applications*, ***6***(1), 15–61.

[5] Kanwal R.P. (1998). *Function theory and technique* (2nd Edition), Birkhauser, Boston, pp. 1-17.

[6] Saichev A.I., and Woyczynski W.A. (1997). *Distributions in the physical and engineering sciences*, Boston, MA, Birkhauser, pp. 1-38.

[7] Khuri A.I. (2004). Applications of dirac's delta function in statistics. *International Journal of Mathematical Education in Science and Technology*, ***35***(2). 185-195.

[8] Brewster R.A., and Franson J.D. (2018.). Generalized delta functions and their use in quantum optics. *Journal of Mathematical Physics*, ***59***, 012102-17.

[9] Chakraborty S. (2008). Some Applications of Dirac-delta Function in statistics for more than one random variable. *Application and Applied Mathematics Journal* ***3***(1), 42-54.

[10] Onural L. (2006). Impulse functions over curves and surfaces and their application to diffraction. *Journal of Mathematical Analysis and Applications*, ***322***(1), 18-27.

[11] Sastry S.S. (2009). *Advanced engineering mathematics*. PHI learning ltd, New Delhi, pp. 36-57.

[12] Zhang L. (2018). On the calculus of dirac-delta function with some applications. *International Journal of Mathematics Trends and Technology*, ***56***(4),.258-270.

[13] Dimitriyadis I., and Oney, U.N. (2009). Deductibles in health insurance. *Journal. of*

Computational and Applied Mathematics, ***233(***1*)*, 51-60.

[14] Woodard J.D., and Yi. J. (2018). Estimation of insurance deductible demand under endogenous premium rates. *Journal of Risk and Insurance*. ***9999***, 1-24.

[15] Zhuang W. Z. C., and Hu T. (2008). Optimal allocation of policy limits and deductibles under distortion risk measures. *Insurance mathematics and Economics*, ***44***(3), 409-414.

[16] Bakar A.S., Hamzah N.A., Maghsoudi M., and Nadarajah S. (2015). Modelling loss data using composite models. *Insurance: Mathematics and Economics*. ***61***, 146-154.

[17] Pacakova V., and Brebera D. (2015). Loss distributions and simulations in general insurance and re-insurance. *International Journal of Mathematics and Computers in Simulation*. ***9***, 159-167.

[18] Thogersen J. (2016). Optimal premium as a function of the deductible: customer analysis and portfolio characteristics. *Risks*. ***4***, 1-19.

[19] Liu H., and Wang R. (2017). Collective risk models with dependence uncertainty. *ASTIN Bulletin*: *The Journal of the IAA, **47***(2), 361–389.

[20] Woodard J.D., and Yi J. (2020). Estimation of insurance deductible demand under endogenous premium rates. *Journal of Risk & Insurance, The American Risk and Insurance Association*. ***87***(2), 477-500.

[21] Ogungbenle, M.G. (2021b). On empirical analysis of Gompertzian mortality dynamics. *Journal of Science-FAS-SEUSL*, ***2***(1), 1-18.

[22] Hua L., and Cheung K.C. (2008). Worst allocation of policy limits and deductibles. *Insurance mathematics and Economics* ***40***(1), 93-98.

[23] Zacaj O., Dhamo E., and Shehu S. (2015). Modelling individual claims for motor third party liability of insurance companies in Albania. *International Journal of innovation in science and mathematics*, ***3***(3), 174-178.

[24] Sakthivel K.M., and Rajitha C.S. (2017). Artificial intelligence for estimation of future claim frequency in non- life insurance. *Global Journal of Pure and Applied Mathematics*. ***13***(6), 1701-1710.

[25] Actuarial Community (2018). *Loss data analytics*, arXiv:1808.06718v1 [q-fin.RM], 74-77.

[26] Garrido J. Genest C. and Schulz J. (2016). Generalized linear models for dependent frequency and severity of insurance claims. *Insurance: Mathematics and Economics*, ***70***, 205 – 215.

[27] Frees E. W. Lee G., and Yang (2016). Multivariate frequency-severity regression models in insurance. *Risks*, ***4***(1), 4.

[29] Park S. C. Kim J. H. and Ahn J. Y. (2018). Does hunger for bonuses drive the dependence between claim frequency and severity? *Insurance: Mathematics and Economics*, ***83***, 32–46.

[28] Valecky J. I. R. I. (2016). Modelling claim frequency in vehicle insurance. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 64(2), 683-689.

[30] Afify A.Z., Gemeay A.M., and Ibrahim N.A. (2020). The heavy tailed exponential distribution risk measures estimation and application to actuarial data. *Mathematics*, ***8***, 1276, 1-28.

RESEARCH ARTICLE

AN ALTERNATIVE METHOD FOR CONSTRUCTING HADAMARD MATRICES

A. P. Batuwita[1], N. T. S. G. Gamachchige[1], P. G. R. S. Ranasinghe[2], A. A. I. Perera[2]*

[1]Department of Science and Technology, University of Uva Wellassa, Sri Lanka.
[2]Department of Mathematics, Faculty of Science, University of Peradeniya, Sri Lanka.

ABSTRACT

Symmetric Hadamard matrices are investigated in this research and an alternative method of construction is introduced. Using the proposed method, we can construct Hadamard matrices of order $2^{n+1}(q+1)$ where $q \equiv 1(\mathrm{mod}\ 4)$ and $n \geq 1$.

This construction can be used to construct an infinite number of Hadamard matrices. For the present study, we use quadratic non-residues over a finite field.

Keywords: *Hadamard matrices, quadratic non-residue, symmetric hadamard matrices*

DOI. *http://doi.org/10.4038/jsc.v12i2.33*

1. INTRODUCTION

A Hadamard matrix $H$of order n whose rows and columns are mutually orthogonal with entries ± 1 and satisfying $HH^T = nI_n$, where H^T is the transpose of H and $I_n$is the identity matrix of order *n* [1]. French mathematician Jacques Hadamard proved that such matrices could exist only if n is 1,2 or a multiple of 4 [2]. Still there are unknown Hadamard matrices of order of multiple of 4. If $H = H^T$, then H is called symmetric Hadamard matrix. These matrices can be transformed to produce incomplete block design, *t*-design, error correcting and detecting codes, and other mathematical and statistical objects [3].

Hadamard matrices can be constructed in many ways. The first construction was published by Sylvester in 1867. A new Hadamard matrix can always be obtained from a known Hadamard matrix using the method known as the Sylvester construction [4]. If H_n is an $n \times n$ Hadamard matrix, then a $2n \times 2n$ matrix H_{2n} can be defined as

$$H_{2n} = \begin{bmatrix} H_n & H_n \\ H_n & -H_n \end{bmatrix}.$$

*Corresponding author: *anushabatuwita@gmail.com*

In 1893, Jacques Hadamard introduced Hadamard matrices of order 12 and 20. He introduced his matrices when studying how large the determinant of a square matrix can be [5]. Another popular construction of Hadamard matrices were due to the English Mathematician Raymond Paley. He gave construction methods for various infinite classes of Hadamard matrices. The Paley construction is a method for constructing Hadamard matrices using finite fields $GF(q)$ [6]. This method uses quadratic residues in $GF(q)$ where q is a power of an odd prime number, $GF(q)$ is a Galois field of order q. An element a in $GF(q)$ is a quadratic residue if and only if there exists b in $GF(q)$ such that $a = b^n$. Otherwise, a is quadratic non-residue. Paley define quadratic character $\chi(a)$ indicates whether the given finite field element a is a perfect square or not.

$$\chi(a) = \begin{cases} 1 & \text{if } a \text{ is a quadratic residue in } GF(q), \\ -1 & \text{if } a \text{ is a quadratic non} - \text{residue in } GF(q), \\ 0 & \text{if } a = 0 \end{cases}$$

Paley construction-I gives Hadamard matrices of order $q+1$, where $q \equiv 3 (mod\ 4)$ and Paley construction-II gives symmetric Hadamard matrices. It has been shown that, if $q \equiv 1 (mod\ 4)$, then by replacing all 0 entries of $H = \begin{bmatrix} 0 & j^T \\ j & Q \end{bmatrix}$ by the matrix $\begin{bmatrix} 1 & - \\ - & - \end{bmatrix}$ and all ±1 entries by the matrix $\pm\begin{bmatrix} 1 & 1 \\ 1 & - \end{bmatrix}$, one can construct a symmetric Hadamard matrix of size $2(q+1)$ (Here, we denote -1 by – sign). Where Q is a symmetric matrix of order q (Q constructed using $\chi(a)$) and j is a column vector of length q with all entries 1. Also, symmetric matrix Q has the properties

$$QQ^T = Q^2 = J - qI \text{ and}$$

$$QJ = JQ = 0,$$

where, J is the $q \times q$ matrix with all entries 1.

Another popular construction was discovered by John Williamson in 1944 which are generalizations of some of Paley's work. He constructed Hadamard matrices of order $4u$ using four symmetric circulant matrices A, B, C, D of order u with entries ± 1 and satisfying both,

$XY^T = Y^T X$, for $X \neq Y \in \{A, B, C, D\}$ and $AA^T + BB^T + CC^T + DD^T = 4uI_u$ [7].

In 1970, Symmetric Hadamard matrices of order 36 were constructed by [8] Bussemaker and Seidel and Symmetric conference matrices of order 46 were constructed by R. Mathon in 1978 [9].

A conference matrix is a square matrix C with 0 on the diagonal and ± 1 on the off diagonal such that $C^T C$ is a multiple of the identity matrix I. Thus, if the matrix has order n, $C^T C = (n-1)I$. There are some relations between conference matrices and

Hadamard matrices of order n. But not all conference matrices represent Hadamard matrices since conference matrices of size $n = 2(\ mod\ 4)$ exist.

In 2014, by modifying Mathon's construction, Balonin and Seberry have constructed symmetric conference matrices of order 46 [10]. It is inequivalent to those Mathon. If two Hadamard matrices (H_1 and $H_2$with same order) are said to be equivalent, if H_1can be obtained from H_2 by permuting rows and columns and by multiplying rows and columns by -1. Up to equivalence a unique Hadamard matrix of order 1, 2, 4, 8 and 12 exists [11]. Matteo, Dokovic and Kotsireas constructed symmetric Hadamard matrices of order $92,116,172$ [12]. All of them are constructed by using the GP array of Balonin and Seberry. Moreover, Kharaghani and Tayfeh discovered Hadamard matrix of order 428 using T-sequences [13]. Now unknown smallest order Hadamard matrix is $668\ 276$ for skew-Hadamard matrices, and 188 for symmetric Hadamard matrices [14].

In this paper we propose an alternative method of constructing symmetric Hadamard matrices using quadratic non-residues over finite fields.

2. MATERIAL AND METHODS

First, we define a function, $\overline{\chi(a)}$ as follows. It indicates whether the given finite field element *a* is a perfect square or not.

$$\overline{\chi(a)} = \begin{cases} -1 & if\ a \text{ is a non zero quadratic residue in } GF(q), \\ 1 & if\ a \text{ is a quadratic non} - \text{residue in } GF(q), \\ 0 & \text{if } a = 0 \end{cases}$$

Let R be the matrix whose rows and columns are indexed by elements of $GF(q)$ and construct using $\overline{\chi(a)}$.

The matrix $R = -Q$ is Symmetric matrix of order q with zero diagonal and ± 1 elsewhere. Also, symmetric matrix R has the properties

$$RR^T = R^2 = J - qI \text{ and}$$

$$RJ = JR = 0$$

Where, J is the $q \times q$ matrix with all entries 1.

Method: Let $q \equiv 1(mod\ 4)$.

For $n \geq 1$

A symmetric Hadamard matrix of order $2^{n+1}(q+1)$ can be constructed by replacing all 0 entries of

$$H_{2^{n+1}(q+1)} = \begin{bmatrix} 0 & j^T \\ j & R \end{bmatrix}$$

by the matrix

$$A_{2^{n+1}} = \begin{bmatrix} A_{2^n} & A_{2^n} \\ A_{2^n} & -A_{2^n} \end{bmatrix},$$

and all ±1 entries by the matrix

$$\pm A'_{2^{n+1}} = \pm \begin{bmatrix} A'_{2^n} & A'_{2^n} \\ A'_{2^n} & -A'_{2^n} \end{bmatrix},$$

where

$$A_2 = \begin{bmatrix} 1 & - \\ - & - \end{bmatrix}, A_2{}' = \begin{bmatrix} 1 & 1 \\ 1 & - \end{bmatrix},$$

and j is a column vector of length q with all entries 1.

Example I (Using proposed method)

Consider $q = 5$ (quadratic non-residues are 2 and 3) and $n = 1$.

A symmetric Hadamard matrix H_{24} of order $2^2(5+1) = 24$ can be constructed by replacing all 0 entries of

$$H_{24} = \begin{bmatrix} 0 & j^T \\ j & R \end{bmatrix},$$

by the matrix

$$A_{2^2} = \begin{bmatrix} A_2 & -A_2 \\ -A_2 & -A_2 \end{bmatrix},$$

and all ±1 entries by the matrix

$$\pm A'_{2^2} = \pm \begin{bmatrix} A_2{}' & A_2{}' \\ A_2{}' & -A_2{}' \end{bmatrix}.$$

$$R = \begin{bmatrix} 0 & - & 1 & 1 & - \\ - & 0 & - & 1 & 1 \\ 1 & - & 0 & - & 1 \\ 1 & 1 & - & 0 & - \\ - & 1 & 1 & - & 0 \end{bmatrix}$$

Then, clearly

$$H_{24}H_{24}{}^T = 24\, I \text{ and } H_{24} = H_{24}{}^T.$$

Therefore, H_{24} is a symmetric Hadamard matrix of order 12, where H_{24} is given by

H_{24}=

1	-	1	-	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
-	-	-	-	1	-	1	-	1	-	1	-	1	-	1	-	1	-	1	-	1	-	1	-
1	-	-	1	1	1	-	-	1	1	-	-	1	1	-	-	1	1	-	-	1	1	-	-
-	-	1	1	1	-	-	1	1	-	-	1	1	-	-	1	1	-	-	1	1	-	-	1
1	1	1	1	1	-	1	-	-	-	-	-	1	1	1	1	1	1	1	1	-	-	-	-
1	-	1	-	-	-	-	-	-	1	-	1	1	-	1	-	1	-	1	-	-	1	-	1
1	1	-	-	1	-	-	1	-	-	1	1	1	1	-	-	1	1	-	-	-	-	1	1
1	-	-	1	-	-	1	1	-	1	1	-	1	-	-	1	1	-	-	1	-	1	1	-
1	1	1	1	-	-	-	-	1	-	1	-	-	-	-	-	1	1	1	1	1	1	1	1
1	-	1	-	-	1	-	1	-	-	-	-	-	1	-	1	1	-	1	-	1	-	1	-
1	1	-	-	-	-	1	1	1	-	-	1	-	-	1	1	1	1	-	-	1	1	-	-
1	-	-	1	-	1	1	-	-	-	1	1	-	1	1	-	1	-	-	1	1	-	-	1
1	1	1	1	1	1	1	1	-	-	-	-	1	-	1	-	-	-	-	-	1	1	1	1
1	-	1	-	1	-	1	-	-	1	-	1	-	-	-	-	-	1	-	1	1	-	1	-
1	1	-	-	1	1	-	-	-	-	1	1	1	-	-	1	-	-	1	1	1	1	-	-
1	-	-	1	1	-	-	1	-	1	1	-	-	-	1	1	-	1	1	-	1	-	-	1
1	1	1	1	1	1	1	1	1	1	1	1	-	-	-	-	1	-	1	-	-	-	-	-
1	-	1	-	1	-	1	-	1	-	1	-	-	1	-	1	-	-	-	-	-	1	-	1
1	1	-	-	1	1	-	-	1	1	-	-	-	-	1	1	1	-	-	1	-	-	1	1
1	-	-	1	1	-	-	1	1	-	-	1	-	1	1	-	-	-	1	1	-	1	1	-
1	1	1	1	-	-	-	-	1	1	1	1	1	1	1	1	-	-	-	-	1	-	1	-
1	-	1	-	-	1	-	1	1	-	1	-	1	-	1	-	-	1	-	1	-	-	-	-
1	1	-	-	-	-	1	1	1	1	-	-	1	1	-	-	-	-	1	1	1	-	-	1
1	-	-	1	-	1	1	-	1	-	-	1	1	-	-	1	-	1	1	-	-	-	1	1

Example II (Using proposed method)

Consider $q = 5$ (quadratic non-residues are 2 and 3) and $n = 2$.

A symmetric Hadamard matrix H_{48} of order $2^3(5+1) = 48$ can be constructed by replacing all 0 entries of $H_{48} = \begin{bmatrix} 0 & j^T \\ j & R \end{bmatrix}$ by the matrix $A_{2^3} = \begin{bmatrix} A_{2^2} & -A_{2^2} \\ -A_{2^2} & -A_{2^2} \end{bmatrix}$, and all ±1 entries by the matrix $\pm A'_{2^3} = \pm \begin{bmatrix} A'_{2^2} & A'_{2^2} \\ A'_{2^2} & -A'_{2^2} \end{bmatrix}$.

We can get, $H_{48} {H_{48}}^T = 48\, I$ and $H_{48} = {H_{48}}^T$

Therefore, H_{48} is a symmetric Hadamard matrix of order 48.

Example III

Now consider $q = 13$ (quadratic non-residues are 2,5,6,7,8 and 11) and $n = 1$.

$$R=\begin{bmatrix}
0 & 1 & - & 1 & 1 & - & - & - & - & 1 & 1 & - & 1\\
1 & 0 & 1 & - & 1 & 1 & - & - & - & - & 1 & 1 & -\\
- & 1 & 0 & 1 & - & 1 & 1 & - & - & - & - & 1 & 1\\
1 & - & 1 & 0 & 1 & - & 1 & 1 & - & - & - & - & 1\\
1 & 1 & - & 1 & 0 & 1 & - & 1 & 1 & - & - & - & -\\
- & 1 & 1 & - & 1 & 0 & 1 & - & 1 & 1 & - & - & -\\
- & - & 1 & 1 & - & 1 & 0 & 1 & - & 1 & 1 & - & -\\
- & - & - & 1 & 1 & - & 1 & 0 & 1 & - & 1 & 1 & -\\
- & - & - & - & 1 & 1 & - & 1 & 0 & 1 & - & 1 & 1\\
1 & - & - & - & - & 1 & 1 & - & 1 & 0 & 1 & - & 1\\
1 & 1 & - & - & - & - & 1 & 1 & - & 1 & 0 & 1 & -\\
- & 1 & 1 & - & - & - & - & 1 & 1 & - & 1 & 0 & 1\\
1 & - & 1 & 1 & - & - & - & - & 1 & 1 & - & 1 & 0
\end{bmatrix}$$

A symmetric Hadamard matrix H_{56} of order $2^2(13+1)=56$ can be constructed by replacing all 0 entries of

$$H_{56}=\begin{bmatrix}0 & j^T\\ j & R\end{bmatrix}$$

by the matrix

$$A_{2^2}=\begin{bmatrix}A_2 & -A_2\\ -A_2 & -A_2\end{bmatrix},$$

and all ±1 entries by the matrix $\pm A'_{2^2}=\pm\begin{bmatrix}A_2{}' & A_2{}'\\ A_2{}' & -A_2{}'\end{bmatrix}$.

We can get, $H_{56}{H_{56}}^T=56\,I$ and $H_{56}={H_{56}}^T$

Therefore, H_{56} is a symmetric Hadamard matrix of order 56.

3. RESULTS AND DISCUSSION

Using the proposed method, we can construct symmetric Hadamard matrix of order $2^{n+1}(q+1)$ where $q\equiv 1(\mathrm{mod}\ 4)$ and $n\geq 1$.

CONCLUSIONS

The proposed alternative method which is our main result, can be used to construct an infinite number of Hadamard matrices. In this work, we used quadratic non-residues over a finite field. Using proposed method, we can construct symmetric Hadamard matrix of order $2^{n+1}(q+1)$ where $q\equiv 1(\mathrm{mod}\ 4)$ and $n\geq 1$. As a future work, planning to implement a computer programme to prove our method and construct large symmetric Hadamard matrices of order $2^{n+1}(q+1)$.

