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Study on antioxidant and antimicrobial activities of methanolic leaf extract of *Glycosmis pentaphylla* against various microbial strains

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Abstract
The purpose of the present study was to investigate the antioxidant and antimicrobial potential of methanolic extract of *Glycosmis pentaphylla* (family: Rutaceae) leaf which possesses significant drug value. *In vitro* antioxidant activity was performed using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity and total phenol content by Folin–Ciocalteu Reagent. The antioxidant activity of different concentrations of plant extracts were found to increase in a concentration dependent manner where the IC_{50} value was 69.64 µg/ml compared to the ascorbic acid (27.02 µg/ml). The total phenol content was found in plant extract was 25.28 mg/g GAE. The antimicrobial assay was performed by disc diffusion method using kanamycin and nystatin as the standard. The highest antimicrobial action was found to be against *Salmonella paratyphi* (zone of inhibition with 22 mm). The MIC was carried out by the serial dilution technique where it showed highest inhibitory effect with minimum concentration of 16 µg against *Salmonella paratyphi* and *Escherichia coli*. The obtained results indicate that investigated plant could be potential sources of natural antioxidants and antimicrobial agents and can be used in the treatment of oxidative disorders and many infectious diseases caused by microorganisms.

Keywords: *Glycosmis pentaphylla*, Antioxidant activity, Antimicrobial activity, Minimum Inhibitory Concentration (MIC)

1. Introduction
Medicinal plants impart an effective place by reducing mortality on participation in the cure of various severe diseases and ensure human wellbeing. According to the recent estimation of WHO more than 80 people of developed country rely on traditional medicines for the health need and 60 people use vitamin and phytomedicine for the health benefits at some points [1].
Many phytochemical compounds like phenolics, polyphenols, terpenoids, quinones act as antioxidants and antimicrobial agents. Antioxidant protects the body cells from highly reactive oxygen species (ROS) called free radicals including superoxide, hydrogen peroxide, hydroxyl (HO), peroxyl (ROO) and alkoxyl (RO) are produced *in vivo* during oxidation [2,3]. These antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS, and therefore can enhance the immune defense and lower the risk of health complications caused by oxidative stress [4]. They have effects on atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, in the aging process, ischemia-reperfusion injury in hind limb, DNA damage, liver damage.
Phenolic compounds, tannins, flavonoid and alkaloids are the most important antimicrobial agent and bioactive constituents in plant [5]. Tannin and flavonoid compounds inhibit the microbial growth by causing the bacterial colonies to disintegrate which probably results from their interference with the bacterial cell wall [6]. They also hasten the healing of wounds, inflamed mucous membrane and arrest bleeding [7, 8]. Multiple drug resistance in human pathogenic microorganism has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. So this work was carried out to determine the antimicrobial and antioxidant potentials of *G. pentaphylla* in advert to search for new source of medicine.
Glycosmis pentaphylla (Bengali name- Ashsaora, Kawatuti, Matmati, Ban Jamir) belongs to the family Rutaceae. It is a shrub or small (1.5–5.0 m) tree widely distributed from India, Malaysia and Southern China to the Philippine Islands and represented by nearly 11 species [9]. Traditionally the leaf is used as juice and paste in the treatment of fever, liver complaints, cough [10], rheumatism, anemia, jaundice, eczema, ascariasis, skin infections bone fracture, fracture induced pain and certain other diseases.

Stem is used as toothbrushes for its fibrous nature [11]. The plant *G. pentaphylla* have some phytochemical constituents like flavonoids, phenols, steroid, tannins, saponin, protein, glycoside [12], glyco-quinone (1) and glycocitrine-III [13], terpenoid [14], amides, imides, coumarins, arborinine, glycozolicine, 3- formylcarbazole, glycosinine, mupamine, varbazole, 3- methyl carbazole, glycolone, glycozolidol, glycozolinine, glycophymoline, glycophymine, glycomide, glycozoline, noracronycine, des- N-methylacrocynine, des-N-methylnoracronycine, furoquinoline bases (kokusaginine and skimmianine), alkaloids (glycosine, arborine, glycosminine, glycosamine, glycerine, glycosmicine, arbinol and isoarbinol), arborinone, two isomeric terpene alcohols, myricyl alcohol, stigmasterol, ß-sitosterol, arbornine, and furthermore glycoric acid, glycozolidine, dictamine [15].

