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STUDIES ON BIOCHEMICAL AND ANTIBACTERIAL ACTIVITIES OF THREE MEDICINALLY IMPORTANT FRUITS

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ABSTRACT: Medicinal plants are essential in our balanced life diet and are in demand in allopathic and herbal medicine. The present study was aimed to evaluate the phytochemical, antioxidant and antimicrobial activities of *Phyllanthus emblica* (Euphorbiaceae), *Averrhoa bilimbi* (Oxalidaceae) and *Phyllanthus acidus* (Euphorbiaceae) fruits. The preliminary phytochemical analysis was carried out using standard methods. Total phenolic, flavonoid, protein, vitamin-C and tannin content were analyzed. Antioxidant and antibacterial activity was assessed by DPPH and agar well diffusion method. Phytochemical screening revealed the presence of alkaloids, flavonoids, glycoside, phenols, proteins, and amino acids, steroids, saponins, terpenoids, tannins, resins and emodols in different fruit extracts. The phenolics, protein and vitamin C content were higher in *Phyllanthus emblica* fruits. While flavonoids and tannin content was higher in *Averrhoa bilimbi* fruits. The free radical scavenging activity was significantly higher in *Phyllanthus emblica* fruits. Maximum inhibition of antibacterial activity was noticed in *Phyllanthus emblica* against *Bacillus subtilis* followed by *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli* respectively. In conclusion, our studies provide evidence that fruit extracts of these medicinal plants can be recommended as potential sources of nutraceuticals and for pharmaceutical industries.

INTRODUCTION: Medicinal plants constitute a source of raw material for approximately 25% of the prescribed drugs, which plays an important role in the field of health care. Medicinal plants in developing countries are used as a formational basis for the maintenance of good health, whereas in the developed countries they are used for the extracts and development of several drugs and chemotherapeutics [1].

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Medicinal plants are rich sources of bioactive compounds such as alkaloids, flavonoids and phenolic compounds [2].

Phyllanthus emblica Linn. is one of the most habitually used herbs and it is widely available in most tropical and subtropical countries of China, India, and Malaysia. The plant is commonly called 'Indian Gooseberry', Amla in Hindi and Nellikai in Kannada and it belongs to the family Euphorbiaceae. Indian gooseberry has been reported to contain several, compounds including alkaloids, diterpene, carbohydrates, furan lactones, *etc.* and it has many pharmacological properties *e.g.* analgesic, anti-inflammatory, hepatoprotective, immunomodulatory, anti-atherogenic, chemo-protective properties [3, 4].

Averrhoa bilimbi Linn. is commonly known as bilimbi or cucumber tree belongs to the family Oxalidaceae. Native from South-East Asia and widely cultivated plants in India, Indonesia, Sri Lanka, Bangladesh, Myanmar, Malaysia, Central, and South America. *A. bilimbi* fruits exhibit good antioxidant and free radical scavenging activity [5]. The fruits are rich in oxalic acid, vitamin C, tannins, and minerals and it contains 53 volatile components consisting mainly of the aliphatic acids, hexadecanoic acid, 9-octadecanoic acid, esters, butyl nicotinate and hexyl nicotinate have been found in the fruits [6].

Phyllanthus acidus is one of the trees with edible small yellow berries fruit in the Phyllanthaceae/ Euphorbiaceae family, found in tropical or subtropical regions of Asia, Caribbean region, Central and South America. The fruits are used to make juice, cooked as relish, syrup and also jam, other than eaten fresh and it is also often eaten as pickle. *P. acidus* contains many chemical compounds such as b-amyrin, lupeol, phyllanthol and it has pharmacological activities like analgesic, antilipoxigenase [7].

Based on the above facts, the present study was designed to evaluate the phytochemical analysis, antioxidant and antibacterial activities of three medicinally important fruits.

MATERIALS AND METHODS: The matured fruits of *Phyllanthus emblica (P. emblica*), *Averrhoa bilimbi (A. bilimbi)* and *Phyllanthus acidus (P. acidus)* were collected from herbal, Bengaluru. Authenticated was done by the Department of Botany, Bangalore University. The voucher specimens (BUB, No. 2301, 2302 and 2303) were deposited in the Department. The fruits are washed thoroughly with tap water and were soaked in bavistin for five minutes then rinsed with distilled water (d.w). The fruits were sliced, shade dried and biomass was recorded.