REFERENCE

[1] H. J., " Résolution d'une Question Relative aux Déterminants," *Bulletin des Sciences Mathématiques,* vol. 17, pp. 240-246, 1893.

[2] K. Horadam, Hadamard Matrices and Their Applications, Princeton University Press, 2006.

[3] A. Hedayat and W. Wallis, "Hadamard matrices and their applications," *The annals of statistics,* vol. 6, pp. 1184-1238, 1978.

[4] J. Sylvester, "Thoughts on inverse orthogonal matrices, simultaneous sign-successions, and tesselated pavenments in two or more colors, with application to Newton's rule, ornamental tile-work, and the theory of numbers," *Phil. Mag.,* vol. 4, pp. 461-475, 1867.

[5] J. Hadamard, "Resolution d'une question relative aux d ´ eterminants," *Bull. Sciences Math,* pp. 240-246, 1893.

[6] R. Paley, "On Orthogonal Matrices," *Journal of Mathematics and Physics,* pp. 311-320, 1933.

[7] J. Williamson, "Hadamard's determinant theorem and the sum of four squares," *Duke Math J.,* pp. 65-81, 1944.

[8] F. Bussemaker and J. Seidel, "Symmetric Hadamard matrices of order 36," Technological University, Eindhoven, 1970.

[9] R. Mathon, "Symmetric conference matrices of order pq2 + 1," *Canad. J. Math,* vol. 30, pp. 321-331, 1978.

[10] N. Balonin and J. Seberry, "A review and new symmetric conference matrices," *Informatsionno-upravliaiushchie sistemy,* vol. 4(71), pp. 2-7, 2014.

[11] H. Kharaghani and B. Tayfeh-Rezaie, "Hadamard matrices of order 32," *Journal of Combinatorial Designs,* vol. 21(5), pp. 212-221, 2012.

[12] O. Matteo, D. Đoković and S. Kotsireas, "Symmetric Hadamard matrices of order 116," *De Gruyter,* vol. 3, pp. 227-234, 2015.

[13] H. Kharaghani and B. Tayfeh- Rezaie, "A Hadamard matrix of order 428," *Journal of Combinatorial Designs,* pp. 435-440, 2005.

[14] N. Balonin, D. Đoković. and D. Karbovskiy, "Construction of symmetric Hadamard matrices of order 4v for v = 47, 73, 113," *De Gruyter,* vol. 6, pp. 11-22, 2017.

RESEARCH ARTICLE

PHYTOCHEMICALS AND ANTIOXIDANT PROPERTIES OF THE LEAVES OF WILD GUAVA VARIETIES GROWN IN SRI LANKA

K. Shanthirasekaram[1], V. P. Bulugahapitiya [1], H. Manawadu[1], C. S. Gangabadage[1]*

[1]Department of Chemistry, University of Ruhuna, Matara 81000, Sri Lanka

ABSTRACT

Guava (*Psidium guajava* Linn.) is well-known throughout the world for its food, nutritional, and medicinal properties. Several guava cultivars/varieties are available in Sri Lanka, which can be classified as common, wild, or introduced. Though common guava has been extensively studied for its phytochemistry and pharmacology, only a few studies on wild varieties has been available so far. Therefore, this study focused on the investigation of phytochemical constituents and antioxidants capacity of two main wild guava varieties grown in Sri Lanka namely, *Psidium guajava* (cv. Getta-pera) and *Psidium guineense* (cv. Embul-pera). An Ultrasound-assisted-extraction technique was used to extract plant constituents, and water was used as the solvent. The phytochemicals were qualitatively and quantitatively analyzed using standard methods whereas the antioxidant capacity was determined using the DPPH and FRAP assays. Phytochemical screening revealed that both varieties contain most of the important phytochemicals. Though both showed higher anti-oxidant activity, Embul-pera had the highest in both the FRAP and DPPH assays, with 612.69±0.50 mg Trolox Eq/g and IC_{50} value of 191.69±0.25 ppm respectively. The highest level of all quantified phytochemicals, particularly polyphenolic content (327.87±0.23 mg GAE/g extract) was found in Embul-pera. As a conclusion, two wild guava varieties considered in the study contain a diverse phytochemical profile and higher antioxidant properties similarity to the common guava. It can be recommended that Getta-pera" and "Embul-pera" are excellent alternatives to be used in functional foods and nutraceuticals preparation and hence to promote the cultivation as economic plants.

Keywords: *Antioxidants, embul-pera, getta-pera, phytochemicals, wild guava*

DOI. *http://doi.org/10.4038/jsc.v12i2.34*

*Corresponding author: *vajira@chem.ruh.ac.lk* *https://orcid.org/0000-0003-1178-1052*

1. INTRODUCTION

The genus *Psidium* (Family: Myrtaceae) is native to the Tropical and Subtropical Americas and contains approximately 92 species worldwide. Brazil is a major *Psidium* diversity hotspot, with approximately 60 species, 47 of which are endemic [1], [2]. The most important species in this genus are guava (*Psidium* spp.) [1]. *Psidium guajava*, *Psidium cattleyanum*, and *Psidium guineense* are the most important commercial species in fruits production and as a source for chemical compounds in the pharmaceutical industry [2]. Different parts of the plant are used in traditional medicine to treat various ailments including wounds, ulcers, bronchitis, eye sores, bowels, diarrhoea, and cholera. Phytochemical studies on different parts of the plant by many researchers has reported diverse phytochemicals with their chemical structures [3]. Pharmacology of commonly available guava has been reported to high extent [4].

Sri Lanka is abundant in guava cultivars/varieties, *i.e.* commonly consumed, wild and introduced varieties. Many cultivars are available in common-guava, *P. guajava*, such as pink, red, and white flesh fruits, and small, middle, and large size fruits; wild verities are getta-pera (a cultivars of *P. guajava*), apple-guava (*P. pomiferum)*, embul-pera/sour-guava (Wild, *P. guineense*) and strawberry-guava (*P. cattleyanum*); introduced varieties are kanthi, pubudu, horana red, horana white, costorican, etc. [5], [6]. The common guava, particularly pink and white - fleshed middle size fruit, is available throughout the country whereas strawberry guava and sour-guava are mostly found in the southern province of Sri Lanka and introduced varieties can primarily be obtained from the Fruit Crops Research and Development Centre (FCRDC), Horana, Sri Lanka [5]. Notably, both getta-pera and common guava are cultivars of *P. guajava*. Despite the fact that both getta-pera and common guava belong to the same genus and species, they can be distinguished by their texture, flavour, and seed yield. Getta-pera has a gritty surface, too many seeds, and a sour taste, whereas common guava has a smooth texture, is delightfully scented, is less seeds, and has a pleasant taste [6]. Though Sri Lanka is a home for a diverse range of guavas, only the common guava has attracted interest by the public [5] and less attention particularly on wild varieties for food and healthcare purposes. Therefore, research on Sri Lankan guava varieties needs to be strengthened for popularizing many verities among the public and to promote them as agricultural crops, and transform them into functional foods.

With this understanding, this study was led to screen and quantify the phytochemicals and evaluate the antioxidant capacity of aqueous extracts of leaves from two wild varieties of guava, getta-pera (*P. guajava*) and embul-pera (*P. guineense*). Non-conventional extraction techniques *i.e.* ultrasound-assisted extraction, was used to make efficient extraction via reducing extraction time, solvent consumption and increase of extraction yield.

2. MATERIAL AND METHODS

2.1 Sample collection

Getta-pera (*P. guajava*, Figure 01) and embul-pera (*P. guineense*, Figure 02) leaves were collected in Matara, Sri Lanka (Sample size-03) and authenticated in the Peradeniya Botanical Garden, Sri Lanka (The authenticated voucher specimens' numbers: *P. guajava*-AHEAD/DOR 05/G1 and *P. guineense*-AHEAD/DOR 05/G3). Healthy leaves were washed in tap water, then distilled water, and air-dried for a day. Dried leaves were ground in a mixture grinder to be used
in the extraction process.

Figure 01: Getta-pera (*P. guajava*)

Figure 02: Embul-pera (*P. guineense*)

2.2 Extraction

Ultrasound-assisted extraction was used to extract bioactive compounds from leaves. The finally grounded leaves of each plant (100.00 g) were sonicated for one hour at 30-35 ºC in an ultrasound-assisted extractor (ROCKER Ultrasonic cleaner, Model: SONER 202H) with distilled water (500 ml) [7]. The extracts were filtered through cotton plugs, followed by filter paper (Whatman No-01) and after removing of water under freeze drying (Model: FE-10-MR, S/No: FD 2020062222), resulted crude extracts were stored at 4ºC until further use.

2.3 Phytochemical qualitative analysis

Qualitative tests for phytochemicals such as polyphenol, flavonoid, tannin, saponin, terpenoid, alkaloid, coumarin, glycoside, anthocyanin, phytosterol, quinones, betacyanin, and chalcones, were performed in triplicates for each aqueous extract of leaves using standard procedures described in the literature [8], [9].

2.4 Phytochemical quantification

The aqueous extract (0.10 g) was dissolved in a small amount of DMSO and diluted with methanol (100 ml) to make a 1000 ppm concentration, which was then used for spectrophotometric quantification of polyphenolics, tannins, flavonoids, terpenoids and saponins as given below.

2.4.1 Total Phenolic content (TPC) and Total Tannin contents (TTC)

The TPC and TTC were estimated using a slightly modified Folin and Ciocalteu method [10], [11]. In brief, a mixture of FC reagent (2.5 ml) was added to the prepared sample extract (0.5 ml) and allowed to stand for 5 minutes. After 30 minutes, 2mL of Na_2CO_3 solution (7.5 percent w/v) was added and incubated and then the absorbance was measured at 765 nm. TPC was calculated using a gallic acid standard curve (0–100 ppm), and TPC of aqueous extracts was expressed in gallic acid equivalents (mg GAE/g extract). TTC was calculated using a tannic acid standard curve (0–100 ppm), and TTC of aqueous extracts was expressed in tannic acid equivalents (mg TAE/g extract).

2.4.2 Total Flavonoid contents (TFC)

TFC was estimated using a slightly modified spectrophotometric method described in [12], [13]. In brief, prepared sample extract (1.0 ml) was mixed with 2 percent $AlCl_3$ solution (0.5 ml) and 0.5 ml of distilled water and allowed to stand for 10 minutes after vigorously shaking the mixture. At 425 nm, the absorbance was measured. TFC was calculated using a quercetin standard curve (0–22 ppm), and TFC of aqueous extracts was expressed in Quercetin equivalents (mg QE/g extract).

2.4.3 Terpenoid contents (TC)

TC was estimated using a slightly modified spectrophotometric method [10]. In brief, 1 ml of 5 percent aqueous phosphomolybdic acid solution was added to 1 ml of sample extract, followed by 1 ml of the con. H_2SO_4 was gradually added. The mixture was thoroughly mixed and left for 30 minutes before being diluted to 5 ml with MeOH. The absorbance was recorded at 700 nm.TC was calculated using a Linalool standard curve (0–2.4 mM), and TC of aqueous extracts was expressed in Linalool equivalents (mg LE/g extract).

2.4.4 Saponin contents (SC)

SC was determined using a spectrophotometric method described in [14], [15]. In brief, 8 percent vanillin (1.0 ml) was mixed with 1 ml of prepared sample extract, then placed in an ice-water bath, followed by 8 ml of 77 % H_2SO_4 (v/v). After shaking, the test tube was placed at 60° C in an oven for 30 minutes. The solution was cooled in an ice-water bath for 10 minutes before being brought to RT for UV analysis and then the absorbance was measured at 540 nm. The SC of the extracts was expressed in Saponin equivalents (mg SE/g extract) (0-500 ppm) using a Saponin standard curve

2.4.5 Alkaloid contents (AC)

AC was determined using a spectrophotometric method described in [16], [17]. A portion of the aqueous extract was dissolved in the HCl solution (2N) before being filtered. One

milliliter of this supernatant was transferred to a separatory funnel, and washed with 10 mL of chloroform (3 times). The pH of this prepared sample was adjusted to neutral using 0.1 N NaOH. The resultant solution was then mixed with prepared BCG solution (5.0 ml) and freshly prepared phosphate buffer solution (pH 4.7, 5.0 ml). It was dynamically shaken, and the complex mixture was re-extracted with $CHCl_3$ (1, 2, 3, and 4 ml). The extracted complex mixture was then poured into a volumetric flask (10 ml), and it was diluted and adjusted with $CHCl_3$. The complex's absorbance in $CHCl_3$ was measured at 470 nm. The AC of aqueous extracts was expressed in Atropine equivalents (mg AE/g extract) (0-10 ppm) using an Atropine standard curve.

2.5 Antioxidant analysis

2.5.1 DPPH Radical Scavenging Assay

With some modifications, the free radical (FR) scavenging activity of guava leaves aqueous extracts were determined using the standard protocol described in the literature [18],[19]. The DPPH solution in MeOH (0.06 mM, 3.9 mL) was carefully mixed with 100 µL of various concentrations of guava leaves aqueous extracts. After 30 minutes in the dark, the absorbance at 517 nm was measured. The IC_{50} value for free radical scavenging activity was calculated using a percentage of scavenging effect vs. concentration plot. As a control, ascorbic acid and Trolox were used.

2.5.2 Ferric Reducing Antioxidant Power Assay (FRAP Assay)

The FRAP value of the aqueous extracts was determined using a standard method described in the literature [20],[21],[22]. About 3 ml of freshly prepared FRAP reagent [300 mM acetate buffer (pH-3.6): 10 mM TPTZ (in 40 mM HCl): 20 mM $FeCl_3$ in a ratio 10:1:1) was mixed with 100 µL of diluted sample. After 30 minutes of incubation at 37 °C, the absorbance at 593 nm was measured. For calibration, a Trolox solution (0–100 ppm) was used.

2.6 Statistical Analysis

Analysis of variance (ANOVA), T-test (LSD) (LSD-Least Significant Difference), and non-parametric statistics Cochran's Q test was used to analyze the data and make comparisons. The statistical analysis was carried out using SAS, R-studio, and Excel. The data were presented as means and standard deviations.

3. RESULTS AND DISCUSSION

3.1 Extraction

Ultrasound-assisted extraction (non-conventional) was used in this study to obtain extract rich in bioactive compounds. The extraction yields for Getta-pera and Embul-pera are 4.3735 ± 0.1878 % and 3.0593 ± 0.4151% respectively. Getta-pera yielded more than Embul-pera, according to the results. The qualitative and quantitative studies of bioactive

Table 01: Statistically analyzed phytochemical screening results of aqueous extracts of leaves of two guava varieties (P: Present, A: Absent).

Phytochemicals	Test method	Wild guava varieties	
		Getta-pera	Embul-pera
Alkaloids	Mayer's Test	P	P
	Wagner's Test	P	P
	Dragendroff's Test	P	P
Glycosides	Keller-kilani Test	P	P
	Modified Borntrager's Test	A	A
	Legal's Test	P	P
Flavonoids	Alkaline reagent Test	P	P
	Shinoda Test/ Mg turning Test	P	P
	Lead acetate Test	P	P
	$AlCl_3$ Test	P	P
	NH_4OH Test	P	P
Saponins	Froth Test	P	P
	Olive Oil Test	P	P
Tannins	Bramer's Test	P	P
	Lead Acetate Test	P	P
Terpenoids	Salkowski's Test	P	P
	Liebermann- Burchardt Test	P	P
	Copper acetate Test	P	P
Polyphenols	Ferric Chloride Test	P	P
Coumarins	UV light Test	A	A
	NaOH Test	P	P
Anthocyanins	HCl & NH3 Test	A	A
Chalcones	NaOH Test	A	A
Phytosterol	Salkowski's Test	P	P
Betacyanin	NaOH Test	P	P
Quinones	H_2SO_4 Test	P	P

compounds derived from plant materials are heavily reliant on the choice of an appropriate extraction method. Over the last 50 years, various extraction procedures have been developed to extract bioactive compounds from plants. The non-conventional extraction method, ultra-sound assisted extraction, used here is an efficient method at a low-cost [23].

3.2 Phytochemical qualitative analysis

Table-01 lists the phytochemicals found in aqueous extracts of Getta-pera and Embul-pera. It revealed the presence of highly important secondary metabolites in Getta-pera and Embul-pera leaves, including alkaloids, glycosides, flavonoids, saponins, tannins, terpenoids, polyphenol, coumarins, phytosterol, betacyanin, and quinones. It was found that anthocyanins and chalcones were absent in aqueous extracts of both varieties. Non-parametric analysis of Cochran's Q test was used to statistically determine the presence and absence of phytochemical availability in each plant sample. Non-parametric analysis Cochran's Q test demonstrated that these phytochemicals are present in both aqueous extracts of Getta-pera and Embul-pera leaves.

This finding lends credence to the outstanding pharmacological activities associated with guava leaves and the use of guava leaves in traditional medicine.

3.3 Phytochemical quantitative analysis

Quantitative analysis of polyphenol, tannin, flavonoid, terpenoid, saponin, and alkaloid, revealed that both Getta-pera and Embul-pera contain varying amounts in the leaves shown in Table-02. Interestingly, TPC, TTC, TFC, TC, SC, and AC levels were higher in Embul-pera (327.87 ± 0.23 mg GAE/g extract, 324.58 ± 0.23 mg TAE/g extract, 36.98 ± 0.03 mg QE/g extract, 10.44 ± 0.01 mM LE/g extract, 505.76 ± 1.65 mg SE/g extract, and 2.36 ± 0.22 mg AE/g extract, respectively) than Getta-pera. These new findings were compared to earlier findings based on a methanolic extract of common guava (*P. guajava)* [4]. The quantities of TPC (479.29 ± 2.16 mg GAE/g extract), TTC (437.54 ± 0.57 mg TAE/g extract), and TC (19.72 ± 0.06 mM LE/g extract) were higher in methanolic extracts of common guava compared to the aqueous extracts of Getta-pera and Embul-pera leaves. In contrast, TFC was higher in aqueous extracts of two wild varieties than the methanolic extract of common guava (28.15 ± 0.09 mg QE/g extract) [4].

T-test (LSD) statistical analysis was performed on each phytochemical quantification data such as polyphenol, flavonoid, tannin, terpenoid, saponin, and alkaloid. According to the T-test (LSD), the quantities of all phytochemicals were significantly ($\alpha = 0.05$) different between the two guava varieties, except for AC, as shown in Figures-03

Table 02: Quantitative Phytochemical Analysis and results of FRAP assay of aqueous extracts of Getta-pera and Embul-pera leaves. Values represent mean ± standard deviation of triplicate sample.

Test Name	Wild guava varieties	
	Getta-pera	**Embul-pera**
Phenolic content (mg GAE/g extract)	261.47 ± 0.23	327.87 ± 0.23
Flavonoid content (mg QE/g extract)	33.13 ± 0.07	36.98 ± 0.03
Tannin content (mg TAE/g extract)	258.84 ± 0.23	324.58 ± 0.23
Terpene content (mM LE/g extract)	9.30 ± 0.03	10.44 ± 0.01
Alkaloid content (mg AE/g extract)	2.15 ± 0.19	2.36 ± 0.22
Saponin content (mg SE/g extract)	462.43 ± 2.86	505.76 ± 1.65
FRAP Assay (mg Trolox Eq/g extract)	441.05 ± 0.88	612.69 ± 0.50

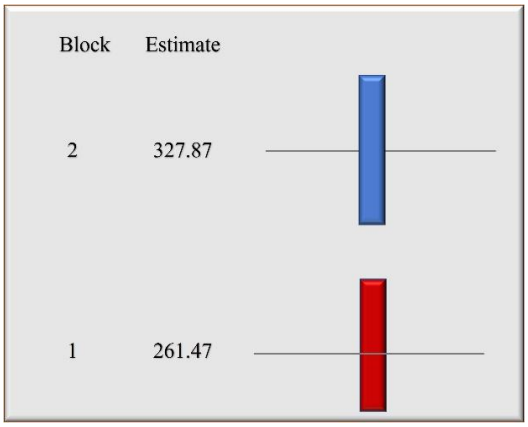


Figure 03: T-test (LSD) for quantification of TPC of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly different).