So guided by the literature review of *G. pentaphylla* the present study has been carried out to determine antioxidant and antimicrobial activity of this plant.

2. Materials and methods

2.1 Sample Collection & Preparation

Sample leafs of *G. pentaphylla* were collected from Gazipur, Bangladesh in January 2014 and authentication of the plant sample has been done by the taxonomist and a voucher specimen (DACB-35914) was submitted to the national herbarium center Mirpur, Dhaka, Bangladesh. After proper identification, leaf samples were placed for sun drying for several days, then oven dried for 24 hours at considerably low temperature (40 °C) for better grinding and the dried samples were grinded into coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Dept. of Pharmacy, Southeast University and stored in an airtight container for further study. The leafs were dried at room temperature, ground into uniform powder to increase the surface area of the sample for extraction. 500g powder of leaf was weighed, poured a sterile flat bottomed flask containing 1000 ml of methanol with vigorous shaking for 7 days to enhance proper dissolution of the bioactive compounds in the samples and then filtered by cotton plug followed by Whatmann filter paper (125mm) at room temperature. The filtrates were concentrated at 45 ºC under reduced pressure using vacuum pump rotary evaporator (Stuart rotary evaporator, RE3022,UK) until the extracts became completely dry. The extracts were stored at 4^0C in a refrigerator until required for further analyses [5].

2.2 Microorganisms

Antibacterial activity was determined by using fourteen microorganisms including four Gram positive bacteria (*Bacillus cereus, Bacillus subtilis, Sarcina lutea and Staphylococcus aureus*), seven Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, Shigella dysenteriae, Shigella boydii, Vibrio parahaemolyticus, Vibrio mimicus*) and three Fungi (*Candida albicans, Aspergillus niger, Saccharomyces cerevisiae*). The microorganisms were obtained from the Institute of Nutrition and Food Sciences (INFS), Dhaka University The bacterial strains were first subcultured in nutrient agar (NA) medium & incubated at 37 ºC for 18 h while the fungal strains were subcultured for 72 h at 25 ºC [16].

2.3 *In vitro* Antioxidant Activity

2.3.1 DPPH radical scavenging activity

DPPH radical scavenging activity of the methanolic extract of *G. pentaphylla* extracts was determined on the stable radical 1, 1-diphenyl-2- picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams *et al.* [17]. 2 ml methanol solution of plant extract or standard ascorbic acid at different concentrations were mixed with 3 ml methanol solution of DPPH in the test tube. The test tube was incubated at room temperature for 30 min in a dark place to complete the reaction. The absorbance of the solution was measured at 517 nm by UV spectrophotometer (UV–1800PC SHIMADZU, Japan) The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extracts as compared to that of ascorbic acid. DPPH free radical scavenging ability (%) was calculated with the following formula:

$(A_0 – A) / A_0 \times 100$

Where A_0 is the absorbance of the control and A is the absorbance of the test or standard. Then % inhibitions were plotted against respective concentrations used and from the graph IC_{50} was calculated. The lower IC_{50} indicates higher radical scavenging activity and vice versa [18].

2.3.2 Total phenol content

Total phenolic content in the extracts was determined by the Folin-Ciocalteu reagent method described by Demiray *et al.* [19]. 1 ml of extract solution (200µg/ml) extracts was mixed with 5 ml Folin- Ciocalteu reagent (previously diluted with water 1:10 (v/v) and 4 ml (75g/L) of sodium carbonate. A series of gallic acid solutions were prepared in the range of 1-100 mg. The tubes were vortexed for few seconds and allowed to stand for 30 min at 26 °C for color development. Absorbance of samples and standard were measured at 765 nm. All the sample analyses were carried out in triplicates (n=3). Total phenol content was expressed as gallic acid equivalents (mg GAE) per gram of dry weight [20].