Preparation of Extracts: The 20 gm of each powdered material was weighed and extracted in methanol using the Soxhlet apparatus. The extraction was carried out continuously for 12-14 h. Then extracts were concentrated for evaporation and stored at low temperature in sterile capped containers till further analysis.

Qualitative Analysis: Preliminary phytochemical screening of methanolic, aqueous and chloroform extracts of the fruits were subjected to various tests by standard methods [8]. Briefly the concentrated extracts were dissolved in respective solvents and screened for the qualitative analysis of alkaloids, flavonoids, proteins & amino acids, phenols, tannins, steroids, & terpenoids, phytosterols, glycosides, coumarins, betacyanin, resins, phlobatannins and emodols.

Quantitative Estimation of Fruits:

Total Phenolic Content: The total phenolic content in methanolic extracts of fruits was determined by using Folin Ciocalteu (FC) method [9]. Every 0.5 ml of fruit extract (1 mg/ml) was mixed with 0.5 ml of FC reagent and allowed to stand for room temperature (RT) for 2-3 min. Followed by 1ml of 7% sodium carbonate was added and the final volume was made up to 5ml with d.w. After 90 min of incubation at RT in dark, the absorbance was read at 725 nm using UV visible spectrophotometer in triplicates. Gallic acid was used for calibration of the standard curve. The results were expressed as mg of gallic acid equivalent (mg GAE/g) of dry weight (DW) of material.

Total Flavonoid Content: Total flavonoid content was determined by aluminum chloride ($AlCl_3$) method [10]. Briefly, 0.5 ml of extracts were taken and the volume was made up to 2 ml with 95% methanol. Then 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1M potassium acetate and 1.8 ml d.w were added consecutively. The test solutions were shaken vigorously and incubated for 30 min in RT. Absorbance was measured at 415 nm with UV-spectrophotometer against blank. The total flavonoid content was expressed as mg of quercetin equivalents per gram sample (mg QE/g).

Total Condensed Tannin (Proanthocyanidin): Total tannins were determined according to the procedure of Liu *et al*. [11] Briefly, to 500 µl of the sample, 3 ml of 4% vanillin solution in methanol and 1.0 ml of concentrated hydrochloric acid were added. The mixture was then shaken and incubated at RT for 15 min. the absorbance was measured at 500 nm against blank. The tannin content was expressed as mg of catechin equivalent per g of dry weight (mg CE/g DW).

Protein Assay: Protein content was measured by the method of Layne [12]. Briefly, 1.0 ml of sample was added to 1.0 ml of biuret reagent (10% NaOH, Sodium potassium tartrate and Copper (II) sulphate pentahydrate). The mixture was incubated for 20 min at RT, the absorbance was read at 540 nm against blank. Bovine serum albumin (BSA) was used as a standard.

Ascorbic Acid (Vitamin-C) Content: Ascorbic content was measured according to the method of Ibrahim *et al.* [13] The fresh fruits were extracted in phosphate-citrate buffer solution (1%), using chilled pestle and mortar. The homogenate was filtered and added to 1 ml of DCPIP (2, 6-dichloroindophenol). The absorbance was read at 520 nm within 10 min. L-ascorbic acid was used as a standard. Vitamin-C content was recorded as mg of L-ascorbic acid in fresh fruits.

DPPH Radical Scavenging Assay: The free radical scavenging activity was measured by DPPH assay [14]. Briefly, different concentration of methanolic extract of the sample (10-100 µg/ml) was added to 3 ml of 0.25 mM (95% methanol) of DPPH solution. After 30 min of incubation at RT in dark, the absorbance was measured at 517 nm. BHT (Butylatedhydroxytoluene) was used as a standard. DPPH radical scavenging activity was calculated by the following formula.

Percentage inhibition = (Absorbance control - Absorbance sample) / Absorbance control × 100

The IC_{50} value is the concentration of the plant extract required to scavenge 50% of the total DPPH radicals available.

Antibacterial Activity: The solvent extracts were assayed against the following organisms *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Bacterial strains were obtained from the Department of Microbiology, Bangalore University, Bengaluru. *In-vitro* antibacterial activity was performed by agar well diffusion method according to the protocol of Varghese *et al.* [15] Wells were bored into Nutrient agar using a sterile 8 mm diameter cork borer. Different solvent extracts (50-200 µl) were added into the wells using sterilized pipettes and allowed to diffuse at room temperature for 2 h. The plates were incubated at 37 ºC for 24 h. After the incubation, diameter of the zone of inhibition was recorded in millimeter and compared with standard antibiotic (streptomycin). The experiments were repeated thrice in triplicates.