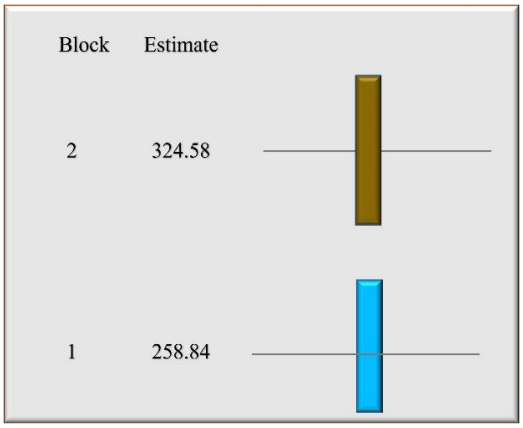


Figure 04: T-test (LSD) for quantification of TTC of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly

In particular, quantified phytochemicals except the alkaloid were present in different levels in aqueous extracts of Getta-pera and Embul-pera leaves, showing higher in Embul-pera extract than Getta-pera extract at a 5% significant level. In contrast, AC was present at the same level in both at a 5% significant level, and the amount was comparatively lower than that of other quantified phytochemicals.

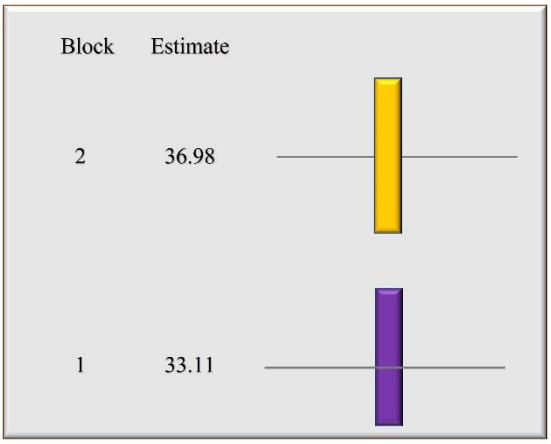


Figure 05: T-test (LSD) for quantification of TFC of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly different).

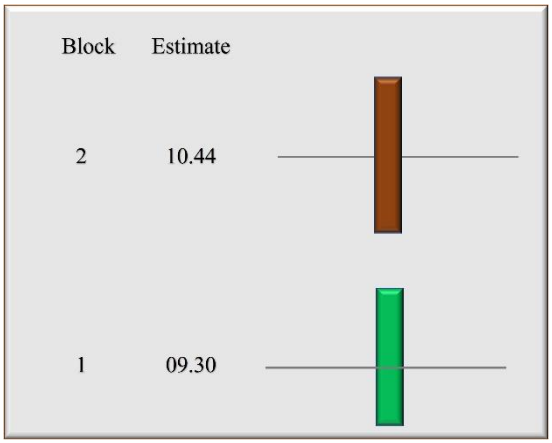


Figure 06: T-test (LSD) for quantification of TC of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly different).

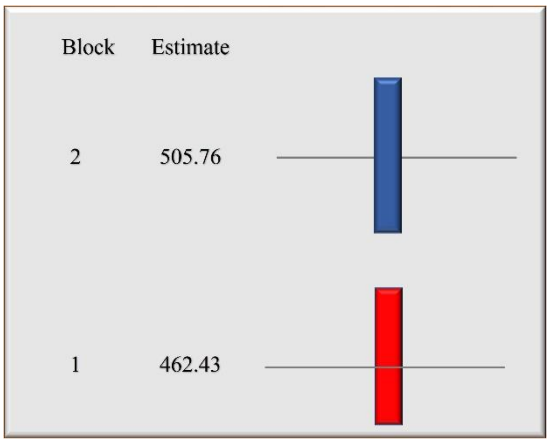


Figure 07: T-test (LSD) for quantification of SC of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly different).

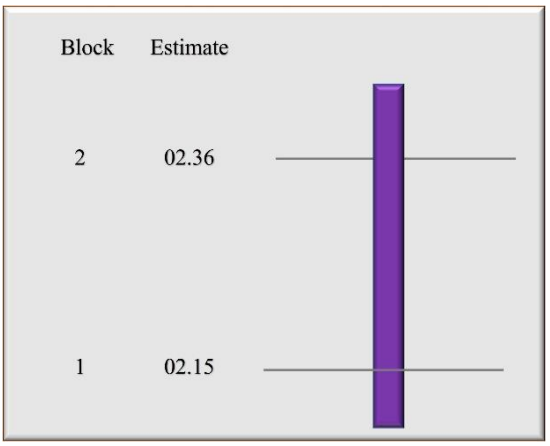


Figure 08: T-test (LSD) for quantification of AC of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly different).

3.4 Antioxidant analysis

3.4.1 DPPH Assay

The DPPH assay results are expressed as IC_{50} values (concentration required to inhibit 50 % of the oxidative reaction). Figure-09 depicts the results of the DPPH assay, and Trolox and Ascorbic acid were used as standards to compare with aqueous extracts of guava varieties' leaves. According to the findings, Embul-pera had the highest radical scavenging activity (IC_{50} value: 191.69 ± 0.25 ppm). These results obtained were compared to past studies on the antioxidant activity of methanolic leaf extracts of guava cultivars' [4]. Getta-pera, Embul-pera and common guava had IC_{50} values of 232.02 ±

0.42, 204.14 ± 0.15, and 192.89 ± 0.07 ppm, respectively in methanolic extracts in our previous study [4]. It showed that aqueous extracts had better antioxidant capacity than methanolic extracts of the same.

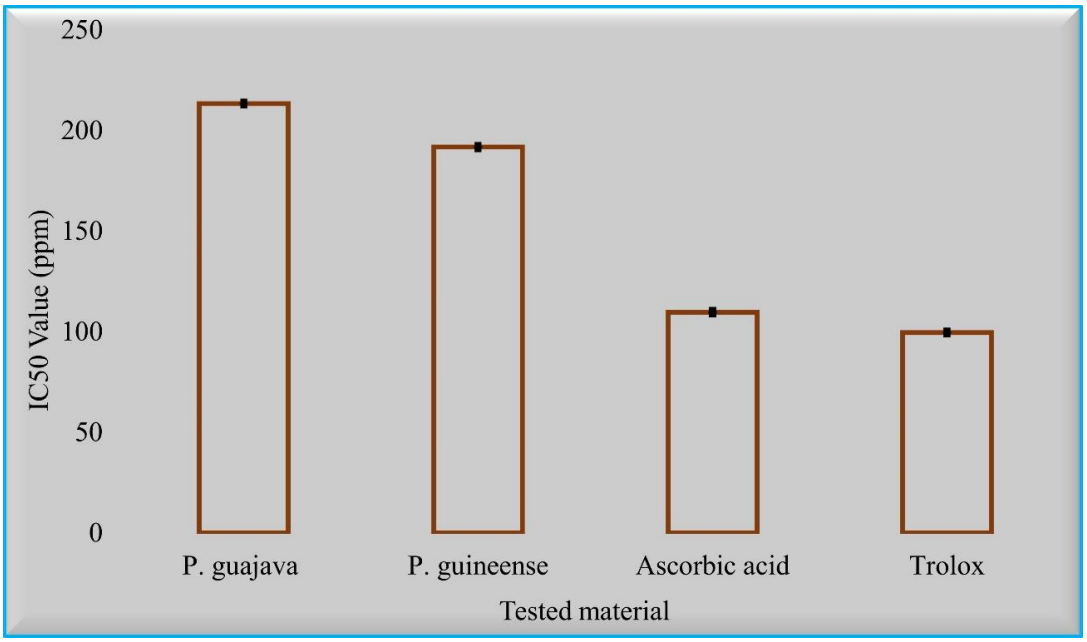


Figure 09: DPPH assay data of aqueous extracts of two wild guava varieties and standards (Error bars indicate the standard deviation).

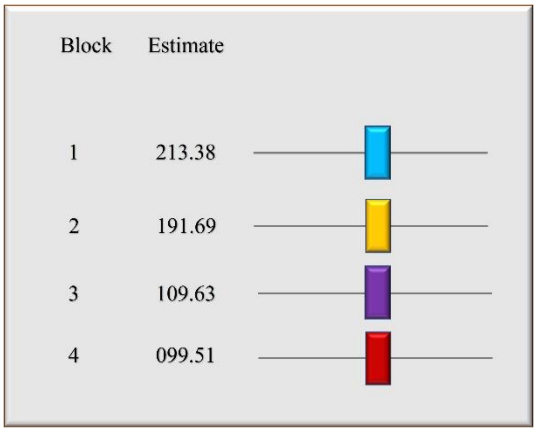


Figure 10: T-test (LSD) for DPPH assay of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, 3: Ascorbic acid, 4: Trolox, means covered by the same bar are not significantly different).

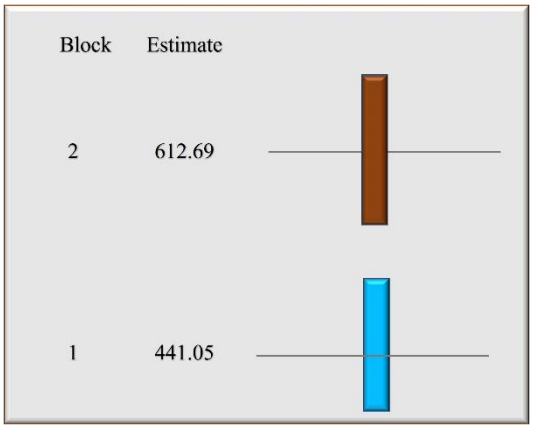


Figure 11: T-test (LSD) for FRAP assay of both guava varieties (α = 0.05, 1: Getta-pera, 2: Embul-pera, means covered by the same bar are not significantly different).

T-test (LSD) of DPPH radical scavenging activity perfectly revealed that both aqueous extracts of Getta-pera and Embul-pera leaves and both standards Ascorbic acid and Trolox are statistically significant difference at 5% significant level, as shown in Figure-

10. This means that the leaves of Getta-pera and Embul-pera each have their own distinct feature in terms of radical scavenging activity. However, of the two guava varieties tested, Embul-pera demonstrated the highest radical scavenging activity.

3.4.2 FRAP Assay

Table-02 displays the Ferric reducing power of aqueous extracts of Getta-pera and Embul-pera leaves. Both extracts exhibit reducing power, but at different extents. The aqueous extract of Embul-pera leaves had the highest reducing power (612.69 ± 0.50 mg Trolox Eq/g) out of both. Furthermore, statistical analysis; T-test (LSD) revealed that there were no significant similarities in ferric reducing power between both aqueous extracts at the 5% significant level, as shown in Figure-11. Furthermore, the results of this study were compared to the data previously published by us for methanolic extracts of the same [4]. The reducing power of methanolic leaf extracts of Getta-pera, Embul-pera, and Common guava is 677.23 ± 2.66, 640.12 ± 3.01, and 722.44 ± 6.58 mg Trolox Eq/g, according to that [4]. All guava species, including common guava, have a higher reducing power than aqueous extracts at a 5% significant level whereas the aqueous extracts of Getta-pera and Embul-pera have acceptable reducing power. The current study found that wild varieties are an excellent source of antioxidants and other phytochemicals.

Natural antioxidants are to reduce the risk of numerous diseases, including atherosclerosis, reperfusion injury, cataractogenesis, rheumatoid arthritis, inflammatory disorders, cancer, the aging process etc. Natural antioxidants secure the human body from harmful free radicals, thereby preventing oxidative stress and the other diseases that it causes [24]. According to our findings, both the leaves of Getta-pera and Embul-pera aqueous extracts are a source of natural antioxidants that could lead to the development of functional foods, nutraceuticals, and to discover novel drugs [24]. As outcomes from the current study, it can be suggested that widely distributed two wild guava varieties in Sri Lanka, namely Getta-pera and Embul-pera, could be used to prepare functional foods, nutraceuticals, or phytomedicine.

CONCLUSION

Wild guava varieties, namely Getta-pera and Embul-pera contain a wide range of important phytochemicals and high antioxidant capacity. As outcome of the study, these two wild guava leaves could be used in preparing of functional foods and nutraceuticals to be used in health enhancement purposes and could be promoted as marketable varieties.

ACKNOWLEDGEMENT

Authors greatly acknowledge AHEAD/RUH/DOR-05 grant for the financial support. Authors extend their sincere thanks to Department of Chemistry, Faculty of Science, University of Ruhuna, Sri Lanka, for providing the necessary laboratory facilities.

REFERENCES

[1] Santos M. A. C, Rêgo M. M, Queiróz M. A, Caproni D. T. R, Dietrich O. H. S, Santos A. F, Rocha D. I, Batista D. S, Otoni W. C, (2020). In vitro growth performance of *Psidium guajava* and *P. guineense* plantlets as affected by culture medium formulations. *Vegetos*, **33**: 435-445.

[2] Marques A. M, Tuler A. C, Carvalho C. R, Carrijo T.T, Ferreira M. F. S, Clarindo W. R, (2016). Refinement of the karyological aspects of *Psidium guineense* (Swartz, 1788): a comparison with *Psidium guajava* (Linnaeus, 1753). *Comp Cytogenet*, **10**(1): 117-128.

[3] Begum S, Hassan S. I, Ali S. N, Siddiqui B. S, (2004). Chemical constituents from the leaves of *Psidium guajava. Natural Product Research*, **18**(2): 135 –140.

[4] Díaz-de-Cerio E, Verardo V, Gómez-Caravaca A. M, Fernández-Gutiérrez A, Segura-Carretero A, (2017). Health Effects of *Psidium guajava* L. Leaves: An Overview of the Last Decade. *International Journal of molecular Sciences*, **18**(4): 897.

[5] Kokilananthan S, Bulugahapitiya V. P, Gangabadagea C.S, Manawadua H, (2020). Comparative account on antioxidant properties, proximate and phytochemical compositions of seven guava varieties grown in Sri Lanka. *Journal of Agriculture and Value Addition*, **3**(2): 1–16.

[6] Menzel, C. M, (1985). Guava: An exotic fruit with potential in Queensland. *Queensland Agricultural Journal*, **111**(2): 93-98.

[7] Musa K. H, Abdullah A, Jusoh K, Subramaniam V. Antioxidant activity of pink-flesh guava (*Psidium guajava* L.): effect of extraction techniques and solvents. *Food Analytical Methods*, **4**: 100-107.

[8] Gayathri V, Kiruba D, (2014). Preliminary phytochemical analysis of leaf powder extracts of *Psidium guajava* L. *International Journal of Pharmacognosy and Phytochemical Research*, **6**(2): 332-334.

[9] Wadood A, Ghufran M, Jamal S. B, Naeem M, Khan A, Ghaffar R, (2013). Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochemistry and Analytical Biochemistry*, **2**(4): 1-4.

[10] Ekwueme F, Nwodo O, Joshua P, Nkwocha C, Eluka P, (2015). Qualitative and quantitative phytochemical screening of the aqueous leaf extract of Senna mimosoides: Its effect in in vivo leukocyte mobilization induced by inflammatory stimulus. *International Journal of Current Microbiology and Applied Sciences*, **4**(5): 1176-1188.

[11] Prabhavathi R, Prasad M, Jayaramu M, (2016). Studies on qualitative and quantitative phytochemical analysis of *Cissus quadrangularis*. *Advances in Applied Science Research*, **7**(4): 11-17.

[12] Fernandes M, Azzolini A, Martinez M, Souza C, Lucisano-Valim Y, Oliveira W, (2014). Assessment of antioxidant activity of spray dried extracts of *Psidium guajava* leaves by DPPH and chemiluminescence inhibition in human neutrophils. *BioMed Research International*, **2014**: 1-10.

[13] Pękal A, Pyrzynska K, (2014). Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Analytical Methods*, **7**: 1776-1782.

[14] Ahmed H. O. A, Wang C, (2015). Determination of tea saponin in Camellia seed oil with UV and HPLC analysis. *World Journal of Engineering and Technology*, **3**(4): 30-37.

[15] Du M, Guo S, Zhang J, Hu L, Li M, (2018). Quantitative analysis method of the tea saponin. *Open Journal of Forestry*, **8**(1): 61-67.

[16] Ajanal M, Gundkalle M.B, Nayak S.U, (2012). Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. *Ancient science of life*, **31**(4): 198-201.

[17] Shamsa F, Monsef H, Ghamooghi R, Verdian Rizi M, (2007). Spectrophotometric determination of total alkaloids in *Peganum harmala* L. using bromocresol green. *Research Journal of Phytochemistry*, **1**: 79-82.

[18] Blois M, (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, **181**: 1199-1200.

[19] Brand-Williams W, Cuvelier M. E, Berset C, (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, **28**(1): 25-30.

[20] Biglari F, AlKarkhi A.F, Easa A.M, (2008). Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. *Food Chemistry*, **107**(4): 1636-1641.

[21] Firuzi O, Lacanna A, Petrucci R, Marrosu G, Saso L, (2005). Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. *Biochimica et Biophysica Acta*, **1721**(1-3): 174-184.

[22] Gliszczyńska-Świgło A, (2006). Antioxidant activity of water-soluble vitamins in the TEAC (trolox equivalent antioxidant capacity) and the FRAP (ferric reducing antioxidant power) assays. *Food chemistry*, **96**(1): 131-136.

[23] Azmir J, Zaidul I.S.M, Rahman M, Sharif K, Mohamed A, Sahena F, Jahurul M, Ghafoor K, Norulaini N, Omar A, (2013). Techniques for extraction of bioactive compounds from plant materials: *A review. Journal of food engineering*, **117**(4): 426-436.

[24] Barros L, Cabrita L, Boas M. V, Carvalho A. M, Ferreira I. C, (2011). Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. *Food Chemistry*, **127**(4): 1600-1608.

RESEARCH ARTICLE

AN ALTERNATIVE APPROACH FOR THE ANTI-MAGIC LABELLING OF A WHEEL GRAPH AND A PENDANT GRAPH

K. M. P. G. S. C. Kapuhennayake [1], A. A. I. Perera [1]*

[1]Department of Mathematics, Faculty of Science, University of Peradeniya, Sri Lanka

ABSTRACT

The Anti-magic labelling of a graph G with m edges and n vertices, is a bijection from the set of edges to the set of integers $\{1, \dots, m\}$ such that all 'n' vertex summations are pairwise distinct. The vertex summation is the summation of the labels assigned to edges incident to a vertex. There is a conjecture that all simple connected graphs except K_2 are anti-magic. In our research, we found an alternative anti-magic labelling method for a wheel graph and a pendant graph. Wheel graph is a graph that contains a cycle of length $n-1$ and for which every graph vertex in the cycle is connected to one other graph vertex known as the "hub". The edges of a wheel, which connect to the hub are called "spokes". Pendant graph is a corona of the form $C_n \Theta K_1$ where $n \geq 3$. We label both wheel graph and pendant graph using the concept of the anti-magic labelling method of the path graph P_{n-1}. For wheel graph, we removed the middle vertex of the wheel graph and created a path graph using the vertices in the outer cycle of the wheel graph. Then the spokes of the wheel graph are represented by adding one edge to each vertex. For Pendant graph, we created a path graph using the cycle of the pendant graph and connect the pendant vertices to every vertex of the path graph. In both cases, we label all the edges using the concept of the anti-magic labelling of path graph P_{n-1}. Finally, we calculated the vertex sum for each vertex and proved that every vertex sums are distinct and in the wheel graph, middle vertex takes the highest value.

Keywords: *Anti-magic labelling, wheel graph, pendant graph, path graph*

DOI. *http://doi.org/10.4038/jsc.v12i2.35*

1. INTRODUCTION

Anti-Magic labelling comes from its connection to magic labelling and magic squares. A magic square is a square array of numbers consisting of the distinct positive integers

*Corresponding author: *sonalichamathka03@gmail.com*

arranged such that the sum of numbers in any horizontal, vertical and main diagonal lines are always the same number. Likewise, motivated from the magic labelling, anti-magic then comes from being the opposite of magic. That is arranging numbers in a way such that the sums of numbers in the horizontal, vertical and main diagonal lines are distinct.

