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EVALUATION OF BIOACTIVITIES OF TWO POLYHERBAL FORMULATIONS FOUND IN SRI LANKAN AYURVEDIC TREATMENTS

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ABSTRACT: Commercially available two polyherbal formulations (Chandra Kalka and Sharkaradi Kalka) were collected from local market. The objective of this study was to determine the Total Phenolic Content (TPC) , Total Flavonoid Content (TFC) of these two 'Kalkas' and to investigate the existence of antioxidant, anti-inflammatory, antibacterial and anti-tyrosinase activities. Total phenolic content and total flavonoid content was determined by Folin Ciocalteu and Aluminium chloride method respectively. Antioxidant activities was evaluated using the DPPH radical scavenging assay and human red blood cell membrane stabilization assay was used to determine the anti-inflammatory activity. Agar well diffusion method and tyrosinase enzyme inhibitory assay was facilitated to investigate the antibacterial and tyrosinase enzyme inhibition properties of the selected polyherbal formulations, accordingly. The results obtained from the study showed that Chandra Kalka has the highest TPC (53.90 ± 948 mg GAE /g extract) and has the highest TFC (119.25 ± 10.11 mg QE/g extract). Sharkaradi Kalka showed higher antioxidant activity for the DPPH radical scavenging, anti-inflammatory activity, and anti-tyrosinase activity for the assays conducted, compared with the Chandra Kalka. Antibacterial assay was conducted against four pathogenic bacteria; *Escherichia coli* [ATCC 25922], *Staphylococcus aureus* [ATCC 25923], *Bacillus subtilis* [MTCC 121] and *Staphylococcus epidermidis* [ATCC 12228] using agar well diffusion method. No antibacterial activity was observed for either polyherbal formulations at 1 mg/mL dose used. Both Chandra Kalka and Sharkaradi Kalka demonstrate bioactivities to an moderate extend for the assays conducted.

INTRODUCTION: Ayurveda or the local indigenous medical system, nourished by the traditional Indian therapeutic concepts has long been valued by the global community as well as the Sri Lankan counterpart. The Ayurveda medicinal system is excessively built upon the usage of herbal plants to make various concoctions, potions and pastes that are used in the treatments of ailments.

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Every part of the plants from leaves to roots is used in variety of ways to make herbal remedies in order to use in the treatment of illnesses [1]. Polyherbal formulations can be explained as a collection of plant materials. Though these plants have been excessively studied individually and evaluated for their medicinal properties and have even isolate the compounds responsible, it could also suggest that as a collection, these phyto-chemicals could behave synergistically to increase the existing potential.

Hence we could propose that the certain biological activities of a polyherbal formulation could be different than its independent counterparts. Two commonly used polyherbal formulations, commonly known as 'Kalka', were studied for their biological

activities. The Sharkaradi Kalka (SK) is used in treatment for fever in children as well as, cough, asthma conditions and constipation. The Chandra Kalka (CK) is also used in treatment for ailments such as respiratory disorders of paraplegia disease and congestion in respiratory system. A total of thirty one items are used to prepare the CK and eighteen ingredients are used in preparation of the SK (**Table 1**).

TABLE 1: INGREDIENTS OF CK AND SK [1]

Chandra Kalka	Sharkaradi Kalka
Santalum album	*Piper nigrum,*
Glycyrrhiza glabra	*Glycyrrhiza glabra,*
Piper longum	*Piper longum*
Myristica fragrance	*Embelia ribes*
syzygium aromaticum	*Sapindus trifoliate*
Nigella sativa	*Piper cubeba*
Cuminum cyminum	*Saussurea lappa*
Thachyspermum ammi	*Aconitum heterophyllum*
Cedrus deodara	*Clerodendrum serratum*
Aconitum heterophyllum	*Allium sativum*
Zingiber officinale	*Rhus succedanea*
Coriandrum sativum	*Anacydus pyrethrum*
Holarrhena antidysentrica	*Picrorhia kurroa*
Embelia ribes	
Saussurea lappa	
Foeniculum vulgare	
Picrorhia kurroa	
Terminalia chebula	
Terminalia belerica	
Embbilica officinalis	
Solanum trilobactum	
Alpinia calcarata	
Kaempferia galangal	
Elataria cardamomum	
Vitex nigando	
Boerhavia diffussa	
Cyperus rotundus.	