Statistical Analysis: The results were expressed as Mean ± Standard Deviation (SD). Data were analyzed by one-way analysis of variance followed by Duncan's multiple range tests using SPSS software. Probability values $P<0.05$ were considered significant.

RESULTS AND DISCUSSION:

Biomass: Fig. 1 represents the fresh and dry weight of *P. emblica*, *A. bilimbi*, and *P. acidus* fruits. The higher biomass was recorded in *P. emblica* fruits followed by *P. acidus* and *A. bilimbi*.

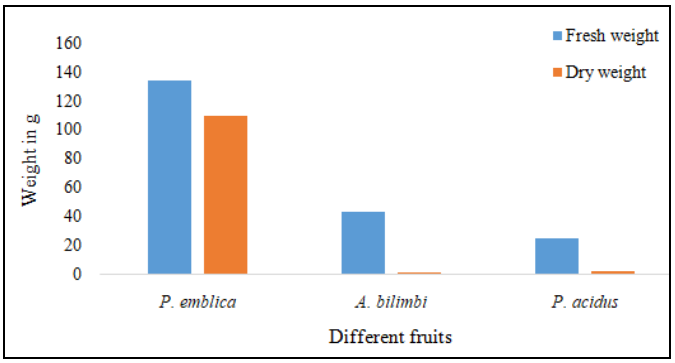

FIG. 1: FRESH AND DRY WEIGHT OF *P. EMBLICA*, *A. BILIMBI*, AND *P. ACIDUS* FRUITS. Values represent the Mean ± SD in triplicates.

Qualitative Analysis: Phytochemical studies revealed the presence of alkaloids, flavonoids, proteins, phenols, tannins, steroids & terpenoids, phytosterols, glycosides, coumarins, betacyanin, resins, phlobatannins, volatile oils. Methanol was proven to be a better solvent for the extraction of major phytochemicals compared to other solvents **Table 1**.

Previous studies by Rahman *et al.*, [16] indicated the presence of glycoside, tannin, and resins in *P. acidus*. Hasanuzzaman *et al.*, [14] showed the presence of alkaloids, tannin, saponins, flavonoids, glycosides, triterpenes, phenols, and carbohydrates in methanolic and aqueous extracts of *A. bilimbi* fruits. Studies by Dhale and Mogle [17] revealed the presence of alkaloids, oil, and fats, glycerides, carbohydrates, phenolics, tannins, lignin, saponins, flavonoids, terpenoids in *P. emblica* alcoholic extract.

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *P. EMBLICA*, *A. BILIMBI* AND *P. ACIDUS* FRUITS

Tests	Methanol			Aqueous			Chloroform		
	A	B	C	A	B	C	A	B	C
Alkaloids	+	+	-	+	-	+	+	+	+
Amino acid	-	-	-	-	-	+	-	-	+
Coumarins	+	-	-	+	+	+	-	+	+
Emodols	+	-	-	+	-	-	+	-	-
Flavonoids	+	+	+	+	+	+	+	-	+
Glycoside	+	-	+	+	+	+	+	-	+
Phenols	+	+	+	+	-	-	+	-	-
Proteins	+	+	+	+	+	+	+	+	+
Reducing sugar	+	+	+	+	+	+	+	+	+
Resins	-	-	+	+	+	+	+	+	+
Saponins	+	+	+	-	-	-	-	-	-
Steroids	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	-	+	+	-	+
Terpenoids	+	+	-	+	+	+	-	-	-
Volatile oils	+	+	-	+	+	+	-	-	-

A =*P. emblica*, B= *A. bilimbi*, C= *P. acidus*, + = Present, - = Absent

Total Phenolic Content: The total phenolic content is estimated using the FC reagent and calculated from regression equation of calibration curve (y=0.0578x - 0.3478, R^2= 0.9907). In the present study, total phenolic content was found to be higher in methanolic extract of *P. emblica* (42.99 ± 4.35 mg/g GAE), *P. acidus* (40.91 ± 2.67 mg/g GAE) and *A. bilimbi* (33.24 ± 3.19 mg/g GAE) respectively **Table 2**. Kumaran and Karunakaran [18] and Hasanuzzaman *et al.*, [14] reported higher phenolic content in *P. emblica* and *A. bilimbi* methanolic fruit extracts. The results were concordance with the reports of Devahastin and Mayachiew [19] and Padmapriya and Poonguzhali [10].