The concept of anti-magic labelling was introduced by Hartsfield and Ringel in 1989 (Hartsfield & Ringel, Pearls in graph theory, 1990). They defined it as follows: An Anti-Magic labelling of a graph G with m edges and n vertices, is a bijection from the set of edges to the set of integers $\{1, \dots, m\}$ such that all 'n' vertex summations are pairwise distinct. The vertex sum is the summation of labels of all edges incident with that vertex. They conjectured that all simple connected graphs except K_2 are anti-magic (Hartsfield & Ringel, Pearls in graph theory, 1990).

There are several applications in anti-magic labelling. It could serve as model of surveillance or security system in civil engineering and circuit design, urban planning, electrical switchboards, communication networks.

In this paper, we introduce an alternative method for anti-magic labelling of wheel graph and pendant graph using the anti-magic labelling of path graph (Chang, Chen, & Li, 2021).

2. MATERIAL AND METHODS

First, we state some important definitions we use to get the result of this work.

Definition 2.1 (Anti-Magic labelling): Let G be a simple graph with V vertices and E edges. Anti-magic labelling of graph G is a one-to-one correspondence between $E(G)$ and $\{ 1,2, \dots, |E| \}$ such that the vertex sum for distinct vertices is different (Sugeng, 2005). Vertex sum is the sum of the labels assigned to edges incident to a vertex.

Definition 2.2 (Wheel Graph): The wheel graph W_n of order n, is a graph that contains a cycle of length $n - 1$ and for which every graph vertex in the cycle is connected to one other graph vertex known as the "hub". The edges of a wheel which connect to the hub are called "spokes" (Weisstein, Wheel Graph, n.d.).

Definition 2.3 (Corona of graphs G_1 and G_2): The corona of graphs G_1 and G_2 is the graph obtained by taking one copy of G_1, which has P_1 vertices and P_1 copies of G_2, and then joining the i^{th} vertex of G_1 by an edge to every vertex in the i^{th} copy of G_2 (Graf, 2014).

Definition 2.4 (Pendant graph): A pendant graph is a corona of the form $C_n \Theta K_1$ where $n \geq 3$ (Graf, 2014).

Definition 2.5 (Path graph): A path graph is a graph which has at least two connected vertices and has at least two terminal vertices (vertices that have degree 1), while all other (if any) have degree two (Weisstein, Path Graph, n.d.).

Since the path graphs are anti-magic we try to convert the wheel graph and the pendant graph into a path graph. (Chang, Chen, & Li, 2021).

First, consider the wheel graph. Represent the vertices in outer cycle as a path graph and then join the first vertex and last vertex by an edge. Spokes of the wheel graph are represented by adding one edge to each vertex.

Theorem 2.1: A path graph P_{n-1} is anti-magic and the wheel graph W_n constructed by path graph P_{n-1} is also anti-magic.

Proof: Represent the vertices in outer cycle as a path graph and join the first vertex and last vertex by an edge. Then spokes of the wheel graph can be represented by adding one edge to each vertex.

Let vertices on the outer cycle be $V_1 = \{(u_i, v_{i)}\} : i = 1,2, \dots, n-1\}$ and the middle vertex (hub) be V_2.

Define the edge label as follows:

Case 1: n is even

Edges in the outer cycle:

$$\vdots$$

$$f((u(\tfrac{n}{2}-3), v_1), (u(\tfrac{n}{2}-2), v_1)) = n-5;$$

$$f((u(\tfrac{n}{2}-2), v_1), (u(\tfrac{n}{2}-1), v_1)) = n-3;$$

$$f((u(\tfrac{n}{2}-1), v_1), (u(\tfrac{n}{2}), v_1)) = n-1;$$

$$f((u(\tfrac{n}{2}), v_1), (u(\tfrac{n}{2}+1), v_1)) = n-2;$$

$$f((u(\tfrac{n}{2}+1), v_1), (u(\tfrac{n}{2}+2), v_1)) = n-4;$$

$$\vdots$$

and Spokes:

$$\vdots$$

$$f((u(\tfrac{n}{2}-2), v_1), (v_2)) = 2n-5;$$

$$f((u(\tfrac{n}{2}-1), , v_1), (v_2)) = 2n-3;$$

$$f((u(\frac{n}{2}),,v_1),(v_2)) = 2n - 2;$$

$$f((u(\frac{n}{2}+1),,v_1),(v_2)) = 2n - 4;$$

$$f((u(\frac{n}{2}+2),,v_1),(v_2)) = 2n - 6;$$

$$\vdots$$

Edge labelling of wheel graph W_n (where n is even) can be represented by Figure 01.

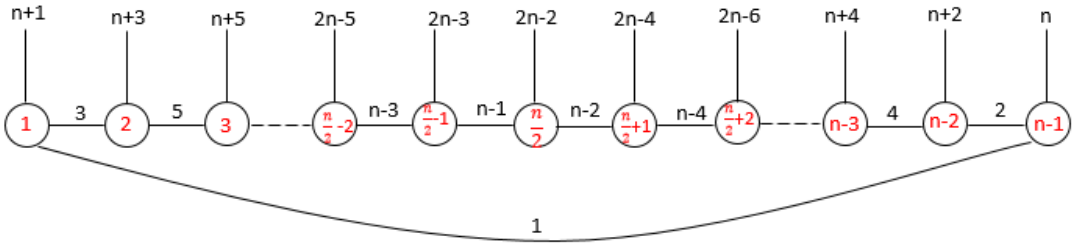


Figure 01: Edge labelling method for even n

Case 2: n is odd

Edges in the outer cycle:

$$\vdots$$

$$f((u\,(\frac{n-1}{2}-3),v_1),(\,u\,(\frac{n-1}{2}-2),v_1)) = n - 6;$$

$$f((u\,(\frac{n-1}{2}-2),v_1),(\,u\,(\frac{n-1}{2}-1),v_1)) = n - 4;$$

$$f((u\,(\frac{n-1}{2}-1),v_1),(\,u\,(\frac{n-1}{2}),v_1)) = n - 2;$$

$$f((u\,(\frac{n-1}{2}),v_1),(\,u\,(\frac{n-1}{2}+1),v_1)) = n - 1;$$

$$f((u\,(\frac{n-1}{2}+1),v_1),(\,u\,(\frac{n-1}{2}+2),v_1)) = n - 3;$$

$$f((u\,(\frac{n-1}{2}+2),v_1),(\,u\,(\frac{n-1}{2}+3),v_1)) = n - 5;$$

$$\vdots$$

and Spokes:

$$\vdots$$

$$f((u(\frac{n-1}{2}-2),v_1),(v_2)) = 2n - 6;$$

$$f\,((u(\frac{n-1}{2}-1),,v_1),(v_2)) = 2n - 4;$$

$$f((u(\frac{n-1}{2}),,v_1),(v_2)) = 2n - 2;$$

$$f((u(\frac{n-1}{2}+1),,v_1),(v_2)) = 2n - 3;$$

$$f((u(\frac{n-1}{2}+2),,v_1),(v_2)) = 2n - 5;$$

$$\vdots$$

Edge labelling of wheel graph W_n (where n is odd) can be represented by figure 02.

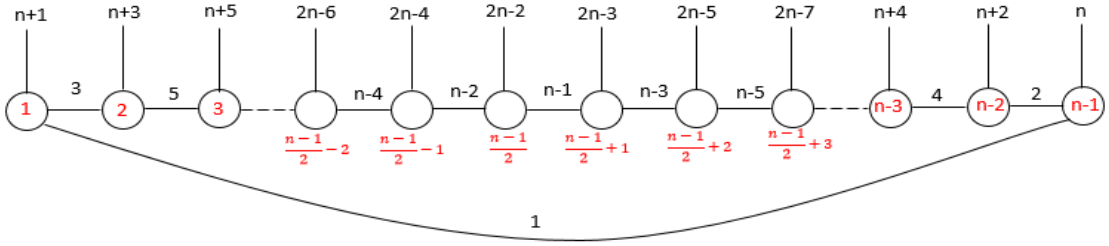


Figure 02: Edge labelling method for odd n

For a counter example, take $n = 6$. Edge labeling of wheel graph (W_6) can be represented by

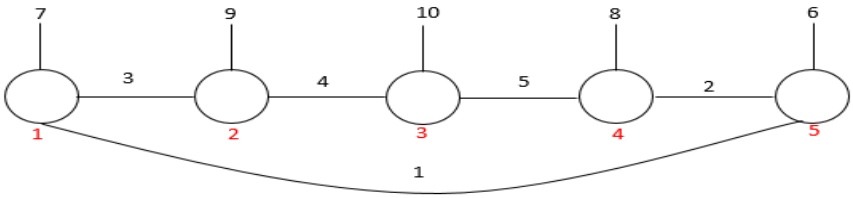


Figure 03: Edge labelling of W_6

Figure 03 shows the path graph constructed by the outer cycle of the wheel graph.

Figure 04 represents the final result of anti-magic labelling of Wheel graph with order 6.

Since the path graphs are anti-magic we try to convert the pendant graph into a path graph (Chang, Chen, & Li, 2021).

Now, let us represent the vertices in cycle graph as a path graph and join the first vertex and last vertex by an edge. Pendant vertices of the pendant graph are represented by adding one edge to each vertex.

Let vertices on the cycle graph be V_1={$(u_i,v_i): i = 1,2,\dots,n$ } and the middle vertex (hub) be $V_2 = \{(u_i,v_i): i = 1,2,\dots,n\}$.

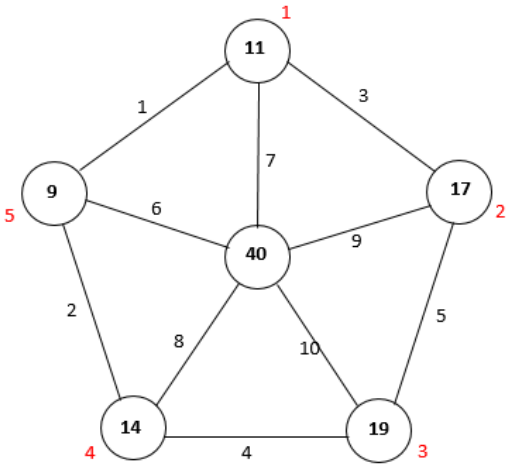


Figure 04: Anti-magic labelling of W_6

Theorem 2.2: A path graph P_{n-1} is anti-magic and the pendant graph W_n constructed by path graph P_{n-1} is also anti-magic.

Define the edge label as follows:

Case 1: *n* is even

Edges in the cycle graph:

$$\vdots$$

$$f((u(\tfrac{n}{2}-3),v_1),(u(\tfrac{n}{2}-2),v_1)) = \tfrac{n}{2}-3;$$

$$f((u(\tfrac{n}{2}-2),v_1),(u(\tfrac{n}{2}-1),v_1)) = \tfrac{n}{2}-2;$$

$$f((u(\tfrac{n}{2}-1),v_1),(u(\tfrac{n}{2}),v_1)) = \tfrac{n}{2}-1;$$

$$f((u(\tfrac{n}{2}),v_1),(u(\tfrac{n}{2}+1),v_1)) = \tfrac{n}{2};$$

$$f((u(\tfrac{n}{2}+1),v_1),(u(\tfrac{n}{2}+2),v_1)) = \tfrac{n}{2}+1;$$

$$\vdots$$

and Spokes,

$$\vdots$$

$$f((u(\tfrac{n}{2}-2),v_1),(v_2)) = \tfrac{3n}{2}+3;$$

$$f((u(\frac{n}{2}-1),,v_1),(v_2)) = \frac{3n}{2}+2;$$

$$f((u(\frac{n}{2}),,v_1),(v_2)) = \frac{3n}{2}+1;$$

$$f((u(\frac{n}{2}+1),,v_1),(v_2)) = \frac{3n}{2};$$

$$f((u(\frac{n}{2}+2),,v_1),(v_2)) = \frac{3n}{2}-1;$$

$$\vdots$$

Edge labelling of pendant graph $C_n \Theta K_1$ (where n is even) can be represented by figure 05.

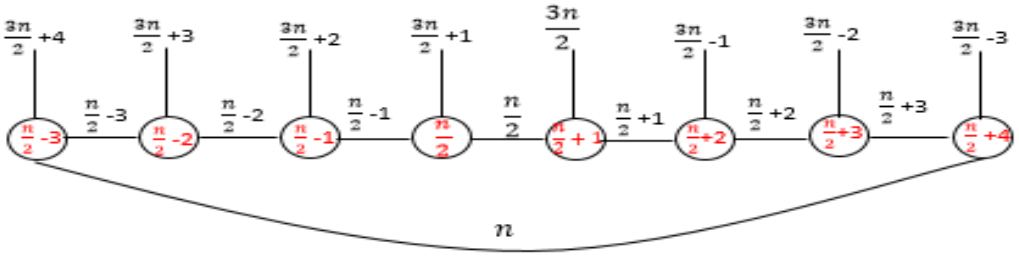


Figure 05: Edge labelling method for even n

Case 2: *n* is odd

Edges in the cycle graph:

$$\vdots$$

$$f((u(\frac{n+1}{2}-2),v_1),(u(\frac{n+1}{2}-1),v_1)) = \frac{n+1}{2}-2;$$

$$f((u(\frac{n+1}{2}-1),v_1),(u(\frac{n}{2}),v_1)) = \frac{n+1}{2}-1;$$

$$f((u(\frac{n+1}{2}),v_1),(u(\frac{n}{2}+1),v_1)) = \frac{n+1}{2};$$

$$f((u(\frac{n+1}{2}+1),v_1),(u(\frac{n}{2}+2),v_1)) = \frac{n+1}{2}+1;$$

$$f((u(\frac{n+1}{2}+2),v_1),(u(\frac{n}{2}+3),v_1)) = \frac{n+1}{2}+2;$$

$$\vdots$$

and Spokes:

$$\vdots$$

$$f((u(\frac{n+1}{2}-2),v_1),(v_2)) = \frac{3n+1}{2}+2$$

$$f((u(\frac{n+1}{2}-1),,v_1),(v_2)) = \frac{3n+1}{2}+1$$

$$f((u(\frac{n+1}{2}),,v_1),(v_2)) = \frac{3n+1}{2}$$

$$f((u(\frac{n+1}{2}+1),,v_1),(v_2)) = \frac{3n+1}{2}-1$$

$$f((u(\frac{n+1}{2}+2),,v_1),(v_2)) = \frac{3n+1}{2}-2$$

$$\vdots$$

Edge labelling of pendant graph $C_n \Theta K_1$ (where n is odd) can be represented by figure 06.

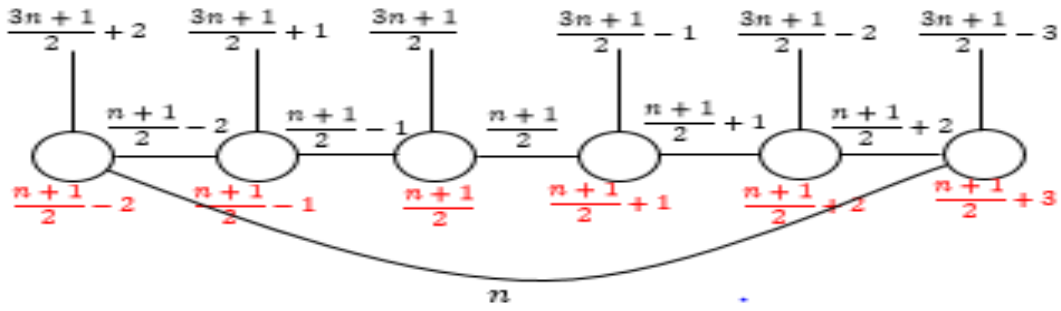


Figure 06: Edge labelling method for odd n

For a counter example, take $n = 5$. Edge labeling of pendant graph ($C_5 \Theta K_1$) can be represented by Figure 07.

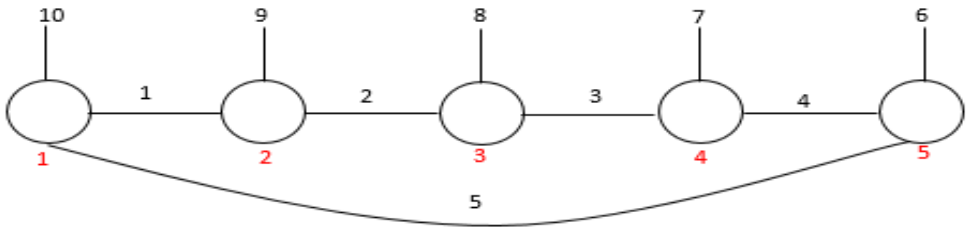


Figure 07: Edge labelling of pendant graph $C_5 \Theta K_1$

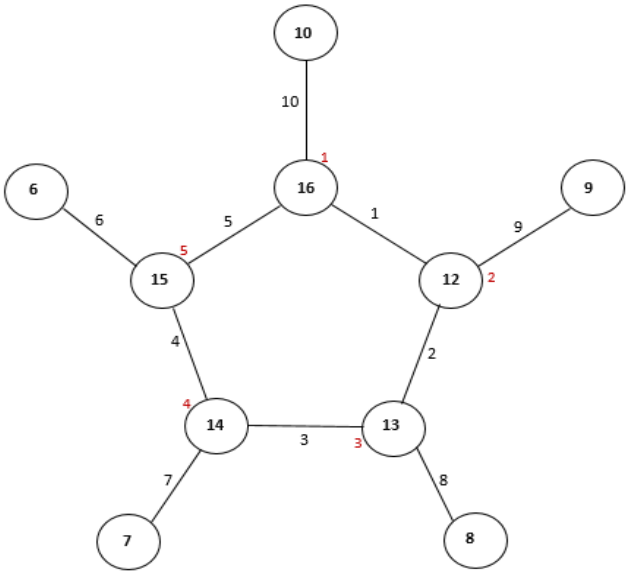


Figure 08: Anti-magic labelling of pendant graph $C_5 \Theta K_1$

3. RESULTS AND DISCUSSION

Consider the vertex summation of each edges of the wheel graph.

The general equation for vertex sums;

For even n

$$(\tfrac{n}{2} - m)^{\text{th}} \text{ vertex; } 4n - [7 + 6(m-1)]$$

$$(\tfrac{n}{2} - 1)^{\text{th}} \text{ vertex; } 4n - 7$$

$$(\tfrac{n}{2})^{\text{th}} \text{ vertex; } 4n - 5$$

$$(\tfrac{n}{2} + 1)^{\text{th}} \text{ vertex; } 4n - 10$$

$$(\tfrac{n}{2} + m)^{\text{th}} \text{ vertex; } 4n - [10 + 6(m-1)];$$

where $n \in \mathbb{Z}^+$, $m = 2,3,4, \ldots$

For odd n

$$(\tfrac{n}{2} - m)^{\text{th}} \text{ vertex ; } 4n - [10 + 6(m-1)]$$

$$(\tfrac{n}{2} - 1)^{\text{th}} \text{ vertex; } 4n - 10$$

$$(\tfrac{n}{2})^{\text{th}} \text{ vertex; } 4n - 5$$

$$(\tfrac{n}{2} + 1)^{\text{th}} \text{ vertex; } 4n - 7$$

$(\frac{n}{2}+m)^{th}$ vertex; $4n-[7+6(m-1)]$;

where $n \in \mathbb{Z}^+$, $m = 2,3,4,\ldots$

Table 01: Vertex summation of outer circle

n even					n odd				
vertex	Left edge	Right edge	spoke	Vertex sum	vertex	Left vertex	Right vertex	spoke	Vertex sum
$\frac{n}{2}$-5	n-11	n-9	2n-11	4n-31	$\frac{n-1}{2}$-5	n-12	n-10	2n-12	4n-34
$\frac{n}{2}$-4	n-9	n-7	2n-9	4n-25	$\frac{n-1}{2}$-4	n-10	n-8	2n-10	4n-28
$\frac{n}{2}$-3	n-7	n-5	2n-7	4n-19	$\frac{n-1}{2}$-3	n-8	n-6	2n-8	4n-22
$\frac{n}{2}$-2	n-5	n-3	2n-5	4n-13	$\frac{n-1}{2}$-2	n-6	n-4	2n-6	4n-16
$\frac{n}{2}$-1	n-3	n-1	2n-3	4n-7	$\frac{n-1}{2}$-1	n-4	n-2	2n-4	4n-10
$\frac{n}{2}$	n-1	n-2	2n-2	4n-5	$\frac{n-1}{2}$	n-2	n-1	2n-2	4n-5
$\frac{n}{2}$+1	n-2	n-4	2n-4	4n-10	$\frac{n-1}{2}$+1	n-1	n-3	2n-3	4n-7
$\frac{n}{2}$+2	n-4	n-6	2n-6	4n-16	$\frac{n-1}{2}$+2	n-3	n-5	2n-5	4n-13
$\frac{n}{2}$+3	n-6	n-8	2n-8	4n-22	$\frac{n-1}{2}$+3	n-5	n-7	2n-7	4n-19
$\frac{n}{2}+4$	n-8	n-10	2n-10	4n-28	$\frac{n-1}{2}+4$	n-7	n-9	2n-9	4n-25

Proof:

Let us assume that any two vertex sums have same number.

