DOI: 10.56511/JIPBS.2021.v8.i01.pp08-13

Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

In vitro comparison of pharmacological properties of *Syzygium jambos*, *Syzygium malaccense* and *Syzygium samarangense* species in Sri Lanka, extracted with different solvents

Chamali Madhushika, Dinusha N. Udukala*

College of Chemical Sciences, Institute of Chemistry Ceylon, Rajagiriya, Sri Lanka.

Received on: 20/11/2020, Revised on: 07/12/2020, Accepted on: 12/12/2020, Published on: 01/01/2021.

***Corresponding Author :** Dinusha N. Udukala, College of Chemical Sciences, Institute of Chemistry Ceylon, Sri Lanka.
Email id: dinusha@ichemc.edu.lk

Copyright © 2021 Dinusha N. Udukala *et al.* This is an open access article distributed under the terms of the Creative Commons Attribution Non Commercial-Share Alike 4.0 International License which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

Keywords: Antioxidant, Antibacterial, Anti-diabetic, Solvents, Alpha amylase, *Syzygium.*

Vol. 8 (1): 08-13, Jan-Mar, 2021.

Abstract

Syzygium jambos (SJ), *Syzygium malaccense* (SM) and *Syzygium samarangense* (SS) are dicotyledonous species with a high distribution density in the wet zone of Sri Lanka. The leaves, barks and seeds are utilized in traditional medicine to treat different diseases. Despite their prevalence, few studies have been conducted in Sri Lanka to screen the bioactive ingredients.
Therefore a comparative study was performed for the first time to investigate the antioxidant, anti-diabetic and antibacterial activities of leaves, fruits and seeds of the three species. Extraction was carried out with hexane, ethyl acetate, methanol and water at room temperature. DPPH free radical scavenging assay, alpha amylase inhibition assay and agar well diffusion method were used for analysis.
Highest antioxidant activity was observed in SS methanol seed extract with a maximum percentage inhibition value of 92.07±0.77% at 34.0 (µg/mL) concentration. A noticeable alpha amylase inhibitory activity was seen in SS methanol leaf extract with a maximum percentage inhibition value of 83.10±1.22% at 2.30 (mg/mL) concentration. Hexane extract of SJ leaves depicted the highest inhibition zone, against *Escherichia coli* which was 21.5±2.5 mm whereas hexane seed extract of SS showed the highest inhibition zones against the bacterial strains *Bacillus subtilis* and *Staphylococcus aureus* which was 20±1 mm in diameter.
Significant antibacterial activity of SS and SJ seeds were noted against the selected strains for the first time through this study. Due to the appreciable antioxidant, antibacterial and alpha amylase inhibitory activity, these species could be a principal source in nutraceutical and pharmaceutical products.

Introduction

Human beings have been utilizing plants for preventive and curative health care since ancient times [1]. The secondary metabolites in plants have been reported to exert biological effects. As a result, many tropical plants are screened as sources of bioactive molecules that could be incorporated into novel drug discovery targets [2]. According to world health organization up to 80% of world's population rely on traditional medicinal systems for some aspect of primary health care [3]. Therefore, there is a rising demand for plant based novel, inexpensive and effective biomolecules [4].
Sri Lanka being a country with a high degree of biodiversity provides a habitat for a vast number of species [5]. It is recorded that 1414 plant species belonging to different families are being used in traditional medicine within the country [6]. Myrtaceae is one such family of evergreen trees and shrubs, which comprises of *Syzygium* species [7]. Three *Syzygium* species selected for this study; SJ, SM and SS have been intimately related with humans due to their seasonal edible fruits and also due to utilization in healthcare

practices. Due to characteristic medicinal properties, these plants have been used for therapeutic purposes not only in Sri Lanka but also in other Asian countries such as India, Bangladesh and Nepal [7-10].

In consideration of previous research based on SJ, SM and SS in different parts of the world; antihyperglycemic activity of the leaf and bark extracts of SM and leaf extracts of SS has been established [8-9]. Moreover the antioxidant activity of the fruit extracts of the three species [11-13] and the leaf extract of SJ and SM has been reported [10, 14]. When focusing on Sri Lanka, very limited research has been conducted based on these three species. In spite of the higher consumer acceptability as fruits and known medicinal value, the pharmacological properties of Sri Lankan species remain underestimated.