Total Flavonoid Content: The flavonoid content was determined using regression equation of calibration curve (y = 0.015x - 0.0645 R^2 = 0.9983) and expressed as Quercetin equivalent (QE). The total flavonoid content was found to be higher in *A. bilimbi* (10.55 ± 1.08 mg QE/g) followed by *P. acidus* (8.32 ± 0.32 mg QE/g) and *P. emblica* (8.27 ± 0.73 mg QE/g) extracts **Table 2**. The results were concordance with the reports of Habib *et al.*, [20], Foyzun *et al.*, [21] and Liu *et al.*, [11] in *Phyllanthus species.*

Total Condensed Tannin: The total condensed tannin was determined using regression equation of calibration curve (y=0.0068 x + 0.0046 R^2 = 0.9979) and expressed as catechin equivalent (CE).The maximum content was recorded in *A. bilimbi* (5.35 ± 0.81 mg CE/g) followed by *P. acidus* (4.42 ± 0.61mg CE/g) and *P. emblica* (4.22 ± 0.25 mg CE/g) extracts **Table 2**. The similar results were reported by Liu *et al.*, [11] where the methanolic extract of *P. emblica* exhibits higher tannin content.

TABLE 2: BIOCHEMICAL ANALYSIS OF *P. EMBLICA*, *A. BILIMBI* AND *P. ACIDUS* FRUITS

Samples	Phenolics mg/g (GAE)	Flavonoids mg /g (QE)	Tannin mg /g (CE)	Protein mg /g	Vitamin-C mg /g
P. emblica	42.99±4.35^b	8.27±0.73^a	4.22±0.25^a	115.00±5.15^b	104.32±2.19^b
A. bilimbi	33.24±3.19^a	10.55±1.08^b	5.35±0.81^a	39.749±4.13^a	91.05±8.87^b
P. acidus	40.91±2.67^b	8.32±0.32^a	4.42±0.61^a	38.499±7.00^a	76.35±8.33^a

Values represent the Mean ± SD in triplicates. Data were analyzed by one-way analysis of variance followed by Duncan's multiple range tests using SPSS software. Probability values P<0.05 were considered significant.

Protein Assay: The protein content was determined using regression equation of calibration curve (y=0.0027 x + 0.0509 R^2 = 0.9959). The protein content was rich in *P. emblica* (115.00 ± 5.15 mg/g) compared to *P. acidus* (38.499 ± 7.00 mg/g) and *A. bilimbi* (39.749 ± 4.13 mg/g). Similar results were reported by Ariharan *et al.*, [22] in *A. bilimbi*.

Ascorbic Acid (Vitamin C) Content: The vitamin C is popularly known as ascorbic acid, it is an L-enantiomer of ascorbic acid, which is water-soluble

involved in various biological reactions. Statistical analysis revealed that vitamin C content of *P. emblica* (104 ± 2.19 mg/g DW) was significantly higher than *A. bilimbi* (91.05 ± 8.87 mg/g DW) and *P. acidus* (76.35 ± 8.33 mg/g DW). The results were in concordance with the Ariharan *et al.*, [22], where they observed higher vitamin C content in *A. bilimbi* fruits.

DPPH Radical Scavenging Assay: *P. emblica* extracts exhibiting scavenging activity compared to standard with an IC_{50} value of 53.55 µg/ml.

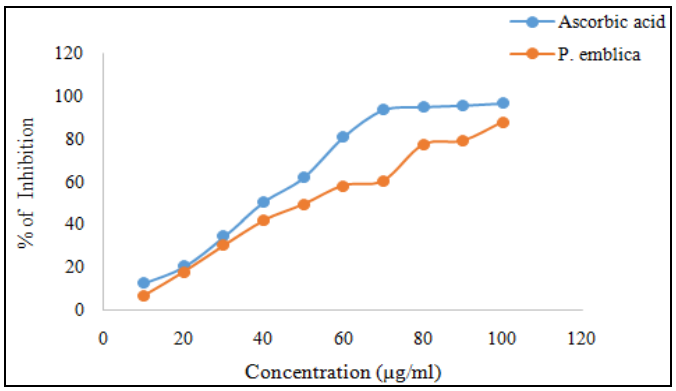

FIG. 2: DPPH RADICAL SCAVENGING ACTIVITY OF*P. EMBLICA* FRUIT EXTRACT

The standard antioxidant Ascorbic acid showed potent radical activity with an IC_{50} value of 11.42 µg/ml. A significant relationship between the antioxidants and total phenolic compounds are the major contributor of antioxidant capacities [23].