2.4 *In vitro* Antibacterial activity

2.4.1 Disc diffusion Method

Antibacterial activity of methanolic extract of *G. pentaphylla* was carried out by disc diffusion method. To prepare agar media solution 28 grams of agar was mixed with 1L of water followed by autoclaving for sterilization for 20 minutes at 121 ºC. With the help of an inoculating loop, the test organisms were transferred from the pure culture to the agar slants under a laminar air flow unit. The inoculated slants were then incubated at 37 ºc for 18-24 hours to ensure the growth of the test organisms. The red heat of loop is required before each and every bacteria inoculation. The agar media was taken in test tube after autoclaving and the organism was transferred from the subculture to the test tube containing 10 ml of autoclaved melted medium with the help of an inoculating loop in an aseptic area. The test tubes were shook by rotation to get a uniform suspension of the organism. The bacterial suspensions were immediately transferred to the sterile petri dishes (120 mm diameters) with and rotated clockwise and anticlockwise for the homogenous distribution of organism in media (depth 4 mm). Dried and sterilized filter paper discs (5 mm diameter) were then impregnated with 10 µl(500 µg/disc) of the test substances using micropipette. Standard antibiotic discs (Kanamycin 30µg/disc), standard antifungal discs (Nystatin 30 µg/disc) and blank disc (impregnated with solvent) were used as a positive and negative control. Positive controls were used to ensure the activity of standard antibiotic against the test organisms along with the comparison of the response between antimicrobial agent and the test samples [21] & negative controls was used to ensure the residual solvent and the filter paper not active themselves [22].

After incorporation petri dishes were transferred to the refrigerator for 1-2 hours at inverted position for the diffusion of drug. After 1-2 hours the petri dishes were transferred to the incubator at 37 ºC for 12-18 hours. The test material having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antibacterial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The measurements were carried out in triplicate to have average zone of inhibition [23].

2.4.2 Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration which resulted in maintenance or reduction of inoculums viability Serial tube dilution technique was used to determine MIC of the extract against these bacteria. Stock solution was prepared by dissolving the plant extract of 1.024 mg/2 ml distilled water (512µg/ml) (3 drops Tween 80 was added to facilitate dissolution). In serial dilution technique, 1 ml prepared stock solution was transferred to test tube containing 1 ml nutrient broth medium (256 µg/ml). Serial dilution was carried out to get 128, 64, 32, 16, 8, 4, 2 µg/ml of concentration. After preparation of suspensions of test organisms (107 organisms per ml) 1 drop of suspension (0.02 ml) was added to each broth dilution. Distilled water with 3 drops of Tween 80 and kanamycin were used as negative and positive control respectively. After 18 h incubation at 37 ºC, the tubes were then examined for the growth. The MIC of the extract was taken as the lowest concentration that showed no growth. Where the concentration of the extract was below the inhibitory level, growth was observed in those tubes with turbid (cloudy) broth medium [18].

3. Result

3.1 DPPH radical scavenging activity

DPPH radical scavenging activity of methanol extract of *G. pentaphylla* is shown in Figure-1 where increasing the concentration of the extract, activity was found to increase remarkably and the inhibitory capacity of the plant extract was found good in comparison to the reference antioxidant ascorbic acid. The percentage of inhibition of methanolic extract of *G. pentaphylla* was 58.33 at concentration of 100 µg/ml. In case of DPPH method for the studying of the antioxidant potential the methanolic extract of *G. pentaphylla* provided IC_{50} value 69.64 µg/ml where IC_{50} value for ascorbic acid was 27.02±4.52 µg/ml.

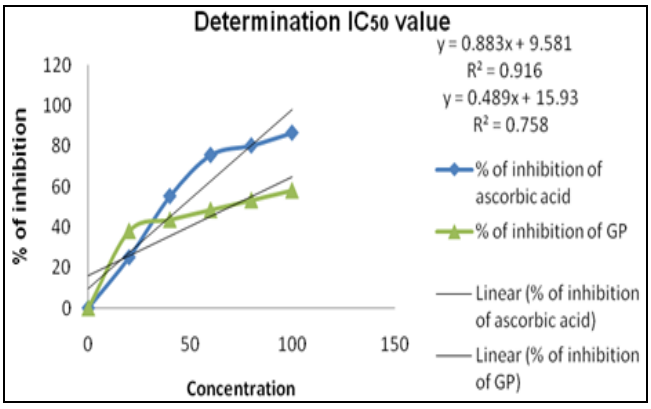

Fig 1: Determination of IC_{50} value of *Glycosmis pentaphylla*

3.2 Total phenolic content

The methanolic extract of *G. pentaphylla* leafs was subjected for the determination of the total phenol content. Total phenol content of the samples was expressed as mg/g GAE (gallic acid equivalent). The amount of total phenol content in *G. pentaphylla* was 25.28 mg/g GAE. Different studies suggest that different types of polyphenolic compounds such as flavonoids, phenolic acids were found in plants have multiple biological effects, including antioxidant activity [24].