Although there is a global appeal for herbal medicine extensive research is needed to validate the safest and effective plant based biomolecules from the repository of medicinal plants. Experimental evidence reflects significant variations in pharmacological activities based on solvent and on used parts of the plant. Therefore, the bioactivities of leaves, seeds and fruits were evaluated comparatively during this study utilizing four different solvents hexane, ethyl acetate, methanol and water for extraction at room temperature. The most appropriate solvents to establish the respective pharmacological properties and the corresponding plant parts were identified.

Experimental procedure

Collection of plant materials

Mature leaves of SJ, SM and SS and ripen fruits of SM and SS were collected from Colombo, Western Province of Sri Lanka. The leaves of the three species were authenticated by National Herbarium at Royal Botanical Garden, Peradeniya.

Materials

Hexane, ethyl acetate, methanol, Folin-Ciocalteu reagent, sodium carbonate, Gallic acid, 1,1-diphenyl-2-picrylhydrazyl(DPPH), Butylated hydroxytoluene (BHT), sodium hydrogen phosphate, potassium dihydrogen phosphate, sodium hydroxide, alpha amylase, corn starch, 3,5-dinitro salicylic acid (DNSA), Dimethyl sulfoxide (DMSO) and sodium potassium tartrate were used.

Preparation of extracts

Freshly harvested, mature leaves were washed with running water and finally with deionized water. Petiole was removed from the leaves. Selected leaves were air dried at room temperature for three days [9]. Dried leaves were ground using a laboratory grinder to obtain a powder. The leaf powder was soaked in hexane for 24 hours at room temperature on a shaker. Solution was filtered using a laboratory filter paper and hexane was removed from the filtrate by rotary evaporation. The residue was again soaked in ethyl acetate for 24 hours at room temperature while shaking. The solution was filtered, and ethyl acetate was removed from the filtrate by rotary evaporation. The resulting leaf powder was again soaked in methanol for 24 hours at room temperature while shaking. Solution was filtered and methanol was removed from the filtrate by rotary evaporation. This procedure was repeated to prepare the leaf and seed extracts for all the three plants [7].

Aqueous extraction was carried out for fruits as follows. Ripen fruits were collected and washed with running water to remove any dirt and finally with deionized water. The seed from each fruit was separated. The remaining fleshy part of the fruits were cut into small cubes. Next it was homogenized with deionized water using a laboratory homogenizer for 10 minutes [15]. The solution was filtered using a cheesecloth and the filtrate was lyophilized [16]. This procedure was used to prepare the aqueous fruit extracts for SS and SM fruits.

Determination of total phenolic content

Folin-Ciocalteu assay was used to determine the total phenolic content of all samples, which was expressed as milligrams of gallic acid equivalent per gram of sample (mg GAE/g). Gallic acid /extract (0.5 mL) was mixed with Folin-Ciocalteu reagent (0.5 mL) and was kept in dark at room temperature. After 05 minutes 6% w/v sodium carbonate (0.5 mL) and distilled water (2 mL) were added to the solution. The resulting mixture was again kept in dark at room temperature. After 01 hour, the absorbance was measured at 765 nm using a UV visible spectrophotometer (Hitachi U-2910 UV/Vis) [17].

Determination of radical scavenging activity

Antioxidant capacity of all extracts were studied through the evaluation of free radical scavenging effect on the DPPH radical [18]. BHT was used as the standard. DPPH solution was made up to 0.5mg/mL concentration with methanol and the solution (2.0 mL) was mixed with sample series /BHT solution series (2.0 mL) in methanol. The final mixture was incubated at room temperature for 10 minutes in dark.

Absorbance of samples were measured at 517 nm using UV visible spectrophotometer (Hitachi U-2910 UV/Vis). The control was prepared as above without the extract and methanol was used as the blank [19, 20]. Radical scavenging activity which was expressed as the inhibition percentage was calculated using the following formula (1);

$$\text{Percentage inhibition} = \frac{\boldsymbol{Ac - As}}{\boldsymbol{Ac}} \times 100\% \qquad (1)$$

Where, Ac is the absorbance of the control and As is the absorbance of the sample.

Alpha amylase inhibition assay

Alpha amylase inhibition assay was performed on all the methanol extracts of leaves and seeds and on lyophilized fruit extracts. Commercially available Acarbose tablets were powdered and used as the standard [14]. Series of Acarbose solutions, series of sample solutions, DNSA solution, Sodium phosphate buffer (pH =6.9), 1% w/v starch solution was prepared.