Antibacterial Activity: The extracts of *P. emblica*, *A. bilimbi*, and *P. acidus* were showed varying degrees of antibacterial activities. The results were evaluated by measuring the diameter zone of inhibition in mm.

The maximum inhibition was observed in *P. emblica* against *B. subtilis* (20.66 ± 2.51 mm) followed by *P. acidus* and *A. bilimbi* respectively. The methanolic extract of *P. emblica* exhibited significant antimicrobial activity than chloroform and diethyl extracts against *S. aureus* and *K. pneumoniae* respectively [24].

Habib *et al.*, [20] reported that *P. acidus* showed moderate inhibition against pathogenic bacterial strains. Studies by Mokhtar and Aziz [25] showed aqueous extract of *A. bilimbi* fruit exhibited maximum inhibition zone against *S. aureus*.

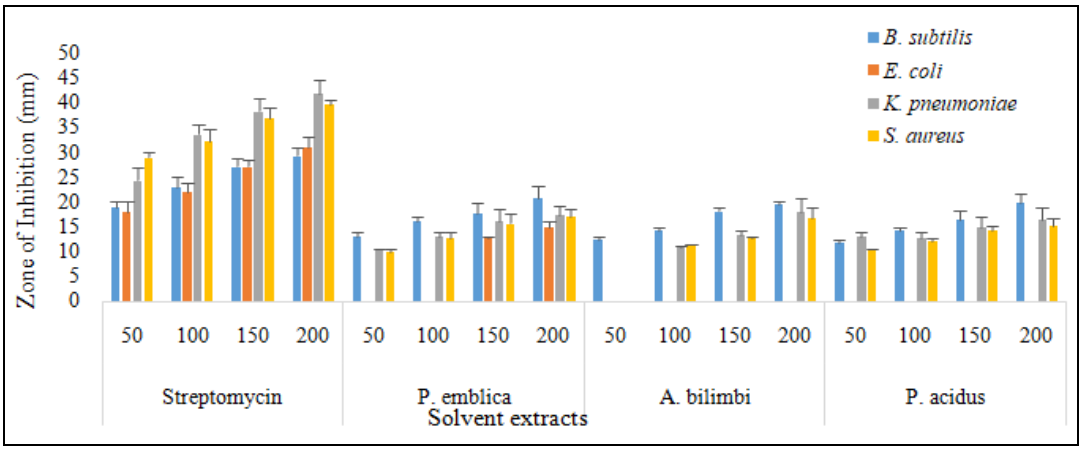

FIG. 3: ZONE OF INHIBITION OF *P. EMBLICA*, *A. BILIMBI* AND *P. ACIDUS* FRUIT EXTRACTS. Values represent the Mean ± SE in triplicates. Means with the different letters in columns indicate significant differences at 5% level.

CONCLUSION: All the fruits are a good source of secondary metabolites, and also rich in protein and vitamin C content which is essentially required in our regular diet. The methanolic extract reveals the presence of higher secondary metabolites such as flavonoids, phenolic, tannins, proteins, saponins, reducing sugar, steroids as compared to aqueous and chloroform extracts. The *P. emblica* extract exhibits higher antibacterial and antioxidant activity. Further studies are needed to isolate the active biochemical constituents.

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REFERENCES:

1. Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohoni DP, Biyani MK and Mohan H: Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry 2003; 63: 97-04.
2. Kannan P, Ramadevi SA and Waheeta H: Antibacterial activity of *Terminalia chebula* fruit extract. Journal of Microbiology 2009; 3(4): 180-84.