Various parts of the each plant such as tender leaves, bark or roots are taken and grinded separately and equal portions of each ingredient is then taken, mixed and heated with sesame oil. This is then again mixed with bee's honey to obtain the paste like nature of the Kalka. Apart from the mentioned fifteen plant materials, Sahinda lunu (Sahinda salt), Yawakshara lunu (Yawakshara salt), Suwasa lunu (Suwasa salt), sugar and Bee's honey is used in preparation of the SK, whereas, seasame oil and bee's honey is used in preparation of CK. It should also note that several parts of the *Myristica fragrance* and *Saussurea lappa* plants, has used in preparation of the CK and counted as two ingredients. Some herbal materials such as, *Glycyrrhiza glabra, Piper longum, Embelia ribes, Saussurea lappa, Aconitum heterophyllum* and *Picrorhia kurroa,* are common in both Kalkas. The *Santalum album*is a well-known plant that is used for fragnance oil production. This plant commonly known as the sandal tree proven to have, hepatoprotective activity, sedative effects and relaxing effect on nerves and *in-vivo* anti-tyrosinase activities. This also have antifungal, antibacterial and anticancer activities too [1].

The literature gives evidence to that common herbs used *Glycyrrhiza glabra, Piper longum* and *Embelia ribes* also have antioxidant, anti-inflammatory, antibacterial, hepatoprotecive, anti-asthmatic and immunomodulatory among other physiological activities [2, 3, 4]. Several other herbs also have been studied individually. In these studies it had concluded that *Nigella sativa* has immuno-modulatory, broncodilatory and gastro-protective activities and *Coriandrum Sativum* also possess numerous health benefits too [5, 6]. *Cuminum cyminum, Zingiber officinale*and *Holarrhena antidysentrica* have demonstrated anti-inflammatory, broncho-dilatory and immunomodulatory [7, 8, 9].

Though there are published investigation that were performed to evaluate the effect of Kalkas[10], however no evidence was found in regards of chemical investigations conducted to justify the biological activities of polyherbal products CK and the SK. Only chemical investigation that were completed was conducted using Nawarathne Kalka [11]. In general, the Kalkas in treatment are prepared before administration it to the patient, but now a days, pre-prepared products are available in the market to purchase. In this study two such commercially available Kalka products were evaluated for their bioactivities.

MATERIALS AND METHODS:

Collections of Samples: Commercially available Chandra Kalka (CK) and Sharkaradi Kalka (SK) were purchased from a local Ayurvedic drug shop for the analysis.

Samples Preparation: A 40 g portion of each Kalka was soaked in 160 mL of EtOAc and kept on an orbital shaker for two days in order to extract the phytochemicals present. The solvent was evaporated under reduce pressure using a rotary evaporator (RV 8V IKA – werke GmbH -Germany) at 40 ^{o}C and the semisolids obtained were carefully

transferred into small vials. The remaining solvent in each vial was evaporated by passing nitrogen gas and extracts were stored at 4 ^{o}C until further use.

Determination of Total Phenolic Content (TPC): The Total Phenolic Content (TPC) of the extracts of CK and SK were determined by Folin Ciocalteu method [12]. The test Kalka sample (0.5 mL) was mixed with 0.5 mL of Folin Ciocalteu reagent (1: 1). The sample was allowed to stand for 5 minutes at room temperature and then 0.5 mL of Na_2CO_3 (6% w/v) was added. After adding 2 mL of distilled water, the mixture this was kept in dark for 60minutes at room temperature. The absorbance of the resulting solution was observed at 765 nm against a blank sample that was prepared in the same way replacing the Kalka extract with distilled water. A series of gallic acid concentrations were used to construct the standard curve (R^2=0.97) and was used to measure the phenolic content. The total phenolic content was express as mg GAE/g of weight of the extract.