Plant extract/Acarbose solution (0.5 mL) was incubated with alpha amylase enzyme solution (0.5 mL) at 37^{0}C for 10 minutes. After incubation, starch solution (0.5 mL) was added and was incubated for another 10 minutes maintaining the temperature at 37^{0}C. DNSA reagent was added to the above solution and was immediately incubated in a water bath at 100^{0}C. After 5 minutes, the reaction mixture was removed from the water bath and was diluted up to 5mL with deionized water after the assay medium reached to room temperature. For each test sample individual blank was performed by replacing enzyme with buffer whereas the control was performed replacing the extract with buffer. Both the plant extract and alpha amylase solution were replaced by buffer for the preparation of control blank.

Absorbance was measured at 540 nm using UV visible spectrophotometer (Hitachi U-2910 UV/Vis). Percentage inhibition was calculated using the following formula (2)[21,22].

$$\text{Percentage inhibition} = \frac{(\text{Ac} - \text{Acb}) - (\text{At} - \text{Atb})}{(\text{Ac} - \text{Acb})} \times 100\% \quad (2)$$

Where Ac is the absorbance of the control, Acb is the absorbance of the control blank, At is the absorbance of the test sample and Atb is the absorbance of the test blank.

Antibacterial assay

All sample extracts were dissolved in DMSO to obtain a solution with a concentration of 6.8 mg/mL. DMSO was used as the negative control. Clinically used antibacterial drug Azythromycin was used as the positive control [20]. The tablet was powdered and dissolved in DMSO to prepare 6.8 mg/mL concentration. Bacteria from the bacterial subcultures were gently scraped and were mixed with sterilized deionized water inside a test tube to adjust to 0.5 MacFarlane standard [23].

Then the bacterial solution (100.00 µL) was withdrawn from the test tube and was evenly spread in the petri dishes containing solidified agar. After 02 minutes, the agar bed was punched to produce 04 wells of 7 mm diameter. Two wells were filled with sample extract (50.00 µL) another well was filled with DMSO solution (50.00 µL) to be used as the negative standard and the final well was filled with Azythromycin solution. All the samples were maintained at room temperature for about 18 hours [24, 25]. All the plates were examined for growth inhibition and the diameter of the inhibition zone was measured in millimeters.

Results and discussion

Determination of total phenolic content

The highest phenolic content among all leaf, seed and fruit extracts was recorded in SS methanol leaf extract, which was 106±1 (mg GAE/g) as shown in figure 1. Except in SM leaf extract the total phenolic content of all hexane, ethyl acetate and methanol leaf and seed extracts were in a progressively increasing order. Whereas in the SM leaf the highest phenolic content was recorded in the hexane extract. The aqueous fruit extracts exhibited a significant low total phenolic content in comparison to other extracts.

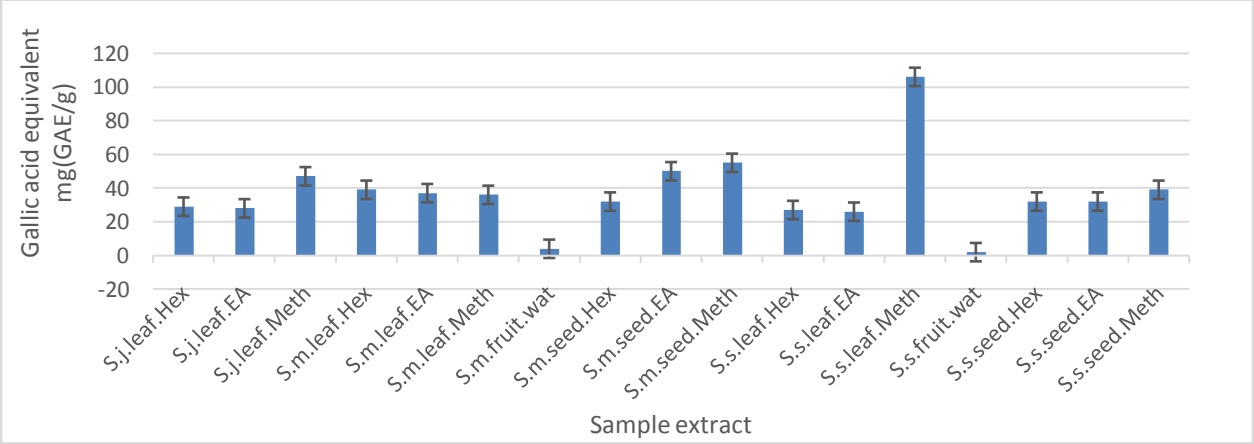


Figure 1. Comparison of total phenolic content of all the extracts expressed as milligrams of gallic acid equivalents per gram of sample.