3.3 Determination of Zone of inhibition

The search for compounds with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistance microorganisms. The antibacterial activity of the methanolic extract of *G. pentaphylla* was studied against gram positive, gram negative bacteria and fungi shown in Table 1

Table 1: Antimicrobial activity of test samples

Test microorganisms		***G. pentaphylla* (500µg/disc)**	**Kanamycin (30µg/disc)**
Gram positive bacteria	*Bacillus cereus*	17	42
	Bacillus subtilis	9	42
	Staphylococcus aureus	15	40
	Sarcina lutea	15	42
Gram negative bacteria	*Escherichia coli*	20	40
	P. aeruginosa	12	40
	Salmonella paratyphi	22	42
	Shigella dysenteriae	12	40
	Shigella boydii	12	42
	Vibrio parahaemolyticus	12	42
	Vibrio mimicus	9	42
Fungi	**Test microorganisms**	***G. pentaphylla* (500µg/disc)**	**Nystatin 30 (µg/disc)**
	Candida albicans	8	40
	Aspergillus niger	17	42
	Saccharomyces cerevisiae	17	40

G. pentaphylla provided a significant antimicrobial effect, producing a diverse range of zone of inhibition where the average diameter is 8-22 mm. The maximum zone of inhibition was 22 mm against *S. paratyphi* and the minimum zone of inhibition is 8 mm against *C. albicans.* It has also provided the zone of inhibition 9 mm against *B. subtilis & V. mimicus;* 12 mm against *V. parahaemolyticus, S. dysenteriae, S. boydii, P. aeruginosa;* 15 mm against *S. aureus, S. lutea* 17 mm against *B. cereus, S. cerevisiae, A. niger* and 20 mm against *E. coli.*

3.4 Minimum Inhibitory Concentration (MIC)

According to the obtaining result from Antimicrobial Activity the minimum inhibitory concentration (MIC) of methanolic extract of *G. pentaphylla* leaf was determined by serial dilution technique against 14 microorganisms. The highest MIC of *G. pentaphylla* was found against *S. paratyphi & E. coli* with 16 µg of MIC.

Table 2: Minimum Inhibitory Concentration of plant extract against several organisms.

Serial no.	Name of the organisms	*G. Pentaphylla* (µg)
1	*Bacillus cereus*	32
2	*Bacillus subtilis*	256
3	*Staphylococcus aureus*	64
4	*Sarcina lutea*	64
5	*Escherichia coli*	16
6	*P. aeruginosa*	128
7	*Salmonella paratyphi*	16
8	*Shigella dysenteriae*	128
9	*Shigella boydii*	128
10	*Vibrio parahaemolyticus*	128
11	*Vibrio mimicus*	256
12	*Candida albicans*	256
13	*Aspergillus niger*	32
14	*Saccharomyces cerevisiae*	32

4. Discussion

Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. In this study, antioxidant potential of methanolic extract of *G. pentaphylla* leaf was evaluated based on the ability to scavenge the DPPH. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule and is usually used as a substrate to evaluate the antioxidant activity of a compound [25]. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet color). As the electron became paired of in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stoichiometrically coincides with the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the test drugs to scavenge the free radicals independently [26]. Based on the data obtained from this study, DPPH radical scavenging activity of *G. pentaphylla* leaf extract was higher compared to that of ascorbic acid. It was revealed that *G. pentaphylla* leaf extract did show the proton donating ability served as free radical inhibitor or scavenger, converted them into more stable products and terminate the radical chain reaction. This may be important in protecting cellular DNA, lipids and proteins from free radical damage [27].

Several studies have also revealed that the phenolic content in the plants are associated with their antioxidant activities for their redox properties that allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. Phenol constituents are well known for their high antioxidant activity based on the absorbance values for the various extract solution, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents. It is understood that the high phenolic content may be responsible for the free radical scavenging activity of the plant extracts [28].