3. Poltanov EA, Alexander N, Shikov AN, Damien Dorman HJ, Pozharitskaya ON, Makarov VG, Tikhonov VP and Hiltunen R: Chemical and antioxidant evaluation of Indian Gooseberry (*Emblica officinalis* Gaertn. syn. *Phyllanthus emblica* L.) supplements. Phytotherapy Research 2009; 23: 1309-15.
4. Khan MTH, Lampronti I, Martello D, Bianchi N, Jabbar S, Choudhuri MSK, Kantidatta B and Gambari R: Identification of pyrogallol as an anti-proliferative compound present in extracts from the medicinal plant *Emblica officinalis*: Effects on *in-vitro* cell growth of human tumour cell lines. International Journal of Oncology 2002; 20: 187-92.
5. Kurup SB and Mini S: *In-vitro* evaluation of free radical scavenging and antioxidant activities of *Averrhoa bilimbi* fruit extracts. Journal of Plant Chemistry and Ecophysiology 2017; 2(1): 01-07.
6. Chowdhury SS, Uddin GM, Mumtahana N, Hossain M and Hasan SMR: *In-vitro* antioxidant and cytotoxic potential of hydro methanolic extract of *Averrhoa bilimbi* L. Fruits. International Journal of Pharmaceutical Sciences and Research 2012; 3(7): 2263-68.
7. Calixto JB, Santos ARS, Filho VC and Yunes RA: A review of the plants of the Genus *Phyllanthus*: their chemistry, pharmacology, and therapeutic potential. Medicinal Research Reviews 1998; 18(4): 225-58.
8. Harbone JB: Phytochemical Methods. Chapman and Hall Ltd., London 1973; 33-80.
9. Asna AR and Noriham A: Antioxidant activity and bioactive components of Oxalidaceae fruit extracts. The Malaysian Journal of Analytical Sciences 2014; 18(1): 116-126.
10. Padmapriya N and Poonguzhali TV: Antibacterial and antioxidant potential of the acetone extract of the fruit of *Phyllanthus acidus* L. International Journal of Current Sciences 2015; 17: 64-72.
11. Liu X, Zhao M, Wang J, Yang B and Jiang Y: Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. Journal of Food Composition and Analysis 2008; 21: 219-28.
12. Layne E: Spectrophotometric and turbidimetric methods for measuring proteins. Methods in Enzymology 1957; 3: 447-54.
13. Ibrahim MH, Jaafar HZE, Karimi E and Ghasemzadeh A: Impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of kacip Fatimah (*Labisia pumila* Benth). Molecules 2013; 18: 10973-88.
14. Hasanuzzaman M, Ali MR, Hossain M, Kuri S and Islam MS: Evaluation of total phenolic content, free radical scavenging activity and phytochemical screening of different extracts of *Averrhoa bilimbi* (fruits). International Current Pharmaceutical Journal 2013: 2(4): 92-96.
15. Varghese LS, Ninan MA, Alex N, Soman S and Jacob S: Comparative antibacterial activity of fruit extracts of *Emblica officinalis* Gaertn against gram-positive versus gram-negative bacteria. Biomedical and Pharmacology Journal 2013; 6(2): 447-52.
16. Rahman MM, Habib MR, Hasan SMR, Sayeed MA and Rana MS: Antibacterial, cytotoxic and antioxidant potential of methanolic extract of *Phyllanthus acidus* L. International Journal of Drug Development and Research 2011; 3(2): 154-61.
17. Dhale DA and Mogle UP: Phytochemical screening and antibacterial of *Phyllanthus emblica* L. Science Research Reporter 2011; 1(3): 138-42.
18. Kumaran A and Karunakaran JR: *In-vitro* antioxidant activities of methanol extracts of five *Phyllanthus species* from India. Food and Agriculture Organisation of the United Nations 2005; 40(2): 344-52.
19. Devahastin S and Mayachiew P: Antimicrobial and antioxidant activities of Indian gooseberry and galangal extracts. Food Science and Technology 2007; 41: 1153-59.
20. Habib RU, Yasin KA and Choudhary MA: Studies on the chemical constituents of *Phyllanthus emblica*. Natural Products of Research 2007; 21(9): 775-81.
21. Foyzun T, Aktar K and Uddin MA: Evaluation of antioxidant, cytotoxic and antimicrobial activity of *Phyllanthus acidus*. International J of Pharmacognosy and Phytochemical Research 2016; 8(11): 1751-58.
22. Ariharan VN, Kalirajan K, Meenadevi VN and Nagendra Prasad P: An exotic fruit which forms the new natural source for vitamin C. Rasayan Journal of Chemistry 2012; 5: 356-59.
23. Li BH, Wong CC, Cheng KW and Chen F: Antioxidant properties *in-vitro* and total phenolic content in methanol extracts from medicinal plants. Food Science and Technology 2008; 41(3): 385-90.
24. Raghu HS and Ravindra P: Antimicrobial activity and phytochemical study of *Phyllanthus emblica* Linn. International Journal of Pharmaceutical Studies and Research 2010; 1(1): 30-33.
25. Mokhtar IS and Aziz NAA: Antimicrobial Properties of *Averrhoa bilimbi* extracts at different maturity stages. J of Medical Microbiology and Diagnosis 2016; 5(3): 1-3.

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