Determination of radical scavenging activity

All hexane, ethyl acetate, methanol and aqueous extracts were tested for DPPH radical scavenging activity. Maximum percentage inhibition value was obtained for the methanol seed extract of SS, which was 92.07±0.77% at 34.00 (µg/mL) concentration. All the extracts which depicted significant free radical scavenging activity with respect to BHT are shown in table 1.

When considering the solvents used for extraction; hexane extracts showed least percentage inhibition values, ethyl acetate extracts showed moderate percentage inhibition values and methanol extracts showed the highest percentage inhibition values with respect to the standard BHT. The lyophilized fruit extracts exhibited percentage inhibition values only in higher concentration with respect to the standard BHT. Hence, the radical scavenging activity was progressively higher in water, hexane, ethyl acetate and methanol extracts respectively.

Alpha amylase inhibition assay

As indicated in table 2, methanol leaf extract of the three plant species showed good alpha amylase inhibition activity in comparison to the standard drug Acarbose, but there was no any significant inhibitory activity in seed extracts and lyophilized fruit extracts.

Determination of anti-bacterial activity

All the leaf, seed and fruit extracts were tested against *Escherichia coli* (gram-negative) *Staphylococcus aureus* (gram positive) and *Bacillus subtilis* (gram-positive) bacteria. Several leaf and seed extracts showed inhibition zones at least against one type of bacterial species as indicated in table 3. No inhibition zones were observed for the lyophilized fruit extracts against any type of bacteria.

Highest inhibition zone against *Escherichia coli* was noticed in the hexane leaf extract of SJ whereas the *hexane* extract of SS seed showed the highest inhibition zone against *Staphylococcus aureus* and *Bacillus subtilis* with respect to standard drug Azithromycin.

Conclusion

Presence of anti-diabetic, antioxidant and antibacterial activities in SJ, SS and SM scientifically validates the use of these plants in traditional medicine to treat diseases including diabetes mellitus. But it was observed that the pharmacological properties vary with different parts of the plant. This may be due to the qualitative and quantitative fluctuation of biomolecule distribution throughout the plant.

Out of the three selected plants, SS depicted the highest biological activity with respect to total phenolic content, free radical scavenging activity and antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* species. Antibacterial activity and free radical scavenging activity were highest in SS seed extracts whereas SS leaves contained the highest phenolic content. Highest alpha amylase inhibition activity was observed in SM leaf.

Table 1. Free radical scavenging activity of extracts.

Sample	Concentration (µg/mL)	Maximum percentage inhibition (%)
BHT	340.00	62.13±1.70
SS-leaf-methanol	30.00	54.56±0.99
SS-seed-hexane	340.00	87.18±0.66
SS-seed-methanol	34.00	92.07±0.77
SM-leaf-methanol	30.00	47.50±0.87
SM-seed-methanol	340.00	92.27±0.74
SJ-leaf-ethyl acetate	30.00	57.40±1.17
SJ-leaf-methanol	30.00	71.00±0.67

Table 2. Percentage inhibition for alpha amylase inhibition assay.

Sample	Concentration (mg/mL)	Maximum percentage inhibition (%)
Acarbose	5.00	46.21±0.98
SS-leaf-methanol	5.00	57.83±0.76
SM-leaf-methanol	2.30	83.10±1.22
SJ-leaf-methanol	5.00	70.13±0.77

Table 3. Antibacterial activity of extracts.