The zone of inhibition obtained of methanolic extract of *G. pentaphylla* and the standard drug for the antibacterial activity were in the range of 8-22 mm against all tested bacteria and fungi. This inhibitory activity may be caused by the presence of some active principles in them which are able to restrict the growth of bacteria by inhibiting protein synthesis of bacterial cell wall or alter the membrane function, inhibit protein synthesis or synthesis of purine and pyrimidines, hinder respiration or antagonize the metabolic pathways of microorganism leading to retardation of growth of bacteria [29]. The highest zone of inhibition found in methanolic extract of *G. pentaphylla* was found against *S. paratyphi* and the lowest zone of inhibition against *C. albicans*. It was found at the antimicrobial activity of the extracts correlated strongly with the DPPH activity due to the availability of the antioxidative compounds to exert different inhibitory effect against tested organisms [30].

The serial dilution assay quantities the antibacterial activity of pure compound by providing the MIC value of the compound for specific susceptible test organisms and important consideration in the further development of bioactive compounds. No inhibition was observed in the test tube containing sample lower than the above concentrations. Methanolic extract of *G. pentaphylla* revealed the inhibition activity with minimum concentration against *S. paratyphi & E. coli* among other microorganisms.

5. Conclusion

The present study exposes *G. pentaphylla* as a promising source of natural antimicrobial and antioxidants agents on various infectious diseases and the aging process of human being to discover of natural-product pharmaceuticals. On the basis of encouraging results of antimicrobial and antioxidant potentials it can be suggested that further studies should be carried out on purifying and isolating the phytochemicals along with evaluation of the antioxidant, antibacterial and antifungal properties *in vivo.*

6. Reference

1. Pandey Govind, Madhuri S. Singnificance of fruits and vegetables in malnutrition cancer, PL arch. 2010; 10(2):517-522
2. Pandey govind. Medicinal plants against liver diseases, International research journal of pharmacy. 2011; 2(5):115-121
3. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J, Role of oxygen radicals in DNA damage and cancer incidence. Mole Cell Biochem. 2004; 266(1-2):37-56.
4. Qu ZQ, Zhou Y, Zeng YS, Lin YK, Li Y, *et al.*, Protective effects of a *Rhodiola crenulata* extract and salidroside on hippocampal neurogenesis against streptozotocin-induced neural injury in the rat. PLoS One.; 2012;7:e29641
5. Ifesan BOT, Fashakin JF, Ebosele F, Oyerinde AS. Antioxidant and Antimicrobial Properties of Selected Plant Leafs. European Journal of Medicinal Plants. 2013: 3(3):465-473,
6. Erasto P, Moleta GB, Majinda RR. Antimicrobial and antioxidant flavonoids from the root wood of *Bolusanthus speciosus*, Phytochemistry. 2004; 65:875-880.
7. Manjunatha BK, Krishna V, Vidya SM, Mankani KL, Manohara YN. Wound healing activity of *Lycopodium serratum*. Indian J Pharm Sci. 2007; 69:283-287.
8. Shivanand B, Sivachandra-Raju S, Chalapathi-Rao AN. Wound healing activity of *Matricaria recutita* L. extract. J Wound Healing. 2007; 16:298-302.
9. Wang J, Di Y, Yang X, Li S, Wang Y, Hao X. Hydroquinone diglycoside acyl esters from the stems of *Glycosmis pentaphylla*. Phytochemistry. 2006; 67:486-491.
10. Rajib Ahsan, Km Monirul Islam, Musaddik A, Haque E. Activity of Methanol Extract of Some Medicinal Plants Against Carbon Tetrachloride Induced Hepatotoxicity in