Determination of Total Flavonoid Content (TFC): The aluminum chloride colorimetry assay was used to evaluate the Total Flavonoid Content (TFC) of the Kalka extracts [12]. Calibration curve was constructed using Quercetine (QE) standards. The total flavonoid content of the evaluated sample were expressed as mg QE/g of weight of extracts.

Evaluation of Bioactivity of Selected Polyherbal Formulation Using Selected Bioassay:

DPPH Free Radical Scavenging Assay: The DPPH radical scavenging activities of CK and SK were determined by following the method reported by Kuganesan *et al*.,[12] The capacity to scavenge DPPH radical was calculated by using following equation;

$$\text{Scavenging activity}(\%) = \left[1 - \left(\frac{As}{Ao}\right)\right] \times 100 \quad (1)$$

Where A_0 is the absorbance of the control and As is the absorbance in presence of extracts or positive standard. The results were plotted as the percentage of scavenging activity against does of the sample.

Anti-inflammatory Assay: Human Red Blood Cell (HRBC) membrane stabilization assay was used to determine the anti-inflammatory activities of the CK and SK [12]. Blood sample used in the assay was obtained from National Blood Transfusion Centre and was stored at 4 ^{o}C in a refrigerator.

A portion of the human blood sample was transferred in to a centrifuged tube and centrifuged at 3000 rpm for 5 minutes, with normal saline. Then the supernatant was carefully removed using a pipette without disturbing the red blood cells packed at the bottom of the centrifugal tube. This process was repeated until the supernatant from the centrifugal process was clear. The 10 % (v/v) suspension was then prepared using a Phosphate Buffered Saline (PBS) solution.

Kalka extract to be tested was weighed and dissolved in 200 µL of dimethyl sulfoxide (DMSO). This was then diluted with PBS to obtain a solution with 1 mg/mL dose and, was further diluted with PBS to obtain a solution series. To a 1.00 mL portion taken from each solution, 100 µL of 10 % (v/v) human red blood cell suspension was added and each sample was incubated at 56 ^{o}C for 30 minutes and was cooled down under a stream of running water for five minutes. Then the test samples were centrifuged at 3000 rpm for five minutes and the absorbance of the supernatant was recorded at 540 nm. Commercially available drug Aspirin was used to construct the positive standard using the same procedure.

The percentage inhibition of hemolysis was calculated using the equation given below.

$$\text{Inhibition of hemolysis}(\%) = \left[1 - \left(\frac{As}{Ao}\right)\right] \times 100 \quad (2)$$

Where, As is the absorbance at 540 nm of the sample and Ao is the absorbance of standard without the presence of the Kalka extract. The percentage stability versus test concentrations was plotted in order to compare the anti-inflammatory activity of extract with the positive standard separately.

Determination of Anti-bacterial Properties Using Agar Well Diffusion Method: Anti-bacterial assay was tested against four pathogenic bacteria; *Escherichia coli* [ATCC 25922], *Staphylococcus aureus* [ATCC 25923], *Bacillus subtilis* [MTCC 121] and *Staphylococcus epidermidis* [ATCC 12228] using Agar well diffusion method for extracts of CK and SK [15, 16]. Test samples were dissolved in 500 µL DMSO to obtain a solution

with a concentration of 1 mg/mL and DMSO alone was used as the negative control. The commercially available anti-bacterial drug azithromycin was purchased from a pharmacy and a solution with a concentration of 0.5 mg/mL in DMSO was prepared and used as the positive standard. The azithromycin was used as the positive standard for all anti-bacterial assays conducted.

Each of the well was filled with 50 µL of the samples to test. After incubation of overnight (approximately 17 - 18 hours) at room temperature, all plates were observed for growth of inhibition and the diameter of these inhibition zones were measured in millimeters.