(i) $4n-[7+6(m-1)] = 4n-7$

$$m = 1.$$

Contradiction since $m = 2,3,4,..$

$$4n-[7+6(m-1)] = 4n-5$$
$$m = 2/3,$$

a contradiction since $m \notin \mathbb{Z}^+$.

$$4n - [7 + 6(m-1)] = 4n - 10$$

$$m = 3/2,$$

a contradiction since $m \notin \mathbb{Z}^+$.

(ii) $$4n - [10 + 6(m-1)] = 4n - 7$$

$$m = 1/2$$

a contradiction since $m \notin \mathbb{Z}^+$.

(iii) $$4n - [10 + 6(m-1)] = 4n - 5$$

$$m = 1/6$$

a contradiction since $m \notin \mathbb{Z}^+$.

(iv) $$4n - [10 + 6(m-1)] = 4n - 10$$

$$m = 1.$$

a contradiction. Since $m = 2,3,4,..$

(v) $$4n - [7 + 6(m-1)] = 4n - [10 + 6(m-1)]$$

$$7 = 10$$

a contradiction.

Therefore, no two vertex values are same. That is, every vertex sum is distinct. Hence, the wheel graphs W_n is anti-magic.

Now consider the vertex summation of each edges in pendant graph, which is shown in Table 2.

Now the general equation for vertex sums;

For odd n

$$(\tfrac{n+1}{2} - m)^{\text{th}} \text{ vertex} \quad ; \quad \tfrac{5n+3}{2} - (m+1)$$

$$(\tfrac{n+1}{2})^{\text{th}} \text{ vertex} \; ; \quad \tfrac{5n+3}{2} - 1$$

$$(\tfrac{n+1}{2} + m)^{\text{th}} \text{ vertex} \quad ; \quad \tfrac{5n+3}{2} - (m-1)$$

where $n, m \notin \mathbb{Z}^+$.

Table 02: Vertex summation of pendant graph

n odd					n even				
Vertex	Left	Right	Pendant	Sum	Vertex	Left	Right	Pendant	Sum
$\frac{n+1}{2}-3$	$\frac{n+1}{2}-4$	$\frac{n+1}{2}-3$	$\frac{3n+1}{2}+3$	$\frac{5n+1}{2}-4$	$\frac{n}{2}-3$	$\frac{n}{2}-4$	$\frac{n}{2}-3$	$\frac{3n}{2}+4$	$\frac{5n}{2}-3$
$\frac{n+1}{2}-2$	$\frac{n+1}{2}-3$	$\frac{n+1}{2}-2$	$\frac{3n+1}{2}+2$	$\frac{5n+1}{2}-3$	$\frac{n}{2}-2$	$\frac{n}{2}-3$	$\frac{n}{2}-2$	$\frac{3n}{2}+3$	$\frac{5n}{2}-2$
$\frac{n+1}{2}-1$	$\frac{n+1}{2}-2$	$\frac{n+1}{2}-1$	$\frac{3n+1}{2}+1$	$\frac{5n+1}{2}-2$	$\frac{n}{2}-1$	$\frac{n}{2}-2$	$\frac{n}{2}-1$	$\frac{3n}{2}+2$	$\frac{5n}{2}-1$
$\frac{n+1}{2}$	$\frac{n+1}{2}-1$	$\frac{n+1}{2}$	$\frac{3n+1}{2}$	$\frac{5n+1}{2}-1$	$\frac{n}{2}$	$\frac{n}{2}-1$	$\frac{n}{2}$	$\frac{3n}{2}+1$	$\frac{5n}{2}$
$\frac{n+1}{2}+1$	$\frac{n+1}{2}$	$\frac{n+1}{2}+1$	$\frac{3n+1}{2}-1$	$\frac{5n+1}{2}$	$\frac{n}{2}+1$	$\frac{n}{2}$	$\frac{n}{2}+1$	$\frac{3n}{2}$	$\frac{5n}{2}+1$
$\frac{n+1}{2}+2$	$\frac{n+1}{2}+1$	$\frac{n+1}{2}+2$	$\frac{3n+1}{2}-2$	$\frac{5n+1}{2}+1$	$\frac{n}{2}+2$	$\frac{n}{2}+1$	$\frac{n}{2}+2$	$\frac{3n}{2}-1$	$\frac{5n}{2}+2$
$\frac{n+1}{2}+3$	$\frac{n+1}{2}+2$	$\frac{n+1}{2}+3$	$\frac{3n+1}{2}-3$	$\frac{5n+1}{2}+2$	$\frac{n}{2}+3$	$\frac{n}{2}+2$	$\frac{n}{2}+3$	$\frac{3n}{2}-2$	$\frac{5n}{2}+3$
$\frac{n+1}{2}+4$	$\frac{n+1}{2}+3$	$\frac{n+1}{2}+4$	$\frac{3n+1}{2}-4$	$\frac{5n+1}{2}+3$	$\frac{n}{2}+4$	$\frac{n}{2}+3$	$\frac{n}{2}+4$	$\frac{3n}{2}-3$	$\frac{5n}{2}+4$

For even n

$$(\frac{n}{2}-m)^{\text{th}} \text{ vertex} \quad ; \frac{5n}{2}-m$$

$$(\frac{n}{2})^{\text{th}} \text{ vertex} \quad ; \frac{5n}{2}$$

$$(\frac{n}{2}+m)^{\text{th}} \text{ vertex} \quad ; \frac{5n}{2}+m$$

Where $n, m \in \mathbb{Z}^+$.

Proof: Let us assume that any two vertex sums have same number.

For odd n

(i) $\frac{5n+3}{2}-1=\frac{5n+3}{2}-(m+1)$

$m=0,$

a contradiction since $m \in \mathbb{Z}^+$;

(ii) $\frac{5n+3}{2}-1=\frac{5n+3}{2}-(m-1)$

$m=0,$

a contradiction since $m \in \mathbb{Z}^+$;

(iii) $\frac{5n+3}{2}-(m+1)=\frac{5n+3}{2}+(m-1)$

$m=0,$

a contradiction since $m \notin \mathbb{Z}^+$.

For even *n*

(iv) $\frac{5n}{2} = \frac{5n}{2} - m$

$m = 0,$

a contradiction since $m \notin \mathbb{Z}^+$;

(v) $\frac{5n}{2} = \frac{5n}{2} + m$

$m = 0,$

a contradiction since $m \notin \mathbb{Z}^+$;

(vi) $\frac{5n}{2} - m = \frac{5n}{2} + m$

$m = 0,$

a contradiction since $m \notin \mathbb{Z}^+$.

Therefore, no two vertex values are same. That is, each vertex sum is distinct. Hence, the pendant graph is anti-magic.

CONCLUSION

In our work, we found an alternative method for anti-magic labelling of wheel graph and pendant graph using the anti-magic labelling of path graph. For a wheel graph, we removed the middle vertex of the wheel graph and created a path graph using the vertices in the outer cycle of the wheel graph. Then spokes of the Wheel graph are represented by adding one edge to each vertex. Using the anti-magic labelling method of the path graph P_{n-1}, we found an alternative method to label the edges of the outer cycle of the wheel graph. Finally found the vertex sum for each vertex and we proved that every vertex sum is distinct and the middle vertex take the highest value. Anti-magic labelling method for a pendant graph was found by creating a path graph using the cycle of the pendant graph and connecting the pendant vertices to every vertex of the path graph. For anti-magic labelling of both pendant graph and wheel graph, we used the concept of the anti-magic labelling of path graph P_{n-1}. Finally, the vertex sum for each vertex was calculated and proved that every vertex sums take different values and the middle vertex take the highest value for the wheel graph.

REFERENCES

1. Chang, F. H., Chen, H. B., & Li, W. T. (2021). Shifted-Antimagic Labelings for Graphs. *Graphs and Combinatorics*, 1065–1082.

2. Graf, A. (2014). Graceful Labelings of Pendant Graphs . *Rose-Hulman Undergraduate Mathematics Journal*, 158-172.

3. Hartsfield, N., & Ringel, G. (1994). *Pearls in graph theory.* Boston: Academic press, INC, Boston.

4. Sugeng, K. A. (2005). *Magic and Antimagic Labeling of Graphs.* Victoria , Australia.

5. Weisstein, E. W. (n.d.). *Path Graph*. Retrieved from MathWorld--A Wolfram Web Resource: https://mathworld.wolfram.com/PathGraph.html

6. Weisstein, E. W. (n.d.). *Wheel Graph*. Retrieved from MathWorld--A Wolfram Web Resource: https://mathworld.wolfram.com/WheelGraph.html

RESEARCH ARTICLE

ANTI-MAGIC LIKE LABELLING OF MARIGOLD GRAPH

K. M. P. G. S. C Kapuhennayake[1], A. A. I. Perera [1]*

[1]Department of Mathematics, Faculty of Science, University of Peradeniya, Sri Lanka

ABSTRACT

In this paper, we present a new family of graphs called Marigold graphs and introduce a new labelling method similar to the anti-magic labelling. The Marigold graph is generated from any number of copies of fully binary trees which are going through concentric circles. All copies of trees are connected to a middle vertex and the height of the Marigold graph is increasing with concentric circles. One copy is considered as one petal in the marigold graph. A Marigold graph with n copies (petals) and height (number of concentric circles) k is denoted by M_k^n . The labelling method is defined as follows: A graph with 'm' edges and 'n' vertices is labelled as an injection from the set of edges to the integers $\{1, \dots, x\}$ such that all 'n' vertex sums are pairwise distinct, where the vertex sum is the sum of labels of all edges incident with that vertex. In our work, for edge labelling, we consider the petals one by one and denote the r^{th} edge at k^{th} level as e_r^k , and define a function to label edges of the first petal. Then define the new labelling method for other petals, such that for n^{th} petal, edge labelling is starting with $J_{n-1}+1$ (where J_{n-1} is the summation of all edge values in $(n-1)^{\text{th}}$ petal, $\sum_{i=1}^{m} e(n-1,i) = J_{n-1}$) and continue the labelling as a monotonically increasing sequence. We discuss some illustrative examples that might be used for studying the Anti-magic like labelling of Marigold graphs.

Keywords: *Anti-magic like labelling, Marigold graph, Full binary trees, Concentric circles*

DOI. *http://doi.org/10.4038/jsc.v12i2.36*

1. INTRODUCTION

Graph labelling is one of the important areas in graph theory. Graph labelling is used to give an identity to all the vertices and edges of it. That means graph labelling is an assignment of labels, represented by integers to edges or integers to vertices of a graph G. Vertex labelling is defined as a function between the set of vertices to the set of labels and a graph with such a function is called a vertex labelled graph. Similarly, an edge labelling is a function of edges to a set of labels. In this case, the graph is called an edge

*Corresponding author: *sonalichamathka03@gmail.com*

labelled graph. On the other hand, if the domain of the mapping is both the set of vertices and edges then the labeling is called total labeling. There is a vast amount of methods to label a graph. Graceful labelling, Prime labelling, Anti-Magic labelling are some examples for special cases of graph labelling. Among these methods, for our work, we use the concept of anti-magic labelling. The idea of anti-magic labelling had come from its connection to magic labelling and magic squares. In magic labelling, we label the set of edges of a graph G using non-negative integers such that the sum of edges around any vertex in G is a constant. (Solairaju & Begam, 2012)

The notion of anti-magic labelling was introduced by Nora Hartsfield and Gerhard Ringel in 1989. After that, so many variations of anti-magic labelling have been studied by referring to their book. They speculated that all simple connected graphs except K_2 are anti-magic.

Adding an extended to the research chain of Anti-magic labelling, in 2010 Hefetz, Mütze, and Schwartz initiated the study of anti-magic labelling of digraphs. (Hefetz, Mütze, & Schwartz, 2010). During their research, they presented that "All orientation is anti-magic" is not possible for directed graphs K_1, K_2, and K_3. And in the last sections of their publication, they conclude that all connected digraphs with at least 4 vertices are antimagic. Also, they conjectured that every connected undirected graph admits an anti-magic orientation. This called "Directed and undirected anti-magicness". Liang and Zhu proved that 3-regular graphs are anti-magic. (Liang & Zhu, 2014). In 2019, Li et.al conjectured that every graph admits an anti-magic orientation (Li, Song, Wang, Yang, & Zhang, 2019). This publication was able to strengthen the ideas of Hefetz and Mütze. K. Venkata Reddy and A. Mallikarjuna Reddy conjectured that the class of trees generated from two copies of full binary trees is anti-magic. (Reddy & Reddy, 2020)

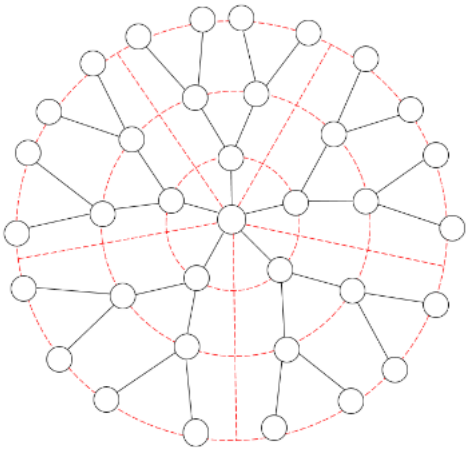


Figure 1: Marigold Graph M_3^5

Furthermore, in this paper, we introduced a new graph named a Marigold graph which is considered as the shape of a Marigold flower. This graph is generated from any number

of copies of full binary trees which are going through concentric circles. All copies of trees are connected to a middle vertex. One such copy of the graph represents one petal.

Moreover, we introduced a new labelling method using the idea of the anti-magic labelling, which is the final result is same as the result of anti-magic labelling. The new labelling is given to this graph, using the result obtained for anti-magic labelling of the full binary tree. The Figure 1 illustrates a Marigold graph with 5 petals and each petal contains full binary tree with height 3.

2. MATERIAL AND METHODS

Some important definitions we used to get the result of this paper are stated below.

Definition 2.1 (Anti-Magic labelling): Let G be a simple graph with V vertices and E edges. Anti-magic labelling of graph G is a 'one-to-one' correspondence between $E(G)$ and $\{1,2,\dots,|E|\}$ such that the vertex sum for distinct vertices are different. Where the vertex sum is the sum of the labels assigned to edges incident to a vertex. (Sugeng, 2005)

Definition 2.2 (The new labelling method similar to the Anti-magic labelling): A graph G with 'm' edges and 'n' vertices, is labelled as an 'injection' from the set of edges to the set of integers $\{1,\dots,x\}$ (where x is any integer) such that all 'n' vertex sums are pairwise distinct. Where the vertex sum is the sum of labels of all edges incident with that vertex.

Definition 2.3 (Full binary tree): A full binary tree is a tree in which every node other than the leaves has two children.

Definition 2.4 (Marigold graph): Marigold graph is a tree with n components, each component, which is a full binary tree of height k, is attached to a common vertex, and all the vertices, other than the middle vertex, belong to the same level are in one circle. If a full binary tree of the Marigold graph has k levels (where $= \{1,2,\dots,k\}$), then there are k concentric circles in the Marigold graph. We denote the Marigold graph as M_k^n; where k is the height of the full binary tree (or the number of concentric circles) and n is the number of copies of full binary trees.

In this section, we prove that the full binary trees are anti-magic.

Theorem 2.1: Full binary trees are anti-magic.

Proof: Let B_k denote the full binary tree of height k.

The maximum number of vertices in $B_k = \sum_{i=0}^{k-1} 2^i = 2k - 1$

Maximum number of edges in $B_k = \sum_{i=1}^{k-1} 2^i = 2k - 2$

Now, let us define a function to label the edges of B_k as follows;

$f\left(e_r^{k-1}\right) = r$; Where $r = \{1,2,\ldots,2k-2\}$ and e_r^k is the r^{th} edge at k^{th} level.

Labelling the edges as a monotonically increasing sequence starting from the bottom to top approach level wise, and approaching the edges connecting the levels k and $k+1$ in the left to right order. That is the function f is a bijection from $E(B_k)$ to $\{1,2 \ldots 2k-2\}$. Label the vertices as the sum of all edges incident with the corresponding vertex. It can be observed that the vertex labelling is a monotonically increasing sequence from bottom to top approach level wise and approaching the vertices in each level in the left to right. That means the vertex sum for distinct vertices are different. Hence, f is anti-magic labelling for a full binary tree. Therefore, full binary trees are anti-magic.

The anti-magic orientation of the full binary trees can be represented by Figure 2.

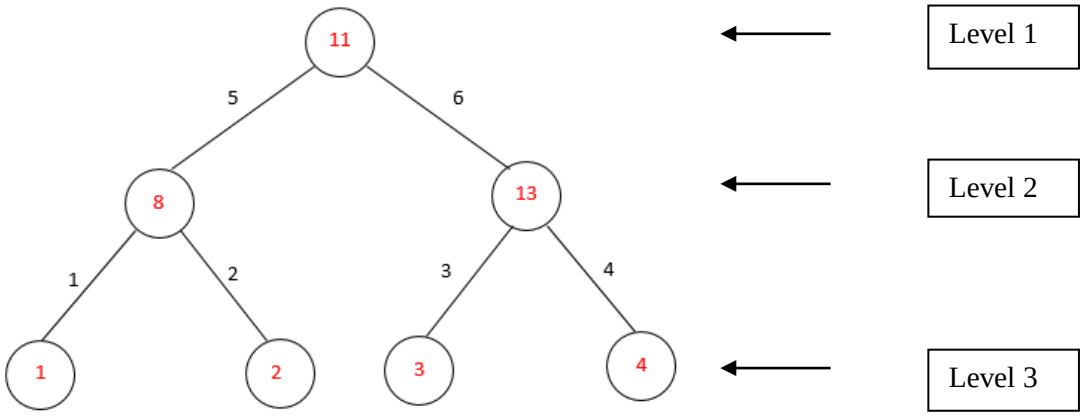


Figure 2: Anti-magic orientation of the full binary tree

Now, consider the Marigold graph.

Let's start labelling with the first copy. Label the edges of that as a monotonically increasing sequence starting from bottom to top approach level wise, and approaching the edges at the same level from left to right. Then it will be the new labelling method similar to the anti-magic labelling according to Theorem 2.1.

Assume the summation of edges in the first copy as;

$$\sum_{i=1}^{m} e(1,\mathrm{i}) = J_1,$$

where m is the number of edges in each full binary tree.

Then label the edges in the second copy starting with $(J_1 + 1)$ and as a monotonically increasing sequence.

Take the summation of edges of the second copy as J_2. Then

$$\sum_{i=1}^{m} e(2,\mathrm{i}) = J_2.$$

Similarly, label the edges in the third copy starting with $(J_2 + 1)$ and as a monotonically increasing sequence. Repeat the procedure for every copy in the Marigold Graph.

Then in the n^{th} copy, edge labelling should start with $(J_{n-1} + 1)$ and the summation would be

$$J_n ; \ \sum_{i=1}^{m} e(\mathrm{n},\mathrm{i}) = J_n.$$

Now, label the edges which are connected those copies to the middle vertex as a monotonically increasing sequence starting with $(J_n + 1)$. Note that we should label those edges starting with the edge which connects the first copy to the main vertex.

As usual, the vertex sum is the sum of labels of all edges incident with that vertex.

When we move on to each copy, the labelling of edges would be increased. Therefore all the vertices in n copies of full binary trees of the Marigold graph are distinct.

Since the edges which are connected to the middle vertex are also in a monotonically increasing sequence the middle vertex is different from all the other vertices in the Marigold graph. And it is the largest vertex in the graph. Therefore all the vertices of Marigold graph have distinct values and its labelling is a ‘onto’ correspondence between $E(G)$ and the set of integers $\{1,2,\ldots,x\}$ where x is any integer.