Bacterial species	Plant extract		Solvent	Diameter of the inhibition zone(mm)		
	Species	Part		Negative control	Positive control	Test
Escherichia coli	SS	Leaves	Hexane	7±1	31±1	-
			Ethyl acetate	7±0	31±1	16.5±1.5
			Methanol	7±1	31±3	10±1
		Seeds	Hexane	7±1	30±1	19±1
			Ethyl acetate	7±1	30±3	-
			Methanol	7±0	30±2	-
	SM	Leaves	Hexane	7±0	31±2	-
			Ethyl acetate	7±0	31±2	10±2
			Methanol	7±1	31±2	-
		Seeds	Hexane	7±0	30±1	-
			Ethyl acetate	7±1	30±1	-
			Methanol	7±2	30±2	-
	SJ	Leaves	Hexane	7±1	31±0	21.5±2.5
			Ethyl acetate	7±0	31±0	15±1
			Methanol	7±1	31±0	13±1
Staphylococcus aureus	SS	Leaves	Hexane	7±0	28±1	-
			Ethyl acetate	7±1	28±1	15±1
			Methanol	7±0	28±1	13±1
		Seeds	Hexane	7±2	28±1	20±1
			Ethyl acetate	7±0	28±2	14±1
			Methanol	7±1	28±1	-
	SM	Leaves	Hexane	7±1	28±0	-
			Ethyl acetate	7±2	28±1	-
			Methanol	7±1	28±0	12±1
		Seeds	Hexane	7±1	28±1	9±1
			Ethyl acetate	7±1	28±0	10±1
			Methanol	7±2	28±1	-
	SJ	Leaves	Hexane	7±2	28±1	17.5±1.0
			Ethyl acetate	7±2	28±0	13±2
			Methanol	7±1	28±0	13.9±1.0
Bacillus subtilis	SS	Leaves	Hexane	7±2	31±3	-
			Ethyl acetate	7±1	31±1	17±2
			Methanol	7±1	31±2	9.5±1.0
		Seeds	Hexane	7±2	26±1	20±1
			Ethyl acetate	7±1	26±2	12±1
			Methanol	7±1	26±2	-
	SM	Leaves	Hexane	7±2	31±3	-
			Ethyl acetate	7±1	31±1	-
			Methanol	7±2	31±2	8±1
		Seeds	Hexane	7±1	26±1	10±1
			Ethyl acetate	7±1	26±1	10.5±1.0
			Methanol	7±1	26±1	-
	SJ	Leaves	Hexane	7±0	31±1	17±2
			Ethyl acetate	7±1	31±2	13±1
			Methanol	7±3	31±1	13±1

When considering about extraction of plant materials; methanol was the most successful solvent for antioxidant and anti-diabetic assays due to the high antioxidant and antidiabetic activity in methanol extracts comparative to the relevant standards. With respect to antibacterial activity, the best solvent was hexane, as the highest inhibition zones against all the selected bacterial species were observed in hexane extract.

Apart from consumption as fruits more attention has to be focused on preparing value added products from seeds and leaves of SJ, SS and SM as it can act as a potent source of anti-diabetic, antioxidant and antibacterial agents. These species have a good potential to be

developed as plant based nutraceutical and pharmaceutical products.

Author contributions

All the authors have contributed equally in designing, drafting the manuscript as per the journal submission format. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that there are no competing conflicts of interest.

Acknowledgement

The authors are thankful to the National Herbarium at Royal Botanical Garden, Peradeniya for conducting the authentication of the plants.