Determination of Anti-tyrosinase Properties Using Tyrosinase Assay: The method reported by Vardhan *et al*., in 2014 was followed to determine the anti-tyrosinase activity with slight modifications [13]. The Kalka extract to be tested was dissolved in Phosphate buffer (pH 6.8). Tyrosinase from mushroom (25 KU, Sigma, USA) was used as enzyme and 3,4-dihydroxy-L-phenylalanine (L-DOPA) was used as the substrate. A does series of the Kalka was prepared (250 µg/mL to 2000 µg/mL) and, to a 40 µL portion taken from this each dose, 50 µL of mushroom tyrosinase (220 Units/mL) and 50 µL of L-DOPA was mixed with extract. All solutions were incubated at room temperature for 15 minutes and the amount of Dopachrome formation was measured at 415 nm using a micro plate reader (Thermo Scientific Multiskan FC).

The percent inhibition of tyrosinase activity was calculated using the equation (1).

Statistical Analysis: All analyses were performed in triplicate and data were reported as mean ± SD. This statistical analysis were carried out using Microsoft Excel. Minitab 15 was used to calculate IC_{50} values.

RESULTS AND DISCUSSION:

Total Phenolic Content (TPC) And Total Flavonoid Content (TFC) of Selected Polyherbal Formulation: Phenolic and flavonoids are two major secondary metabolites produced by the plants and have proven to show potent biological activities. These physiological activities of the phenolic compounds include antioxidant, anti-bacterial, anti-inflammatory and anticancer activities [15]. Not only phenolic compounds are known to give visual and fragrance characteristics of fruits and vegetables, they are also contribute in growth of the plants as well as providing protections against pathogens and predators. The high phenolic content and flavonoid content of the SK and CK can be attribute to the fact that large number of herbs that are used in preparation of these products.

Table 2 flavonoids found in nature have a great structural diversity and includes flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols and chalcones. Over 4,000 different flavonoids have been identified and many processes biological properties including antibacterial, antiviral, anti-inflammatory and anti-allergic activities [16].

TABLE 2: THE TOTAL PHENOLIC AND FLAVONOID CONTENTS OBTAINED FOR EtOAc EXTRACT OF TWO SELECTED KALKA

	TPC mg GAE/g of extract	TFC mg QE/g of extract
CK	53.90 ± 9.48 [a]	119.25 ± 10.11[a]
SK	32.55 ± 4.31[b]	54.42 ± 17.90 [b]

All data are presented as mean ± SD of the three replicates. GAE - gallic acid equivalent, QE - Quercetin equivalent. Mean followed by different letter in the same column differs significantly ($p \leq 0.05$)

Though it's expected to have a comparably high flavonoid content much similar to the phenolic content this could be due to the solvent used to extract the phytochemicals. Since many flavonoids exist in sugar form, their solvent solubility could depend on the structures of the compounds [17].

In this study a cold extraction was used using a mildly polar solvent rather than using a hot extraction using polar solvents (water, methanol *etc*). The expectation was to extract not only the most polar components, but molecules that do not dissolve in polar solvents too. The intention of avoiding a hot extraction process was on the basis that, the thermally unstable phytochemical constituents present in the Kalkas could either lost their functionality or endure structural changes under prolong heat treatment [18, 19]. Thus, phytochemicals extracted using a hot extraction may not entirely represent an accurate bioactivity of the original composition.

Evaluation of Bioactivity of Selected Polyherbal Formulation Using Selected Bioassay:

Determination of Antioxidant Activity Using DPPH Radical Scavenging Activity: Atoms, molecules or ions with unpaired electrons are known as free radicals and are highly reactive towards chemical reactions with other molecules. Free radicals could derived from oxygen, nitrogen and sulfur, create reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulfur species (RSS) respectively [20]. Though, free radicals are generated as a normal part of metabolism within the mitochondria, certain external factors such as smoking, radiation, drugs, pesticides, industrial solvents and ozone could accelerate this processes and excessive formation of these reactive chemical species could lead to oxidative stress. Antioxidants are compounds that have the capability to delays, prevents or removes oxidative damage to a target molecule. Antioxidants tend to react with free radicals and minimize the damage they could cause in the presence of oxidative stress. Flavonoids and phenolic compounds could be classified as natural non-enzymatic antioxidants. DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical scavenging assay was used to determine hydrogen donating capability of the Kalka extracts. The IC_{50} values obtained from the DPPH assay were 133.19 ± 18.46 µL/mL, 388.10 ± 5.48 µL/mL and 10.97 ± 0.22 µL/mL for CK, SK and L-Ascorbic acid respectively. **Fig. 1** shows the DPPH radical scavenging activity of CK and SK. This results suggested that the radical scavenging ability of SK was higher compared to the extract of CK. But has a lesser activity compared with the positive standard, L-Ascorbic acid.