Albino Rats. Global Journal of Pharmacology. 2009; 3(3):116-122,
11. Sayed Mamur Hossain, Farhana Islam, Tasnuva Sharmin, Hasib Sheikh, A.M. Rashedul Hasan and Mohammad A. Rashid, *In vitro* Antioxidant, Membrane Stabilizing and Thrombolytic Activities of *Glycosmis arborea*. Bangladesh Pharmaceutical Journal. 2012; 15(2):141-43
12. ebenezer T, kadamban d, ms shekhawat s. justin packia jacob, phytochemical evaluation and antioxidant activity of *glycosmis mauritiana* (lam.) tanaka leaf extract; international journal of pharma research & review. 2013; 2(9):16-23
13. Chihiro ITO, Yuichi Kondo K, Sundar Rao, Harukuni Tokuda, Hoyoku Nishino, Cand Hiroshi Furukawa, Chemical Constituents of *Glycosmis pentaphylla*. Isolation of A Novel Naphthoquinone and A New Acridone Alkaloid, Chem. Pharm. Bull. 1999; 47(11):1579-1581
14. Sivakumar M, Chamundeeswari D. Pharmacognostic studies and preliminary phytochemical screening on the root of *Glycosmis pentaphylla* (retz.,) dc., (rutaceae), iajpr. 2014; 4(7):3337-3342
15. Sreejith PS, Praseeja RJ, Asha VV, Sreejith PS *et al*., A review on the pharmacology and phytochemistry of traditional medicinal plant, *Glycosmis pentaphylla* (Retz.) Correa / Journal of Pharmacy Research. 2012; 5(5):2723-2728.
16. Sanjana Safi, Farhad Hossen, Rubaida Ahasan, Mahfuza Maleque, Khondoker D Alam, Mohammad S Ali. Study of in-vitro antioxidant potential and antimicrobial activity of Jatropha curcas- an important medicinal plant of the Indian subcontinent, Pharmacologyonline. 2012; 1:1-7.
17. Kumaran A, Karunakaran RJ. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT-Food Sci Tech. 2007; 40(2):344-352.
18. Mohammad Mustakim, Md. Mizanur Rahman, Prawej Ansari1, Anaytulla, Md. Foyez Ahmad and Mohammed Abu Sayeed, Investigation of Antioxidant and Cytotoxic Activities of Methanolic Extract from *Curculigo recurvata* Leafs International Journal of Biochemistry Research & Review. 2015; 8(2):1-8
19. Demiray S, Pintado ME, Castro PML. Evaluation of Phenolic Profiles and Antioxidant Activities of Turkish Medicinal Plants: Tilia Argentea, Crataegi Folium Leafs and Polygonum Bistorta Roots'. World Academy of Science, Engineering and Technology. 2009; 54:312-31.
20. Iyawe1 HOT, Azih MC. Total Phenolic Contents and Lipid Peroxidation Potentials of Some Tropical Antimalarial Plants, European Journal of Medicinal Plants. 2011; 1(2):33-39.
21. Rahman M, Rashid M. Antimicrobial activity and cytotoxicity of *Eclipta prostrata.* Oriental Pharm. Exp. Med. 2008; 8:47-52.
22. Encarnacion Dimayuga R, Keer Garcia S. Antimicrobial screening of medicinal plants from *Baja California* Sur, Mexico. J Ethnopharmacol. 1991; 31(2):181-92.
23. Alam khan, Moizur rahman, Shariful islam, Antibacterial, Antifungal and Cytotoxic Activities of Tuberous Roots of *Amorphophallus campanulatus*. Turk J Biol 31. 2007, 167-172
24. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002; 10(3):178-182.
25. Anup K Chakraborty, Harikrishna Roy, Shailaja Bastia. Evaluation of Antioxidant Activity of the Leafs of *Eupatorium Odoratum* Linn. Int J Pharm Pharm Sci. 2010; 2(4):77-79.
26. Ercisli S, Coruh I, Gormez A, Sengu M. Antioxidant and Antibacterial Activities of *Portulaca Oleracea* L. Grown Wild in Turkey. Ital J Food Sci. 2008; 4:20.
27. Adedapo AA, Jimoh FO, Koduru S, Masika PJ, Afolayan AJ. Evaluation of the medicinal potentials of the methanol extracts of the leafs and stems of *Halleria lucida.* Bioresour Technol. 2008; 99(10):4158-63.
28. Food Yee Chye, Kheng Yuen Sim. Antioxidative and Antibacterial Activities of *Pangium edule* Seed Extracts. International Journal of Pharmacology. 2009; 5(5):285-297.
29. Mukesh Sharma, Vimal Mohan, Maneesha Abraham, Meenakshi Sharma. Antimicrobial potential of the phytoextracts of some *Nyctaginaceae* members. African Journal of Biotechnology. 2010; 9(46):7942-7947.
30. Anitha roy, geetha rv, lakshmi t. Evaluation of the antibacterial activity of ethanolic extract of *dioscorea villosa* tubers, an *in vitro* study research Article**,** International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 4(1):314-316.