References

1. Rani A, Sulakshana G, Patnaik S: Costus *speciosus*, an antidiabetic plant-Review. FS J Pharm Res. 2012; 1:52-53.
2. El-Far A, Shaheen H, Alsenosy A, El-Sayed Y, Al Jaouni S, Mousa S: *Costus speciosus*: Traditional uses, phytochemistry, and therapeutic potentials. Pharmacognosy Reviews. 2018; 12(23):120.
3. Pavar V, Pavar P: *Costus speciosus*: An Important Medicinal Plant. International Journal of Science and Research (IJSR). 2014; 7(7):28-33.
4. Benherlal P, Arumughan C: Chemical composition and in vitro antioxidant studies on *Syzygium cumini* fruit. Journal of the Science of Food and Agriculture. 2007; 87(14):2560-2569.
5. Cincotta R, Wisnewski J, Engelman R: Human population in the biodiversity hotspots. Nature. 2000; 404(6781):990-992.
6. Gunatilleke N, Pethiyagoda R, Gunatilleke S: Biodiversity of Sri Lanka. Journal of the National Science Foundation of Sri Lanka. 2017; 36:25.
7. Nigam V, Nigam R, singh A: Distribution and medicinal properties of *Syzygium* species. Current Research in Pharmaceutical Sciences. 2012; 02:73–80.
8. Bairy KL, Sharma A, Shalini A: Evaluation of the hypoglycemic, hypolipidemic and hepatic glycogen raising effects of *Syzygium malaccense* upon streptozotocin induced diabetic rats. Journal of natural remedies. 2005; 5(1):46–51.
9. Shahreen S, Banik J, Hafiz A, Rahman S, Zaman A, Shoyeb A, et al: Antihyperglycemic Activities of Leaves of Three Edible Fruit Plants (*Averrhoa carambola*, *Ficus hispida* and *Syzygium samarangense*) of Bangladesh. African Journal of Traditional, Complementary and Alternative Medicines. 2012; 9(2):287–291.
10. Sharma R, Kishore N, Hussein A, Lall N: Antibacterial and anti-inflammatory effects of *Syzygium jambos* L. (Alston) and isolated compounds on acne vulgaris. BMC Complementary and Alternative Medicine. 2013; 13(1):292.
11. Ikram EHK, Eng KH, Jalil AMM, Ismail A, Idris S, Azlan A, et al: Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. Journal of Food Composition and Analysis. 2009; 22(5):388–93.
12. Nunes PC, Aquino JdeS, Rockenbach II, Stamford TL: Physico-Chemical Characterization, Bioactive Compounds and Antioxidant Activity of Malay Apple [*Syzygium malaccense* (L.) Merr. & L.M.Perry]. 2016; 11(6):1–11.
13. Silva KDRR, Sirasa MSF: Antioxidant properties of selected fruit cultivars grown in Sri Lanka. Food Chemistry. 2018; 238:203–8.
14. Arumugam B, Manaharan T, Heng CK, Kuppusamy UR, Palanisamy UD: Antioxidant and antiglycemic potentials of a standardized extract of *Syzygium malaccensse*. LWT - Food Science and Technology. 2014; 59(2):707–712.
15. Ishrad, Ifran A, Mehedi SJ, Goel HC, Rizvi MA: Antioxidant Capacity and Phenolic Content of the Aqueous Extract of Commonly Consumed Cucurbits. International Journal of Food properties. 2013; 17(1):179–86.
16. Koczka N, Stefanovits-Bányai É, Ombódi A. Total Polyphenol Content and Antioxidant Capacity of Rosehips of Some Rosa Species. Medicines. 2018; 5(3):84.
17. Kurian A, Thiripuranathar G, Paranagama PA: Determination of Total phenolic content and antioxidant activity of *Borassus Flabelliffer* Linn. Fruit pulp collected from several parts of Sri Lanka. International Journal of Pharmaceutical Sciences and Research. 2017; 8(6):2701–2705.
18. Nimse SB, Pal D: Free radicals, natural antioxidants, and their reaction mechanisms. RSC Advances. 2015; 5(35):27986–8006.
19. Kuganesan A, Thiripuranathar G, Navaratne AN, Paranagama PA: Antioxidant and anti-inflammatory activities of peels, pulps and seed kernels of three common mango *(Mangiferaindica* L.)Varieties in Sri Lanka. International Journal of Pharmaceutical Sciences and Research. 2017; 8(1):70–78.
20. Ratnayake KRMSH, Deraniyagala SA, Udukala D: Antioxidant and ant-microbial activity, total phenolic and total flavonoid contents of the peel and seed of *Spondias duclcis* (Amberalla) fruit. Pharmaceutical Society of Sri Lanka. 2018; 8(1):20-27.
21. Samidha K, Vrushali K, Vijaya P: Estimation of Phenolic content, Flavanoid content Antioxidant and Alpha amylase Inhibitory Activity of Marketed Polyherbal Formulation. Journal of Appied Pharmaceutical Science. 2014; 4(9):61-62.
22. Thrikawala VS, Deraniyagala SA, Fernando CD, Udukala DN: In Vitro α-Amylase and Protein Glycation Inhibitory Activity of the Aqueous Extract of *Flueggealeucopyrus* Willd. Journal of Chemistry. 2018; 2018:1–7.
23. Balouiri M, Sadiki M, Ibnsouda SK: Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016; 6(2):71–77.
24. Ramakrishnan G, Kothai R, Jaykar B, Rathnakmar V: *in vitro* Antibacterial Activity of different extracts of Leaves of *Coldenia procumbens*. Internatonal Journal of Pharm Tech Research. 2011; 3(2):1000-1004.
25. Antra S, Amla B: Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach* L. International Journal of Current Pharmaceutical Research.2012; 4(2):67-73.