Determination of Anti-inflammatory Activity of Human Red Blood Cell (HRBC) Membrane Stabilization Assay: Inflammation could be described as a defense response that is activated upon harmful stimuli to tissues. Uncontrolled inflammatory could also occur as a result of allergies, cardiovascular dysfunctions, metabolic syndrome, cancer and autoimmune diseases. During inflammation, lysosomal hydrolytic enzymes are released into cites which causes damages of the surrounding organelles and tissues with attendance variety of disorders. Several methods are employed to screen and study drugs or herbal preparations that exhibit anti-inflammatory properties and erythrocyte membrane stabilization method here is used due to its convenience and reproducibility.

As most of the human cellular organelles have a similar membrane structure, in this assay the erythrocyte membrane has taken as an analogous to the lysosomal membrane. Thus ability to stabilize the erythrocyte membranes implies that the extract may as well stabilize lysosomal membranes too. Stabilization of lysosomal membrane is significant in harnessing the inflammatory actions because, deterrence the release of lysosomal constituents which cause further tissue inflammation and damage upon extra cellular release [14]. Aspirin, anti-inflammatory drug was used as the positive standard to compare the anti-inflammatory properties of CK and SK extracts. **Fig. 2** shows the anti-inflammatory activity of selected Kalkas compared with positive standard aspirin. CK and SK showed moderate anti-inflammatory activity and both extracts' activities were similar to each other.

Determination of Anti-bacterial Properties Using Agar Well Diffusion method: The selected Kalka, once prepared could be stored for several months without detecting of any bacterial contaminations. This gives an indication of some antibacterial activity associating with the Kalka used for evaluations. Ingredients such as Bee's honey have the possibility to reduce the bacteria activity as well as some plant material used in making of the Kalkas [21]. Literature gives the evidence to that many plants demonstrate antibacterial activity for pathogenic bacteria species. However, no antibacterial activity was observed for either of Kalka used at the 1 mg/mL does.

Determination of Anti-tyrosinase Activity Using Tyrosinase Enzyme: Tyrosinase enzyme react with substrate L-DOPA and subsequently oxidized to dopaquinone. Tyrosinase catalyzes the conversion of L-tyrosine to L-DOPA and then to melanin. Melanin formation can stop by inhibit the formation of dopachrome. The results obtained in the present study indicate that two selected extracts have moderate anti-tyrosinase potency. However, SK has the higher anti tyrosinase activity than CK extract **Fig. 3**.