3 RESULTS AND DISCUSSION

Figure 3 illustrate the new labelling method similar to the anti-magic labelling, for the Marigold graph with 5 copies of full binary trees and 3 concentric circles; M_3^5, where

$$J_1 = 21, J_2 = 147, J_3 = 903, J_4 = 5439, J_5 = 32655$$

When the new labelling method applied, the vertex values of the Marigold graph get increased when we go through the first petal to the last petal in order, and also they are increased when go inside to the graph, through the concentric circles. That is the vertices in the outer circle have less values in each copy of perfect binary trees. Then, the middle vertex has the highest vertex value.

CONCLUSION

In our work, we defined a new graph called the Marigold graph which is generated by any number of copies of full binary trees, and the height of the graph is increasing with concentric circles. Furthermore, we introduced a new labelling method which is similar to the anti-magic labelling for the Marigold graph. The vertex sums of the final labelling

graph are pairwise distinct. As our future work, we are planning to apply this new labelling method to reduce the tendency of Gold grabbing game.

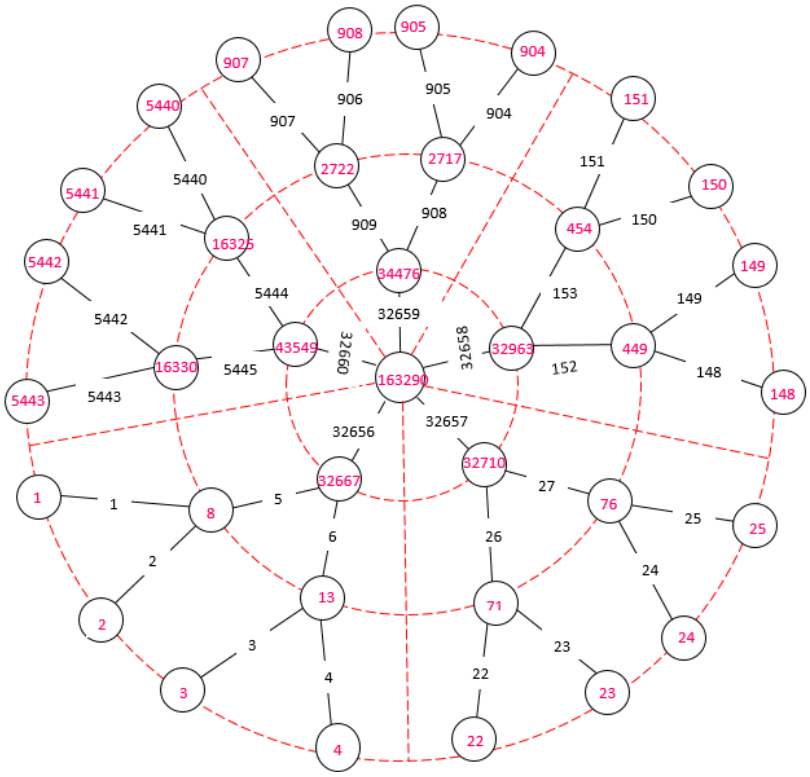


Figure 3: New labelling for M_3^5 which has a result same as the anti-magic labelling

REFERENCES

1. Hartsfield, N., & Ringel, G. (1994). *Pearls in graph theory*. Boston: Academic press, INC, Boston.
2. Hefetz, D., Mütze, T., & Schwartz, J. (2010). *On antimagic directed graphs*. Journal of Graph Theory, 219-232.
3. Li, T., Song, Z.-X., Wang, G., Yang, D., & Zhang, C.-Q. (2019). *Antimagic orientations of even regular graphs*. Journal of Graph Theory, 46-53.
4. Liang, Y.-C., & Zhu, X. (2014). *Antimagic Labeling of Cubic Graphs*. Journal of graph theory, 31-36.
5. Reddy, K. V., & Reddy, A. M. (2020). *A new class of Anti-magic Trees*. Journal of Xi'an University of Architecture & Technology, 118-123.
6. Solairaju, A., & Begam, R. R. (2012). *Edge-Magic Labeling of some Graphs.* International Journal of Fuzzy Mathematics and Systems. , 47-51.
7. Sugeng, K. A. (2005). *Magic and Antimagic Labeling of Graphs.* Victoria , Australia

RESEARCH ARTICLE

A MONTHLY EVALUATION OF MICROBIOLOGICAL AND CHEMICAL QUALITY OF BOTTLED DRINKING WATER

A. T. Herath[1]*, *C. L. Abayasekara*[2]

[1]*Department of Botany, Faculty of Natural Sciences, Open University of Sri Lanka*
[2]*Department of Botany, Faculty of Science, University of Peradeniya, Sri Lanka*

ABSTRACT

Use of bottled water has flourished in Sri Lanka, owing to its high demand, however, the quality of commercially available bottled drinking water is questionable. The objective of the current study was to assess the monthly variation of microbiological and chemical quality of bottled water. Three brands were selected, twenty-four bottles of each brand were collected for analysis. Microbiological and chemical analysis were carried out monthly. Though the shelf life of bottled drinking water is one year, the experiment was terminated in eight months as bacterial counts showed a steady decline with time as follows. The results indicated that Total Coliform [TC], Fecal Coliform [FC] and Heterotrophic Plate Count [HPC] bacteria decreased throughout the shelf life. There were significant differences in TC ($P<0.05$) and FC ($P<0.01$) between microorganisms initially present and after eight months of storage. Even though SLSI permitted levels for presumptive TC is less than zero cfu per 100ml, the average count of TC was 139 cfu per 100 ml at the end of the first month. According to national and international standards, the FC count should be zero per 100 ml for drinking water. However, at the first month some bottled water samples exceeded this limit for presumptive FC, with an average count of 32.5 cfu per 100 ml. For HPC bacteria, only one brand exceeded the WHO guidelines (50 cfu/ml). No algal species were detected. Fungal colonies showed a reduction in number over an eight-month duration. *Penicillium* sp. and *Aspergillus* sp. were dominant. Chemical parameters were within the permitted levels, except hardness in water samples. The result of this study reveals that the bottled water industry needs to be monitored closely and continuously by relevant authorities, intending to provide safe bottled water to the public.

Keywords: *Bottled water, Microbiological and chemical quality, Monthly variation*

DOI. *http://doi.org/10.4038/jsc.v12i2.37*

*Corresponding author: *whher@ou.ac.lk*

1. INTRODUCTION

Bottled water can be defined as any potable water that is bottled and distributed or offered for sale and specifically intended for human consumption. Sales of bottled water are increasing for many reasons. In many developing countries, tap water is contaminated or thought to be contaminated with various forms of pollutants. In regions, where reliable, low-cost, and high quality tap water is not available, bottled water provides a safe alternative. Bottled water is regulated as a 'food' by the US Food and Drug Administration (FDA). The FDA requires that bottled water to be clean and safe for human consumption, that they are processed and distributed under sanitary conditions and they are produced in compliance with FDA good manufacturing practices (https://www.fda.gov/food/buy-store-serve-safe-food/fda-regulates-safety-bottled-water beverages-including-flavored-water-and-nutrient-added-water).In applying the guidelines to bottled waters, certain chemical constituents and microbial contents may be more readily controlled than in tap water. Some microorganisms that are normally of little or no public health significance may grow to higher levels in bottled water [1].

The bottled water industry has become a vital and vigorous sector in developed and developing countries worldwide. In Europe, The United States, North America, Canada and in other developed countries bottled water is one of the most important food items of high demand. As a result, bottled water consumption has significantly increased [2, 3, 4, 5, 6]. Further, the consumption of bottled water in Iran has increased particularly among the urban population and travelers [7], as there is a general belief among consumers that bottled water is safe and free of all impurities, including bacteria [8]. This reflects consumer concerns about tap water, since bottled water is often regarded as safer as and healthier than tap water.

However, microbial surveys carried out worldwide indicated various problems with bottled water such as high Heterotrophic Plate Count (HPC) levels [2, 9], *Vibrio cholerae* presence and infections [10], fungal spoilage [11], *Pseudomonas aeruginosa* [3, 12]. Therefore, the bottled water industry has to exhibit strict quality standards in terms of microbial parameters, production processing, bottling, transportation and storage [13, 14], while Hazard Analysis Critical Control Points (HACCP) systems should be implemented in the bottling process [3].

In Sri Lanka use of bottled water has been increasing over the last two decades. However, due to the increased demand and consumption of bottled water in Sri Lanka, there has been a growing concern about the microbiological quality of this product. Several studies done in Sri Lanka concerning the quality of the bottled water have reported that they have exceeded the levels permitted by the Sri Lankan Standard Institution for the presumptive total coliforms [15, 16]. With this background, the main objective of this study was to assess the monthly variation of microbiological and chemical quality of bottled water during storage.

2. MATERIAL AND METHODS

2.1 Sample Collection

To determine the microbiological and chemical quality of bottled water three bottled water brands were collected. Twenty-four bottles of each brand were collected for analysis. All bottles were collected from the Kandy district, of the central province of Sri Lanka. The samples were stored at room temperature (27±2 ºC) and analyzed monthly after the date of manufacture throughout the first eight months of the shelf life of the bottled water, while the shelf life of bottled water is one year. (The experiment was terminated after 8 months as bacterial counts showed a steady decline and FC counts reduced to zero as early as in the 2nd month.)

2.2 Microbiological analysis

Total and fecal coliforms were counted by membrane filtration method [17] passing 100 ml volumes of each sample through the membrane filtration apparatus (Pyrex, Germany) using sterilized membrane filters (Sartorius, Germany) with 0.45 µm pore sizes. Membrane filters were placed on pre sterilized absorbent pads (Sartorius, Germany), saturated with 3 ml of M-Endo broth (HI-media, India) (for TC) and 3 ml of M-FC broth base (HI-media, India) (for FC). Plates were incubated for 24-48 hours at 36 ± 1 °C and at 44.5 °C for the detection of total coliforms and fecal coliforms respectively. Sterilized distilled water and typical coliforms (*Serratia marcescens* -NCTC 11935, *Escherichia coli* -ATCC 25922) were used as a negative control and a positive control respectively in detection of coliform bacteria. Typical red color colonies with a green metallic sheen were counted as total coliforms on M-Endo medium. In addition, red- pink colonies with a sheen were counted. Typical blue color colonies formed on M-FC medium were counted as fecal coliforms. Numbers of yellow colour atypical colonies on M-FC were also recorded. Heterotrophic Plate Count (HPC) bacteria present in bottled water samples were determined by the pour plate method [18]. The number of bacterial colonies was reported as colony-forming units per milliliter (cfu/ml). Analyses were carried out in triplicate for all determinations. Fungi were isolated by spreading 0.1 ml aliquots of bottled water samples on Potato Dextrose Agar plates, and the plates were incubated at room temperature for 4-5 days. Identification was done by observing colony characteristics, reproductive morphology through microscopic observations and with the aid of reference materials [19, 20]. Algae in bottled water samples were estimated in a zigzag pattern using a Sedgwick rafter cell, under a light microscope.

Confirmation of presumptive coliform bacteria

Selected colonies isolated on M-Endo and M-FC media were confirmed for total coliforms and fecal coliforms respectively. Presumptive total and fecal coliforms colonies were sub cultured by streaking on Tryptone Soy Agar (TSA) (OXOID-

CM0131). Subsequently, well isolated colonies from TSA plates were subjected to confirmation. Total coliforms and fecal coliforms were confirmed by inoculating colonies into Brilliant Green Lactose Broth (BGLB) (HI-media, India) tubes and peptone water tubes respectively.

2.3 Chemical analysis

Physiochemical parameters were analyzed following the standard guidelines and procedures [21]. The alkalinity, hardness and the chloride (Cl^-) contents were determined by titration methods using Hach digital titrator and Hach standard reagent cartridges. Calcium (Ca), iron (Fe), manganese (Mn) and zinc (Zn) were measured by the atomic absorption spectrometer (Varian 240FS Inc., Australia) and spectrophotometer (Hach DR-2400 with standard reagents) was used to determine nitrate (NO^{3-}), nitrite (NO^{2-}), phosphate (PO_4^{3-}), fluoride (F^-), ammonium (NH_4^+), sulphate (SO_4^{2-}) and sulfide (S^{2-}). All instruments were calibrated using commercially available standard solutions (BDH, Fulka) before performing the measurements.

3. RESULTS AND DISCUSSION

Over the shelf life of the bottled water samples, a reduction in number of colony forming units were observed. Table 1 shows the analysis of variance applied to microbiological quality of bottled water samples. For all three bacteriological parameters (TC, FC, HPC bacteria), bacterial counts decreased with time during the eight months of the shelf life of bottled water. There were significant differences ($P < 0.05$) in total coliforms numbers between the numbers of microorganisms initially present, with an average count of 139 cfu per 100 ml at one month in the water and those present after eight months of storage, which decreased to 4 cfu per 100 ml, while the level permitted by SLSI for TC is zero cfu per 100 ml [22]. According to national and international standards, the FC count should be zero per 100 ml for drinking water. However, at the first month some bottled water samples exceeded this limit for presumptive fecal coliforms, with an average count of 32.5 cfu per 100 ml, which declined to zero cfu per 100 mL at eight months, exhibiting a significant difference ($P < 0.01$) in the number of presumptive FC between one month and eight months after storage (Table 1). This reduction may be due to nutrients depletion during storage. Fecal coliforms were absent in all bottles of one brand analyzed in the study.

Coliform bacteria are used as an indicator organism in assessing drinking water quality. Coliforms constitute a large portion of human intestinal microflora. These ensure the presence of indicator organisms providing evidence for contamination of water with human faeces. However, there are some coliforms found in plant and soil samples. Therefore, the numbers of TC should be higher than FC, generally present as fecal contaminants. Presence of FC in water is considered as an accurate indication of contamination from fecal matter rather than presence of TC [23]. Coliform organisms

have long been recognized as a good microbiological indicator of drinking water quality, due to their ease of detection and quantification in water [1].

Table 1: Analysis of variance (ANOVA) of microbial quality of bottled water stored during 8 months after the date of manufacture. (the data were converted to arcsin values to minimize gaps [Zar, 1999]); Mean values followed by the same superscript (a & b) within a column, do not differ significantly ($p<0.05$) for TC, ($p<0.01$) for FC and ($p<0.05$) for HPC

Time Period (months)	Mean of presumptive TC cfu per 100 ml	Mean of presumptive FC cfu per 100 ml	Mean of HPC cfu per ml
1	139.00[a]	32.50[a]	84.00[a]
2	42.83[b]	0.00[b]	60.50[a]
3	23.33[b]	0.50[b]	38.67[a]
4	15.00[b]	0.00[b]	30.83[a]
5	11.33[b]	0.00[b]	19.83[a]
6	7.33[b]	0.00[b]	12.83[a]
7	7.33[b]	0.00[b]	11.83[a]
8	4.00[b]	0.00[b]	8.33[a]

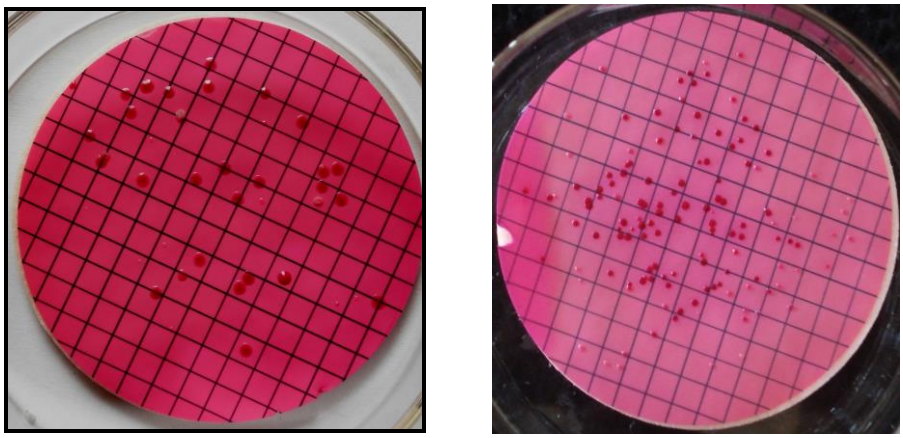
Several studies done in different countries have revealed that bottled water were inappropriate for human consumption due to the presence of different microorganisms. A study done in Nepal stated that out of 100 samples, 48% of samples were found to be contaminated with total coliform such as, *E. coli*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa* [24]. Another study done in Nepal has shown bottled water brands commercially available were found to be contaminated with indicator organisms in drinking water and the counts exceeded WHO standards [25]. Further, a study done in Mexico stated that total coliforms, fecal coliforms, and *E. coli* were found in bottled water samples tested and certain water samples exceeded the maximum allowable limit imposed by Mexico's standard guidelines [26]. Moreover, many studies done in Sri Lanka stated that bottled water brands tested exceeded permitted level for microbiological parameters [9, 10, 16, 27].

It is important to conduct confirmation test for presumptive coliform in assessing drinking water quality as confirmation. In the current study twenty percent of presumptive total coliform and fecal coliform colonies were used for confirmation testing. With respect to total coliforms confirmation was done for typical red colonies with a green metallic sheen and for red-pink colonies with a sheen (Figure 1). With respect to fecal coliforms confirmation was done for typical blue colonies and atypical green/yellow colonies (Figure 2). During the current study, typical red colonies with a green metallic sheen on M-Endo medium were confirmed as total coliforms while red/

pink colour colonies with a sheen were not confirmed as total coliforms. Furthermore, certain typical blue colonies on M-FC medium were confirmed as fecal coliforms (Table 2). However, according to [28] both pink colour colonies and atypical cream colour colonies (total of colonies isolated from well water, river water, effluent water and bottled water) isolated on M-Endo medium have been confirmed as total coliforms ranging from a confirmation rate of 55 % – 88 %. The difference of these confirmation results may probably be due to the fact that, bottled water being subjected to UV radiation before bottling, altering the cell characteristics of these pathogens and not showing a typical reaction in selective media or during the confirmation. This also raises the question of the pathogenicity of these organisms. This hypothesis will have to be examined further.

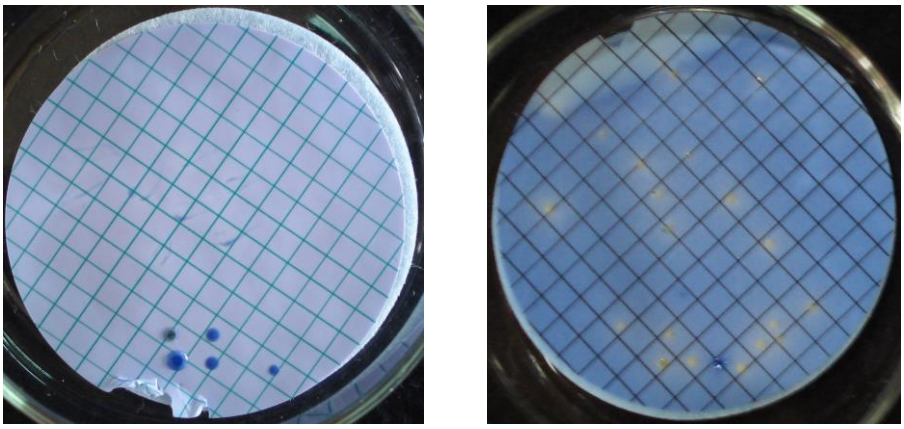
During storage, HPC bacteria was detected in all three brands tested, although only one brand exceeded the permitted level for drinking bottled water. According to the results, during the eight months of the shelf life of bottled water samples, they showed a reduction in number of colony forming units for HPC bacteria (Table 1). The presence of high number of heterotrophic bacteria in bottled water is probably due to microorganisms naturally occurring in source water.

Many studies done in different countries have revealed that bottled water may contain a range of microorganisms, including *Aeromonas*, *Alcaligenes*, *Arthrobacter*, *Caulobacter*, *Corynebacterium*, *Flavobacterium*, and *Pseudomonas* [29, 30, 31, 32]. It is not reported that these HPC bacteria represent a health affect through water consumption by the general public. However, severely immunocompromised persons are not recommended to consume drinking water contaminated with these microorganisms.