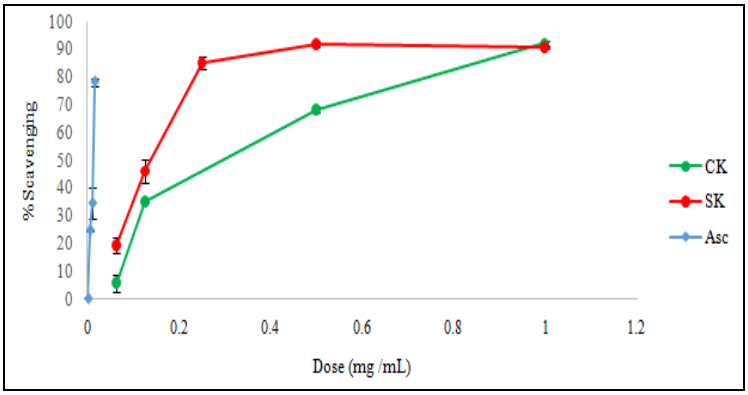

FIG. 1: THE DPPH FREE RADICAL SCAVENGING ACTIVITY OF SK AND CK COMPARED WITH L-ASCORBIC ACID

Each data point presents the mean of duplicates. asc-L-Ascorbic acid

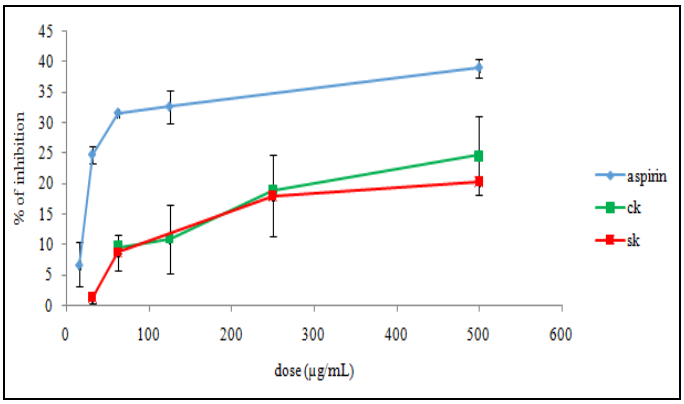

FIG. 2: HRBC ACTIVITY OF CK, SK AND POSITIVE REFERENCE STANDARD ASPIRIN

Each data point presents the mean of 3 replicates

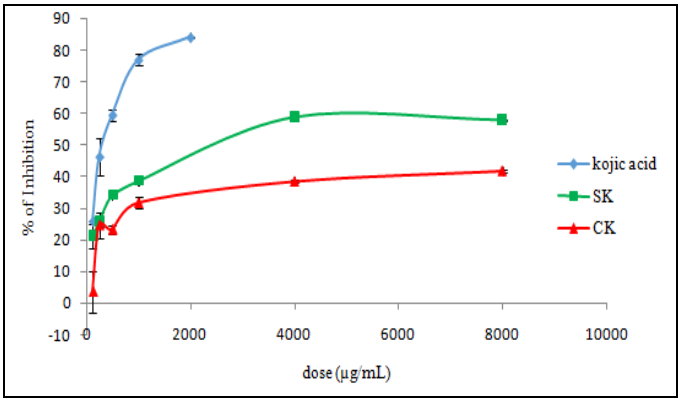

FIG. 3: COMPARISON OF INHIBITION ACTIVITY OF POLYHERBAL FORMULATION AND POSITIVE STANDARD KOJIC ACID

Each data point presents the mean of 3 replicate

CONCLUSION: From the conducted assays and statistical calculation it was concluded that, EtOAc extract of the Sharkaradi Kalka and Chandra Kalka used in Ayurvedic treatments show moderately low anti-inflammatory activities compared with the positive standard used. Both polyherbal products analyzed have moderate antityrosinase activities and have a considerable content of phenolic content and flavonoid content. The flavonoid and phenolic content could be attribute to the antioxidant and anti-inflammatory activity of the Kalkas. No antibacterial activity was observed at the dosage used. It can be justify that the moderate activities of the Kalkas exhibit are in the beneficial range as these are mostly used in the treatment of young or infant children.

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CONFLICT OF INTEREST: Nil

REFERENCES:

1. Kumar R, Anjum N and Tripathi YC: Phytochemistry and pharmacology of *Santalum Album* Linn.: A review. World Journal of Pharmaceutical Research. 2015; 4(10): 1842-1876.
2. Asl MN and Hosseinzadeh H: Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. Phytheraphy Research.2008; 22: 709-724.
3. Preetham J, Sarath R, Prasanna K, Sujan G and Kiran S: Evaluation of *in-vitro* antioxidant activity of piper-longumine isolated from *Piper longum* Linn. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2014; 5(5): 174-178.
4. Harish GU, Danapur V, Jain R, Patell V.M: Endangered medicinal plant *Embelia ribes* Burm. F. - A review. Pharmacognosy Journal. 2012; 4 (27): 6-19.
5. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, Damanhouri ZA and Anwar F: A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asia Pacific Journal of Tropical Biomedicine. 2013; 3(5): 337-352.

6. Momin AH, Sawapnil SA and Gajjar AV: *Coriandrum sativum* - review of advances in phytopharmacology. International Journal of Pharmaceutical Sciences and Research. 2012; 3(5): 1233-1239.
7. AlSnafi AE: The pharmacological activities of *Cuminum cyminum* - a review. IOSR Journal of Pharmacy. 2017; 6(2): 45-65.
8. Ali BH, Blunden G, Tanira MO and Nemmar A: Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber Officinale* Roscoe). A review of recent research. Food and Chemical Toxicology. 2008; 46: 409-420.
9. Sinha S, Sharma A, Reddy PH, Rathi B, Prasad NVSRK and Vashishtha A: Evaluation of phytochemical and pharmacological aspects of *Holarrhena antidysenterica* (Wall.): A comprehensive review. Journal of Pharmacy Research. 2013; 6(4): 488-492.
10. Amarasinghe APG: Scientific Studies of a popular Sri Lankan indigenous therapeutic agent "Rathakalka" used in paediatric practice. Sri Lanka Journal of Indigenous Medicine. 2011; 1(1): 44-48.
11. Fernando CD, Karunaratne DT, Gunasinghe SD, Cooray MCD, Kanchana P, Udawatte C and Perera PK: Inhibitory action on the production of Advanced Glycation End Products (AGEs) and suppression of free radicals *in-vitro* by a Sri Lankan polyherbal formulation Nawarathne Kalka. BMC Complementary and Alternative Medicine. 2016; 16(1): 197.
12. Kuganesan A, Thiripuranathar G, Navaratne AN and Paranagama PA: Antioxidant and anti-inflammatory activities of peels, pulps and seed kernels of three common mango (*Mangifera indika* Linn.) varieties in Sri Lanka. International Journal of Pharmaceutical Sciences and Research. 2017; 8(1): 70-78.
13. Vardhan A, Khan S and Pandey B: Screening of plant parts for anti-tyrosinase activity by tyrosinase assay using mushroom tyrosinase. Indian Journal of Scientific Research. 2014; 4(1): 134-139.
14. Venkatalakshmi P, Brindta P and Vadivel V: *In-vitro* antioxidant and anti-inflammatory studies on bark, wood and fruits of *Terminalia catappa* Linn. International Journal of phyto medicine. 2015; 7(3): 246-25.
15. Ronald LP, Xianli W and Karen S: Standardized method for determination of antioxidant capacity and phenolics in food and dietary supplements. Journal of Agricultural and Food Chemistry. 2015; 53(3): 4290-4302.
16. Cook NC and Samman S: Flavonoids-Chemistry, metabolism, cardioprotective effects, and dietary sources. The Journal of Nutritional Biochemistry. 1996; 7(2): 66-76.
17. Chebil L, Humeau C, Anthony J, Dehez F, Engasser JM and Ghoul M: Solubility of flavonoids in organic solvents. Journal of Chemical and Engineering Data. 2007; 52(5): 1552-1556.
18. Sharma K, Young E, Assefa AD and Ha S: Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities and sugar content in six onion varieties. Journal of Food and Drug Analysis. 2015; 23 (2): 243-252.
19. Aoyama S and Yamamoto Y: Antioxidant activity and flavonoid content of welsh onion (*Allium fistulosum*) and the efect of thermal treatment. Food Science and Technology Research. 2007; 13 (1): 67-72.
20. Singh D, Mishra M, Gupta M, Singh P, Gupta A and Nema R: Nitric oxide radical scavenging assay of bioactive compounds present in methanol extract of *Centella asiatica*. International Journal of Pharmacy and Pharmaceutical Science Research. 2012; 2(3): 42-44.
21. Tan HT, Rahman RA, Gan SH, Halim AS, Hassan SA, Sulaiman SA and Kirnpal-Kaur B: The anti-bacterial properties of malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. BMC Complementary and Alternative Medicine. 2009; 9(1): 34.

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