(a) (b)

Figure 1: Presumptive total coliforms species on a M-Endo plates with typical red colonies with a green metallic sheen (a) and red-pink colonies with sheen (b)



(a) (b)

Figure 2 : Presumptive fecal coliforms species on a M-FC plates with typical blue colonies (a) and atypical colonies (yellow colour) (b)

Table 2: Confirmation test results for detecting presumptive total and fecal coliforms

Number of colonies used for confirmation		Number confirmed by testing	Confirmation rate %
Total coliforms			
Typical red colour with a green metallic sheen colonies	23	23	100
Red/pink colour colonies with a sheen	35	0	0
Fecal coliforms			
Typical blue colonies	15	15	100
Atypical yellow colonies	10	0	0

During the current study, no algal species were detected in any of the tested bottled water samples throughout the eight-month duration. All the water samples examined were contaminated with fungal species. Among the fungal species isolated, *Penicillium* sp. and *Aspergillus* sp. were dominant while, *Cladosporium* sp. and *Trichoderma* sp. were detected in lesser numbers. In considering the result of fungal colonies, a reduction in number over the eight-month duration was observed. Some of the fungi isolated from bottled water samples are species commonly found in the environment, while some of these fungi can cause diseases in humans. eg; chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis [33]. *Alternaria alternata* and *Penicillium citrinum* have some toxigenic potential and could constitute some health risk. It is therefore advisable to assess fungal propagules in routine microbiological studies of bottled drinking water to establish baselines [34].

Chemical parameters are important factors of drinking water. Drinking water may contain several minerals (e.g., fluoride, potassium, zinc) and trace constituents (e.g., zinc, arsenic, manganese. iron, cyanide, lead) that are associated with both benefits and risks for public health [4].

Table 3: Summary of results of chemical quality of bottled water analysis during 1st and 8th months after manufacturing. Parameter concentrations are given in mg/l unless otherwise specified. SD – Standard Deviation

Parameter	Initial (1st month)				Final (8th month)				Permitted level
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	
pH	5.75	6.8	6.37	0.54	5.32	6.89	6.22	0.81	6.5 to 8.5
EC (µS/cm)	15	146	83.33	65.68	17	148	85	65.64	2500
Alkalinity	4	14	9.73	5.16	5.6	60	36.53	27.96	200.0
Hardness	9.2	34.8	24.93	13.37	8.8	48.8	33.73	21.75	10-20
Chloride	7.5	22.5	13	8.05	11.25	39.5	21.17	15.89	250.0
Fluoride	0	0.35	0.23	0.2	0.12	0.21	0.17	0.05	1.5
Sulfate	0	4	1.67	2.08	1	1	1	0	250.0
Sulfide (µg/l)	0	32	11	18.19	0	0	0	0	50
Phosphate	0.01	0.12	0.06	0.06	0.12	0.19	0.16	0.04	5
Nitrate-N	2.7	3.6	3.3	0.49	0.3	0.6	0.47	0.15	50.0
Nitrite-N	0.003	0.007	0.005	0.002	0.005	0.007	0.006	0.001	3.0
Ammonium-N	0	0.04	0.01	0.02	0	0.02	0.007	0.011	0.5
Zn	0	0.1	0.003	0.006	0	0.04	0.01	0.02	3.0
Ca	0.49	5.12	3.32	2.482	0.11	4.85	2.87	2.46	150
Mn	0	0.1	0.007	0.006	0.01	0.03	0.02	0.01	0.05
Fe	0	0.1	0.007	0.006	0.01	0.03	0.02	0.01	0.2

Chemical analysis of bottled water samples collected showed that most of the parameters investigated slightly deviated from the permitted levels of WHO and SLS at the first month and at the 8th month.

In the present study, the minimum and maximum values of pH in bottled water were 5.32 and 6.89 respectively. The minimum value was slightly below the WHO and SLS permitted levels. At the beginning and the end of the analysis maximum values of hardness in water samples exceeded the permitted levels. Values of alkalinity and electric conductivity did not exceed the permitted levels recommended by the Health Ministry, Sri Lanka, and the values were very low (Table 3). However, NO_2^-, Cl^- and PO_4^{3-} levels were higher at the 8th month than that of the initial values for most of the samples, though within the permitted levels. NO_3^-, SO_4^{2-}, S^{2-}, NH_4^+, F and Ca levels were higher at the beginning and decreased with time and were all below their respective permitted levels.

According to the information given on the labels of bottled water, most of the source water for bottling in Sri Lanka, is from the central hill regions or wet zone of the country, where lower levels of fluoride is observed in water compared to that of water from the dry zone. In bottled water samples tested in the current study, the maximum fluoride level was 0.35 mg/l, which does not have any detrimental health effects accordingly to WHO guidelines.

CONCLUSION

The results of the current study indicate concerns over the bottled water industry in Sri Lanka, according to the results, some bottled water samples were not safe for drinking according to the Health Ministry regulation in Sri Lanka (The Gazette of the Democratic Socialist Republic of Sri Lanka, 1420/4, 21/11/2005) as they exceeded the permitted levels for one or more of the microbial parameters analyzed. When considering the tested chemical parameters, the chemical quality of bottled water was within permitted levels, except hardness in water samples. It is also revealed that both bacteriological and chemical parameters can change during the storage of bottles. Therefore, bottled water industries need to be closely and continuously monitored by relevant authorities, intending to provide safe bottled water to the public.

REFERENCES

[1] WHO (2011). Guidelines for Drinking-Water Quality (4th ed.) Microbiological Methods. World Health Organization, Geneva.

[2] Warburton, D. W. (2000). Methodology for screening bottled water for the presence of indicator and pathogenic bacteria. Food Microbiology 17: 3-12.

[3] Venieri, D., Vantarakis, A., Komninou, G. and Papapetropoulou, M. (2006). Microbiological evaluation of bottled non-carbonated ("still") water from domestic brands in Greece. International Journal of Food Microbiology 107(1): 68-72.

[4] Azoulay, A., Garzon, P. and Eisenberg, M.J. (2002). Comparison of the mineral content of tap water and bottled waters. Journal of General Internal Medicine 16 (3): 168-75.

[5] Pintar, K. D. M., Waltner-Toews, D., Charron, D., Pollari, F., Fazil, A. and McEwen, S. A. (2009). Water Consumption Habits of a South-Western Ontario Community. Journal of Water and Health 7: 276-92.

[6] Ward, L., Cain, O., Mullally, R., Holliday, K., Wernham, A. and Baillie, P. (2009) Health Beliefs about Bottled Water: A Qualitative Study. BMC Public Health 9: 196-210.

[7] Mardani, M., Gachkar, L., Peerayeh, S. N., Asgari, A., Hajikhani, B. and Amiri, R. A. (2007). Surveying common bacterial contamination in bottled mineral water in Iran. Iranian Journal of Clinical Infectious Diseases 2: 13-15.

[8] Tamagnini, L. M. and Gonzalez, R.D. (1997). Bacteriological stability and growth kinetics of *Pseudomonas aeruginosa* in bottled water. Journal of Applied Microbiology 83: 91-94.

[9] Diduch, M., Polkowska, Z., and Jacek, N. (2016). The role of heterotropic plate count bacteria in bottled water quality assesment. Food control, 61: 188-195.

[10] Blake, P. A., Rosenberg, M. L., Florencia, J., Costa, J. B. and Gangarosa, E. J. (1977). Cholera in Portugal, 1974. II. Transmission by bottled mineral water. American Journal of Epidemiology 105: 344-48.

[11] Carbal, D. and Fernandez-Pinto, V. E. (2002). Fungal spoilage of bottled water. International Journal of Food Microbiology 72: 73-6.

[12] Herath, A. T., Abayasekara, C. L., Chandrajith, R. and Adikaram, N. K. B. (2014). *Pseudomonas aeruginosa* in bottled drinking water in Sri Lanka: a potential health hazard. Water Supply 14: 1045-1050.

[13] Cowman, S. and Kelsey, R. (1992). Bottled water. In C. Vanderzant & D. F. Splittstoesser (Eds.), Compendium of methods for the microbiological examination of foods (3rd ed.) Washington, DC: American Public Health Association, 1031-36.

[14] Hunter, P. R. (1993). The microbiology of bottled mineral waters. Journal of Applied Bacteriology 74: 345-52.

[15] Herath, A. T., Abayasekara, C. L., Chandrajith, R. and Adikaram, N.K.B. (2012). Temporal variation of microbiological and chemical quality of noncarbonated bottled drinking water sold in Sri Lanka. Journal of food Science77: 160-164.

[16] Sasikaran, S., Sritharan, K., Balakumar, S. and Arasaratnam, V. (2012). Physical, chemical and microbial analysis of bottled drinking water. Ceylon Medical Journal, 57: 111-116.

[17] Obiri-Danso, K. and Jones, K. (1999). The effect of a new sewage treatment plant on faecal indicator numbers, campylobacters and bathing water compliance in Morecambe Bay. Journal of Applied Microbiology 86: 603-14.

[18] FAO (Food and Agricultural Organization) (1992). Manual of Food Quality Control 4. Rev. 1. Microbiological Analysis. FAO, Rome, pp. 27– 48.

[19] CMI (Commonwealth Mycological Institute) (1964). Description of Pathogenic Fungi and Bacteria. Kew, Surrey, England No. 91, 96.

[20] Barnett, H. L and Hunter, B. B. (1972). Illustrated Genera of Imperfect Fungi. 3rd ed. Burgess Publishing Company, 241.

[21] Hach (2002). Water analysis handbook. 4th Edition. Hach Company, Colorado, USA.

[22] Sri Lanka Standards 894: Part 2: (2020). Specification for Bottled (Packaged) Drinking water (3rd Revision), SLSI.

[23] Downes, F. P. and Ito, K. (Eds) (2001). Compendium of methods for the microbiological examination of foods. 4th ed. American Public Health Association, 676.

[24] Gautam, B. (2021). Microbiological quality assessment (including antibiogram and threat assessment) of bottled water. Food Scirnce & Nutrition, 9: 1980-1988.

[25] Pant N. D., Poudyal N. and Bhattacharya S.K. (2016). Bacteriological quality of bottled drinking water versus municipal tap water in Dharan municipality, Nepal. Journal of Health, Population and Nutrition, 35: 17. (doi:10.1186/s41043-016-0054-0)

[26] Soria-Herrera, R. J., Dominguez-Gonzalez, K. G., Rumbo-Pino, R., Piña-Lazaro, A., Rivera-Gutierrez, S., Salas-Rangel, L. P., Ortiz-Alvarado, R., Gonzalez-y-Merchand, J. A., Yahuaca-Juarez, B. and Cerna-Cortes, J. F. (2020). Microbiological quality of bottled water obtained from mexican small water purification plants: A pilot study, carried out in Morelia (central Mexico). Food Protection Trends, 40: 314-319.

[27] Ragila, K., Abiman, P., Thavaranjit, A. and Iyngaran, P. (2017). Quality analysis of bottled drinking water in Jaffna Peninsula. Vingnanam Journal of Science, 13:78-83.

[28] Mannapperuma, C. K. (2010). Comparison of Bacteriological Methods for Detecting Total Coliforms and Escherichia coli in water. Ph.D. thesis, University of Peradeniya, Sri Lanka, 207.

[29] Manaia, C. M., Nunes, O. C., Morais, P. V. and Dacosta, M. S. (1990). Heterotrophic plate counts and the isolation of bacteria from mineral waters on selective and enrichment media: Journal of Applied Bacteriology 69: 871-76.

[30] Kassenga and Gabriel, R. (2007). The health-related microbiological quality of bottled drinking water sold in Dar es Salaam, Tanzania. Journal of water health, 5: 179-185.

[31] Majumder A. K., Islam K. M. N., Nite R. N. and Noor R. (2011). Evaluation of Microbiological Quality of Commercially Available Bottled Water in the City of Dhaka, Bangladesh. Stamford Journal of Microbiology, 1(1), 24-30.

[32] Rai R., Kumal B., Rai D., Keshari A. and Bhandari R. (2016). Bacteriological Evaluation of Bottled Water Commercially Available in Eastern Nepal. Sunsari Technical College Journal, 2: 54-57. (doi:10.3126/stcj.v2i1.14801)

[33] Hedayati, M. T., Pasqualotto, A. C., Warn, P. A., Bowyer, P. and Denning, D. W. (2007). *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer, Microbiology 153: 1677–92.

[34] Carbal, D. and Fernandez-Pinto, V. E. (2002). Fungal spoilage of bottled water. International Journal of Food Microbiology 72: 73-6.

RESEARCH ARTICLE

ASSESSMENT OF MICROBIOLOGICAL AND CHEMICAL QUALITY OF SPRINGWATER IN RIVERSTON OF KNUCKLES MOUNTAIN RANGE IN SRI LANKA

*A. T. Herath**

Department of Botany, Faculty of Natural Sciences, Open University of Sri Lanka

ABSTRACT

Safe and readily available water is important for public health, whether it is used for drinking, domestic use, food production or recreational purposes. The main objectives of this study were to assess the microbiological and chemical quality of spring water in Riverston, situated in the Knuckles Mountain range, Sri Lanka and to identify bacteria isolated from spring water. Water samples were collected from ten springs from different locations. Microbiological and chemical analysis were carried out according to standard protocols. Isolated bacteria were identified using biochemical tests and API identification system. According to the results, Total coliforms (TC) (ranged from 0-27 per 100 ml) and Fecal coliforms (FC) (ranged from 38-326 per 100 ml) bacteria were detected in all water samples tested, and the detected numbers exceeded permitted levels for drinking water. There are four TC species, *viz; Escherichia vulneris, Serratia marcescens*, *Serratia liquefaciens* and *Proteus mirabilis* and one FC species, *viz; Escherichia coli* (dominant species), were identified during the study. All chemical parameters tested were within the permitted levels. This study reveals that spring water in Riverston, Knuckles Mountain Range is not a safe drinking water source. Hence, it is important to take necessary precautions, especially as spring waters from these areas are consumed by many and is the main source water for the bottling industry in Sri Lanka.

Keywords: *Drinking water quality, fecal coliforms, Riverston, spring water, total coliforms*

DOI. *http://doi.org/10.4038/jsc.v12i2.38*

1. INTRODUCTION

Natural freshwater is the most valuable resource which is distributed unevenly around the globe. A major portion of the available water is trapped in areas where humans cannot utilize it for their daily needs. Globally, around 785 million people do not have access to

*Corresponding author: whher@ou.ac.lk

adequate water supply sources [1]. Drinking water is defined as one which does not contain disease producing organisms and chemical substances deleterious to health [2]. Drinking water comes from two major sources; surface water and groundwater. The natural surface water sources include rivers, streams and lakes and groundwater sources are wells and springs.

In Sri Lanka, it is challenging to realize the trend of water quality in public water bodies due to a shortage of monitoring data [3]. As the natural water bodies are polluted with toxic substances and microorganisms, this unsafe water can cause health issues in humans and numerous adverse impacts on aquatic organisms. The Sri Lanka National Water Development Report stated a variety of quality concerns in Sri Lanka, including contamination by nitrate and bacteria in underground and surface waters mainly due to poor sanitation and untreated wastewater or inadequate wastewater treatment, toxic substances from industrial and agricultural activities, and eutrophication in lakes and reservoirs [3, 4].

Groundwater is a major vital natural resource because of its purity and availability. They provide most of the water for individual homes in small towns and rural areas in Sri Lanka. Further, natural springs, shallow and deep wells are commonly used as sources for bottling, natural springs from the mountain regions being the most popular source in Sri Lanka. Most bottled water and mineral water brands in Sri Lanka indicate natural spring water as their water sources. The public believes that these waters originate from protected underground water sources and must be safe to drink at source, in their natural state, without disinfection or chemical treatment. Natural mineral water can only come from specific designated groundwater sources, such as natural exiting or boreholes.

Knuckles mountain range in Sri Lanka is a popular area for spring water. It lies in central Sri Lanka, in the district of Matale and Kandy (33.6819° S, 150.8594° E). Moreover, some bottled water companies are situated at the Knuckles Mountain range. With this background, the main objectives of this study were to assess microbiological and chemical quality of springwater in Riverston, situated in the Knuckles Mountain range, Sri Lanka and to identify bacteria isolated from springwater.

2. MATERIALS AND METHODS

2.1 *Sample collection*

Water samples were collected from ten springs from different locations in Riverston, in the Knuckles Mountain range, Sri Lanka to determine the microbiological and chemical parameters. Water samples were collected in sterilized bottles, brought to the laboratory, and stored at refrigerated temperature (4^{o} C) until the time of analysis. Analysis was carried out within 24 hours after collection.

2.2 *Sample analysis*

Microbiological analysis

Total coliform (TC) and fecal coliform (FC) were enumerated by membrane filtration method [5] passing 100 ml volumes of each sample through the membrane filtration apparatus (Pyrex, Germany) using sterilized membrane filters (Sartorius, Germany) with a pore size of 0.45 µm. Membrane filters were aseptically placed on pre sterilized absorbent pads (Sartorius, Germany), saturated with 3 ml of M-Endo broth (HI-media, India) and 3 ml of M-FC broth base (HI-media, India) and were incubated for 24-48 hours at 36 ± 1 °C and at 44.5 °C for the detection of total coliforms and fecal coliforms respectively. Sterilized distilled water and typical coliforms (*Serratia marcescens* -NCTC 11935, *Escherichia coli* -ATCC 25922) were used as a negative control and positive controls respectively in the detection of coliform bacteria.

Isolation of bacteria for identification

Selected presumptive coliforms colonies were sub cultured by streaking on Tryptone Soy Agar (TSA, Oxoid, UK). Subsequently, well isolated colonies from TSA plates were subjected to identification. The three basic standard preliminary tests viz; Gram's test, oxidase test and catalase test were conducted for each isolate. Stock cultures of all isolates were made for further identification as follows. Pure colonies of isolates were inoculated into microcentrifuge tubes, containing Brain Heart Infusion Broth (BHIB, Oxoid, UK) and the tubes were incubated for 24 hours at 37^{o} C. Three replicate stock cultures were prepared from each isolate and stored at -20^{o} C, after overlaying with 60 % glycerol.

Identification of bacteria

One or two microcentrifuge tubes from each stock culture were thawed, and the tubes were centrifuged for a few seconds to obtain a concentrated cell mass. Subsequently, the TSA (Oxoid, UK) plates were streaked from the concentrated cell mass and the plates were incubated at 37^{o} C for 24 h to obtain pure colonies required for identification tests. The three basic preliminary tests, the Gram's test, Oxidase test and the Catalase test were repeated. Subsequently, other standard biochemical tests used for identification of Gram's negative bacteria were performed. These tests included Triple sugar iron (TSI), Urease, Citrate utilization, Methyl Red and Voges-proskauer tests (MR-VP), Indole production and Motility tests. Further, identification of bacteria was performed by using commercially available Analytical Profile Index (API) 20E (bioMeriex) rapid identification strips as follows.

Identification using API identification strips

Pure colonies (well isolated) on Tryptone Soy Agar (TSA) plates were selected and emulsified in 5 ml sterilized distilled water, in a sterilized tube and mixed well using a vortex machine (VELP Scientifica, Europe) to obtain a homogenous suspension. Using a

micropipette, 2 ml of this bacterial suspension was inoculated into twenty mini test tubes of the API 20E strips.

API 20E strips

Both the tube and the cupule were filled for the tests marked as CIT, VP and GEL. Only the tube was filled in the remaining tubes; anaerobiosis was created for the tests ADH, LDC, ODC, H_2S and URE by overlaying with sterilized mineral oil. To create a humid atmosphere, 5 ml of sterilized distilled water was distributed in the honey-combed wells on the tray. The incubation box was closed with the lid and incubated at 37^o C for 18-24 hours. After incubation, the strip was referred to the 'reading table' provided by the Biomerieux, Inc, USA and spontaneous reactions were recorded. The TDA, VP and IND tests were performed by addition of TDA, VP 1 + VP 2 and James reagents respectively. On the result sheet, the tests were separated into groups of 3 sets and values were summarized as instructed. By adding together, the values corresponding to positive reactions within each group, a 7-digit profile number was obtained for the 20 tests of the API 20 E strips. The numerical profile was entered into the identification software and submitted, and identification performed.

Chemical Analysis

Physiochemical parameters were analyzed following the standard guidelines and procedures [6]. The alkalinity, hardness and the chloride (Cl^-) contents were determined by titration methods using Hach digital titrator and Hach standard reagent cartridges. Calcium (Ca), iron (Fe), manganese (Mn) and zinc (Zn) were measured by the atomic absorption spectrometer (Varian 240FS Inc., Australia) and spectrophotometer (Hach DR-2400 with standard reagents) was used to determine nitrate (NO^{3-}), nitrite (NO^{2-}), phosphate (PO_4^{3-}), fluoride (F^-), ammonium (NH_4^+), sulphate (SO_4^{2-}) and sulfide (S^{2-}). All instruments were calibrated using commercially available standard solutions (BDH, Fulka) before performing the measurements.

3. RESULTS AND DISCUSSION

Microbiological analysis

Pollution of water is a major national and global issue, and billions of people do not have access to water that is safe to drink [1]. There is no pure water in nature as water is naturally polluted by-products of rock, deposition of leaf and animal wastes, and solution of minerals [7]. Coliforms have been identified as reliable indicator organisms in water quality testing. They are present in and throughout the environment. Coliforms are found in soil, water, and human or animal waste. Therefore, the numbers of TC should be higher than FC, which are generally present as a contaminant from fecal matter [8]. When animal and human fecal matter enter the water bodies, water can get contaminated with harmful pathogens such as bacteria, viruses and parasites. *Escherichia coli*, a member of the coliform group, had been found as an ideal indicator organism to detect faecal contamination of any water source. The presence of *E. coli* indicates that the water

is fecally contaminated and thus has the possibility of the presence of other pathogenic microorganisms such as, *Salmonella* spp., *Shigella* spp. and *Vibrio* spp., which may pose an immediate health risk to anyone consuming the water.

In the current study, total coliforms and fecal coliforms bacteria were detected in all water samples tested. M-Endo and M-FC media were used to detect total and fecal coliforms respectively. Both red colonies with a green metallic sheen, and red colour colonies with a sheen were enumerated separately for TC. Red colonies with a sheen were too numerous to count (TNTC) in all samples analysed, while the red colonies with a green metallic sheen ranged from 0-27. Typical blue clones indicating fecal coliforms ranged from 38-326 per 100 ml of water (Table 1). According to Sri Lankan Standards [9] and the Health Ministry regulation in Sri Lanka [10], the TC and FC counts should be zero per 100 ml for drinking water which indicated that the water samples tested are not suitable for drinking purposes in its present state.

Bacterial Identification

Results obtained for bacteriological identification, with conventional biochemical test and the API 20E rapid identification systems are shown in Table 2. As depicted in the Table 2 by using the two identification systems five bacterial species were identified including four total coliforms species and one fecal coliform species.

Table 1 Summary of total and fecal coliform counts in Riverston, the Knuckles Mountain range water samples

Sample no	Location (Mile posts/ culvert no; along the road)	Average counts of presumptive TC / 100 ml		Average counts of presumptive FC / 100 ml
		Red colonies with a green metallic sheen	Red colonies with sheen	Blue colonies
1	23/2	0	TNTC	38
2	23/5	1	TNTC	66
3	24/6	23	TNTC	229
4	26/2	14	TNTC	238
5	28	9	TNTC	128
6	28/2	5	TNTC	140
7	29/3	8	TNTC	247
8	29/4	9	TNTC	326
9	29/5	27	TNTC	127
10	29/6	0	TNTC	47

Escherichia vulneris, *Serratia marcescens*, *Serratia liquefaciens* and *Proteus mirabilis* were identified as total coliform species (Figure 1 – a, b, c and d), while *Escherichia coli* which was the dominant species, was identified as the fecal coliform species (Figure 1 – e). The presence of these pathogenic species in drinking water may cause diseases such

as, diarrhoea, bacterial infection and urinary infections. Similarly, research done in Sri Lanka by [11] indicated that ground water samples of Jaffna peninsula were contaminated with total coliform and *E. coli.* Further it revealed that 38% sampling locations were positive for *Salmonella* spp. and out of them six sampling sites were used for drinking purposes. Another study done in Jaffna peninsula reported

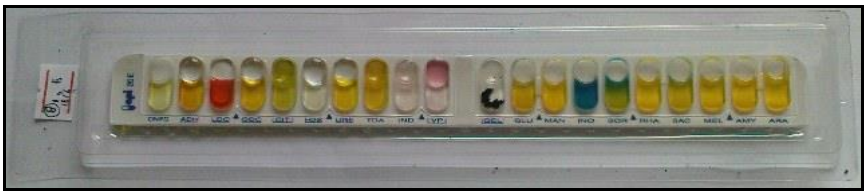
Table 2 Bacterial identification: Biochemical tests and API 20 identification system

No of isolates	Gram test	Oxidase test	Catalase test	TSI test	Citrate test	Urease test	Indole test	Motility test	MR test	VP test	Identification API 20E	Bacterial type Total coliforms (TC) Fecal coliforms (FC)
3	-	-	+	+	-	-	-	-	-	-	*Escherichia vulneris*	TC
6	-	-	+	+	-	-	-	-	-	+	*Serratia liquefaciens*	TC
4	-	-	+	+	+	+	-	-	-	+	*Serratia marcescens*	TC
3	-	-	+	+	-	-	-	-	+	+	*Proteus mirabilis*	TC
9	-	-	+	+	-	-	+	-	+	-	*Escherichia coli*	FC
Total 25												

that most (90%) public water sources were microbiologically unacceptable [12]. Further, a study revealed that surface water and groundwater of the Kelani River Basin were contaminated with total coliform and *E. coli* bacteria and also it is stated that all the sampling locations exceed the permitted values for drinking water given by the SLS guideline [13]. As most bottled water and mineral water brands in Sri Lanka indicate natural spring water as their water sources, the results of the current study are alarming. Though filtration and UV radiation are employed before bottling, many studies undertaken in Sri Lanka revealed that even bottled water samples exceeded permitted levels for microbiological parameters [14, 15, 16].

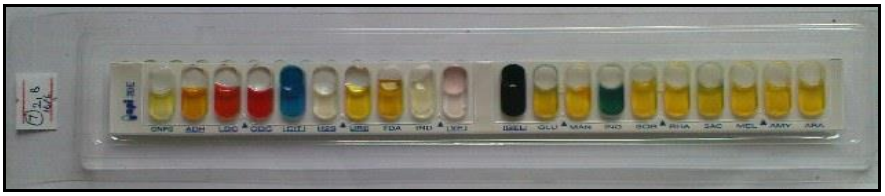
Chemical Analysis

When considering chemical parameters of drinking water, pH is one of the most important water quality parameters. According to WHO guidelines and Sri Lanka



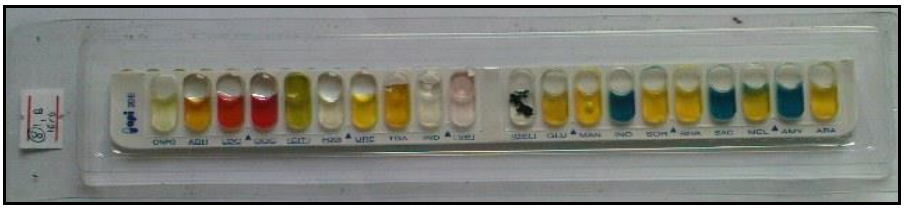
(a)Escherichia vulneris

(b) Serratia macescens



(c) Serratia liquefaciens

(d) Proteus mirabilis



(e) Escherichia coli

Figure 1 API profiles of Total coliforms (TC) and Fecal coliforms (FC) identified.
(a) *Escherichia vulneris* (TC) (b) *Serratia macescens* (TC) (c) *Serratia liquefaciens* (TC)
(d) *Proteus mirabilis* (TC) and (e) *Escherichia coli* (FC)

Standards (SLS), the optimum required pH in drinking water is in the range of 6.5 to 9.5 and 6.5–8.5 respectively [17, 18]. In the present study, the minimum and maximum values of pH in spring water was 6.5 and 7.75, respectively. The values were within the WHO and SLS permitted levels. According to SLS, the maximum permitted level for electric conductivity (EC) for drinking water is 750.0 $\mu S \cdot cm^{-1}$ [18]. All water samples tested were within the permitted level for EC, however the values were very low. Hardness and fluoride are also two important water quality parameters of drinking water, and both parameters were within the permitted levels in all samples tested. Considering the other water quality parameters investigated, alkalinity, anions (Cl^-, SO_4^{2-}, S^{-2}, PO_4^{3-},

NO_3^-, NO_2^-) and cations (Zn^{2+}, Ca^{2+}, Mn^{2+} and Fe^{2+}) were found within the Sri Lanka drinking water standards (Table 3).

Table 3 Summary of chemical parameters of spring water in Riverston, the Knuckles Mountain range water samples. Parameter concentrations are given in mg/l unless otherwise specified.

Parameter	Min.	Max.	Mean	SD	Permitted level
pH	6.5	7.75	6.93	0.37	6.5 to 8.5
EC (µS/cm)	12.3	22.1	16.43	3.62	750
Alkalinity	15.2	53.6	21.6	11.48	200.0
Hardness	1.6	7.2	3.28	1.54	10-20
Chloride	6.25	9.75	7.5	1.26	250.0
Fluoride	0	0.09	0.021	0.02	1.5
Sulfate	0	1	0.6	0.46	250.0
Sulfide (µg/L)	3	17	8.3	5.1	50
Phosphate	0.01	0.07	0.034	0.02	5
Nitrate-N	0.9	2.8	1.64	0.56	50.0
Nitrite-N	0.004	0.008	0.0056	0.001	3.0
Ammonium-N	0.03	0.11	0.078	0.03	0.5
Zn	0	0.01	0.002	0.004	3.0
Ca	0.03	0.35	0.191	0.08	150
Mn	0	0.02	0.004	0.01	0.05
Fe	0	0	0	0	0.2

CONCLUSION

While the chemical parameters of spring water were within permitted levels, the results of the current study indicate concerns over the microbiological quality of the water of the spring water in Riverston, in the Knuckles Mountain range, as they exceeded the permitted levels of coliform bacteria for drinking water according to the Health Ministry regulation in Sri Lanka, Sri Lanka standards and WHO guidelines. Hence, it is important to take necessary precautions, especially as spring waters from these areas are consumed by many and in addition is the main source water for the bottling industry in Sri Lanka.

REFERENCES

[1] WHO (2019). Drinking water – key facts, Report 16th June 2019, World Health Organization, Geneva.

[2] WHO (2016). Drinking water fact sheets, reviewed November 2016. https://reliefweb.int/report/world/drinking-water-fact-sheet-reviewed-november-2016.

[3] UNESCO (2006). Sri Lanka National Water Development Report. Case Study: Sri Lanka. Report. Imbulana, K. A. U. S., Sri Lanka, National Water Development Report (Ed. by Wijesekara, N. T. S. and Neupane, B. R.), MAI&MD, UN-WWAP, UNESCO and University of Moratuwa, Sri Lanka, Paris and New Delhi.

[4] Athukorala, S. W., Weerasinghe, L. S., Jayasooria, M., Rajapakshe, D., Fernando, L., Raffeeze, M. and Miguntanna, N.P. (2013). Ananlysis of Water Quality Variation in Kelani River, Sri Lanka Using Principal Componet Analysis. *In Proceedings of the SAITM Research Symposium on Engineering Advancements*, Malabe, Sri Lanka **27**, 129–135.

[5] Obiri-Danso, K. and Jones, K. (1999). The effect of a new sewage treatment plant on faecal indicator numbers, campylobacters and bathing water compliance in Moreca- me Bay. *Journal of Applied Microbiology* **86**, 603-14.

[6] Hach (2002). Water analysis handbook. 4th Ed. Hach Company, Colorado, USA.

[7] Fawell, J. and Nieuwenhuiisen, M. J. (2003). Contaminants in drinking water: Environmental pollution and health. *British Medical Bulletin* **68**, 199-208.

[8] Downes, F. P. and Ito, K. (2001). Compendium of methods for the microbiological examination of foods. Washington, DC: APHA.

[9] Sri Lanka Standards 894: Part 2: (2020). Specification for Bottled (Packaged) drinking water (3rd Revision), SLSI.

[10] The Gazette of the Democratic Socialist Republic of Sri Lanka, Extraordinary, Part 1: Section (1) –General. Government Notifications, Food Act, No.26 of 1980. No. 1420/4- Monday November 21, 2005, 1A-9A.

[11] Mahagamage, M.G.Y.L., Manage, P.S. and Manage, P.M. (2019). Water quality and microbial contamination status of groundwater in Jaffna peninsula, Sri Lanka. *Journal of Water and Land Development* **40**, 3–12.

[12] Arulnesan, C.A., Kamsan, S., Rajeshkannam, N., Sivapalan, K. and Murugananthan, K. (2015). Contaminated public well water as a source of sporadic outbreak of enteric infection in Northern Sri Lanka. *Sri Lankan Journal of Infectious Diseases* **5**(2), 64–72.

[13] Mahagamage, M.G.Y.L., Pathirage, M.V.S.C. and Manage, P.L. (2020). Contamination status of *Salmonella* spp., *Shigella* spp. And *Campylobacter* spp. In surface and ground water of the Kelani river basin, Sri Lanka. *Water* **12**(8), 2187.

[14] Herath, A.T., Abayasekara, C.L., Chandrajith, R. and Adikaram, N.K.B. (2012). Temporal variation of microbiological and chemical quality of noncarbonated bottled drinking water sold in Sri Lanka. *Journal of food Science* **77**, 160-164.

[15] Sasikaran, S., Sritharan, K., Balakumar, S. and Arasaratnam, V. (2012). Physical, chemical and microbial analysis of bottled drinking water. *Ceylon Medical Journal* **57**, 111-116.

[16] Herath, A.T., Abayasekara, C.L., Chandrajith, R. and Adikaram, N. K.B. (2014). *Pseudomonas aeruginosa* in bottled drinking water in Sri Lanka: a potential health hazard. *Water Supply* **14**, 1045-1050.

[17] WHO (2011). Guidelines for Drinking-Water Quality (4^{th} edn.) Microbiological Methods. World Health Organization, Geneva.

[18] Sri Lanka Standards 614: part 1: (1983). Specification for potable water – pt. 1: Physical and chemical requirement. Colombo. SLSI. 1–19.

GUIDELINES FOR AUTHORS/CONTRIBUTORS

Manuscripts for the Journal

The Journal of Science publishes the results of research in all aspects of science and technology, in the form of articles, Short communications and Correspondence. The Journal has a wide coverage of subject areas, which include Physical Science, Biological Science, Environmental Science, Computer Science and Applied Science.

Types of papers considered for publication

Articles are full- length papers presenting complete description of original research or technological achievements. Short communications are brief descriptions on new findings that are significant enough to publish without more complete description as a full paper. Correspondence about one or more article in a recent issue of the journal may also be published. All papers are subject to refereeing procedures.

Guide to preparation of manuscripts

The manuscript should be free of corrections. The language of publication is English. The style of writing should conform to acceptable English usage, Slang, jargon, unauthorized abbreviated phrasings and the like should be avoided. The length of the article is restricted to ten printed pages including figures and tables or its equivalent manuscript pages. To avoid unnecessary delays, authors should adhere strictly to the following instructions.

Manuscript Layout: Although a rigid format is not insisted, it is usually, convenient to divide the papers in to sections such as ABSTRACT, KEY WORDS, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION, CONCLUSIONS, ACKNOWLEDGEMENT AND REFERENCES.

Title: The title should be concise and specific. When the length of the title exceeds 45 characters, a *running title* should be indicated. The name of the institute where the research was carried out should be stated with author's name. The corresponding authors name should be underlined. If the present address is different from the given address then it should be given a foot note.

Abstract: A self-contained abstract of approximately up to 200 words should be provided, summarizing in a single paragraph the new information and the author's conclusions. For plant and animals use both common and scientific names in this section unless they are given in the title.

Materials and methods: The materials and methods should be described in sufficient detail for the protocol to be replicated. Repetition of methodological details can sometimes be avoided by referring to previous studies. If any special equipment (with details of model type/number), chemicals and reagents used then it should be included in the reference, after mentioning for the first time. Animal information should include:

bred, breeder, sex, age, weight and the maintenance of conditions. For drugs, generic names should be used: trade names may be given in brackets where the drug is first mentioned. In the case of new drugs or chemicals, a full chemical description (formula) should be given. This section may also contain description of the statistics used and the activities that were recorded. *Ethical note*: where ethical considerations arise from the study, these should be addressed either in the main methods section itself (where the additional discussion is relatively minor) or in a separate subsection of the methods headed ethical note. Any ethical implication of the experimental design and procedures should be identified, and any license acquired to carry out the work specified. Procedures that were taken to minimize the welfare impact on subjects, including choice of sample sizes, use of pilot tests and predetermined rules for invention, should be described.

Key words: A list of 3 to 5 key words chosen within the paper should be provided.

Figures and Tables: Figures and Tables should be numbered consecutively and given suitable caption with reference in the text.

If you send the figures and tables in separate sheets, please label each figure and table with their numbers and the name of the author.

For the final submission it is essential to produce the figures in either in .jpg or .bmp format. This will help us to type set the final printing.

Foot notes to tables should be typed below the table and should be referred to by superscript symbols #, ₸ and ¤. No vertical rules should be used. Tables should not duplicate results presented elsewhere in the manuscript, (e.g., in graph)

Photographs: Photographs must be of good quality, printed on glossy paper. Photocopies and the photocopies of the photomicrographs are not acceptable. Colour photographs are accepted only when it is very essential to describe the text.

References: Special care must be taken in citing references correctly. The accuracy of the citation is entirely with the author(s). It is the author's responsibility to obtain written permission to produce material, which has appeared in another publication. Reference in the text should be numbered and listed under reference section stating the name of the author(s), year of publication (in brackets), title of the paper journal (in italic fonts), volume and issue and the page range (e.g., 80-85).

Example:

Book:

Gregory, G., and Parry, T. (2006). Designing brain-compatible learning (3rd Ed.). Thousand Oaks, CA: Corwin.

Chapter of a Book:

Bergquist, J. M. (1992). German Americans. In J. D. Buenker& L. A. Ratner (Eds.), Multiculturalism in the United States: A comparative guide to acculturation and ethnicity (pp. 53-76). New York, NY: Greenwood.

Journal Article with DOI:

Paivio, A. (1975). Perceptual comparisons through the mind's eye. Memory & Cognition, **3**:635-647. doi:10.1037/0278-6133.24.2.225

Journal Article without DOI

Becker, L. J., and Seligman, C. (1981). Welcome to the energy crisis. Journal of Social Issues, **37(2)**: 1-7

Online Report:

Kenney, G. M., Cook, A., and Pelletier, J. (2009). Prospects for reducing uninsured rates among children: How much can premium assistance programs help? Retrieved from Urban Institute website: http://www.urban.org/ url.cfm? ID=411823

Units: The SI system of units should be used throughout the article.

Refereeing

Every paper will be referred to at least two referees familiar with the field of research covered by the paper, who will be nominated by the editorial board.

Proofs

The corresponding author will receive galley proofs by mail for correction, which must be returned to the editor within one week of receipt. Please note that alterations in the text cannot be permitted during the proof reading.

Copyright

Once the paper is accepted a copyright-transfer form will be sent along with the proofs. Duly filled and signed form should be sent to the editor along with the proofs as mentioned above.

Submission of Manuscript

The manuscript should be type written using Microsoft word with 1.15 spacing on one side of A4, paper with 3 cm margin in all sides and fond size of 12 pt Times New Roman. Manuscripts in LaTex with 12pt will also be accepted. The length of the article is restricted to ten printed pages including figures and tables or its equivalent manuscript pages. **Manuscript will be accepted only in electronic format**.

Address of the corresponding author

It should be given in a separate page as an attachment. It is important to keep the editor informed any change in your address.

Address for submission: jsc@esn.ac.lk

The Editor-in-Chief
Journal of Science
Faculty of Science
Eastern University, Sri Lanka.
Chenkalady,

Telephone: +94 65 2240755,
Fax: +94 65 2240758