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Research Article

IN-VITRO ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *EMBLICA OFFICINALIS* LEAVES EXTRACT

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ABSTRACT

Emblica officinalis **(**Amla) has been considered the best of the ayurvedic rejuvenative herbs, because it is tridosaghna. The aim of the present study was to investigate antimicrobial and antioxidant activity of hydro-methanol (20:80) extract of leaves of *Emblica officinalis* (HMLEO). HMLEO was evaluated for its antimicrobial activity by using agar well diffusion method & their possible antioxidant assay by three complementary test systems, namely DPPH, hydrogen peroxide and reductive potential scavenging activity. The bacteria used in the study were *Bacillus subtilis, Salmonella typhi, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli.* The extract exhibited antibacterial activity with zone of inhibition ranging from 8.10 ± 0.65 to 23.37 ± 0.12 mm at different concentrations. Screening of crude extract showed notable minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) at concentrations of 12.50 to 50 and 200 to 300 mg/ml respectively. Crude extract showed maximum relative percentage inhibition against *Escherichia coli* (70.53 %) and lowest relative percentage inhibition against *Salmonella typhi* (33.58%). For antioxidant activity, DPPH & hydrogen peroxide scavenging activity, the IC_{50} value of extract was 45.38µg/ml & 42.87µg/m respectively. Preliminary phytochemical tests of hydro-methanolic extract of leaves of *Emblica officinalis* revealed the presence of flavonoids, terpenoids, tannins, and phenolic compounds. The organisms were sensitive to extract of the *Emblica officinalis* leaves, and could be considered as a potential source of natural antimicrobial and antioxidants.

Keywords: *Emblica officinalis,* Hydro-methanol extract, Antimicrobial, Antioxidants.

INTRODUCTION

Plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies[1]. This plant-based, traditional medicine system playing an essential role in health care, and about 80% of the world's population relying mainly on traditional medicines for their primary health care[2]. The use of plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments. The antimicrobial properties of plants have been investigated by a number of researchers worldwide, especially in Latin America[3]. One problem of microbial resistance is growth and the other is outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem to continue studies for development of new drugs, either synthetic or natural.

Antioxidant refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent or repair damage done to the body's cells by oxygen[4]. It act by several mechanisms such as, inhibition of scavenging activity against reactive oxygen species (ROS), reducing power, metal chelation, activity as antioxidative enzymes, inhibition of oxidative enzymes[5]. In recent years, there has been a considerable interest in finding natural antioxidants from plant materials. The antioxidant phytochemicals from plants, particularly flavonoids and other polyphenols, have been reported to inhibit the propagation of free radical reactions, to protect the human body from disease[4]. In addition, the use of synthetic antioxidants has been questioned because of their toxicity[6].

The objective of this research was to evaluate the potential of plant extracts on standard microorganism strains as well as multi-drug resistant bacteria and also effective antioxidants to replace the synthetic ones. The ultimate goal is to offer appropriate and efficient antimicrobial and antioxidant to the patient so that such plants should be investigated for better understand their properties, safety and efficacy *Emblica officinalis* belongs to the family Euphorbiaceae. In traditional Indian medicine, all parts of plant including the fruit, seed, leaves, root, bark and flowers are used in various herbal preparations. The plant is used in many forms. One of the most popular is as a decoction and infusion of leaves and seeds. Traditional use of *Emblica officinalis* leaves against cold, in anaemia, dysentery, fever, gravel, sores *(agya ghao, rokoc ghao);* Decoctions of the leaves used in the treatment of diabetes mellitus and chemical-free bactericidal mouthwash in the treatment of aphthae.[7-8] An infusion of the leaves with fenugreek seed is given for chronic diarrhea[9]. Green fresh leaves combined with curds have carminative and stomachic effect[8].

Leaves have been used for anti-inflammatory and antipyretic treatments[10]. The milky juice of the leaves is a good application to sores[7]. Infusion of the leaves is applied to sore eyes[11]. .*Emblica officinalis* leaves are rich in tannin, amlaicacid, astragalin, ellagic acid, gallo-tannin, kaempferol, kaempferol-3-o-glucoside, phyllantidine , phyllantine and rutin[12].

MATERIAL AND METHOD

Plant material

Leaves of *Emblica officinalis* were collected in month of June from National Park, Yamunanagar and authenticated from NISCAIR, (Ref no. NISCAIR/RHMD/CONSULT/-2010/1336/138) New Delhi. Leaves were air dried, & pulverized (coarse power).

Chemicals & instruments

DMSO (vehicle), DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical, hydrogen peroxide, ascorbic acid were obtained from Sigma-Aldrich, USA. All other chemicals and reagents used were of analytical grade. UV-Visible spectrophotometer (Shimadzu, Japan).

Preparation of the hydro-methanolic extract

The coarse powder (250 g) was extracted with 500 ml of hydro-methanol (20:80) using a soxhlet extractor for 7 h at a temperature (64°C) not exceeding the boiling point of the solvent. The extract was filtrated using whatman filter paper (no-1) and then concentrated at 40°C using a rotary evaporator. The residue obtained was stored in freezer at -80°C until further tests.

Preliminary phytochemicals studies

The extracts were subjected to various phytochemicals tests to determine the active constituents present in the crude hydro-methanolic extracts. The slightly modified method of Okerulu and Ani was used[13].

Evaluation of antimicrobial activity

Test microorganisms - A total of six bacterial species were tested including *Bacillus subtilis, Salmonella typhi, Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* that were isolated from medical samples and confirmed at the research laboratory of the department of microbiology, M.M. Medical Institute and Research, Mullana, Ambala (Haryana). They were identified using standard biochemical tests stock cultures of gram-negative organisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi)* and gram-positive organism (*Staphylococcus aureus, Streptococcus faecalis, Bacillus subtilis*). They were maintained on Muller-Hinton agar (Himedia, Mumbai) slope at 4^{o}C and subcultured into Muller-Hinton broth by a picking off technique[14]. Twenty-four hour old pure cultures were prepared for each time use.

Antibacterial activity- *In-vitro* antibacterial activity of the crude extract was studied against gram-negative and gram positive bacteria by the agar well diffusion method[1]. The extract was dissolved in 10% aqueous dimethylsulfoxide (DMSO) to a final concentration of 100, 200, 300 & 400 mg/ml. Pure DMSO was taken as the negative control. Gentamycin and Gatifloxcin as the positive control. Muller-Hinton agar was used as the bacteriological medium. It was prepared according to the manufacturer's instructions, autoclaved and dispensed at 20 ml per plate in 12 x 12 cm petri dishes. Set plates were incubated overnight to ensure sterility before use. Suspension of micro-organisms was made in sterile normal saline and adjusted to 0.5 ml McFarland standards inoculum (size was 108 cells/ml as per McFarland standard)[15]. Each labelled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed. A sterile cork borer of 5 mm diameter was used to make wells on the medium. 100µl of the various concentration of extract and control compound were dropped into each, appropriate labeled well. The inoculated plates were kept in the refrigerator for 1 hour to allow the extracts to diffuse into the agar[16]. The Muller-Hinton agar plates were incubated at 37^{o}C for 24 hours. After 24 hrs, the antibacterial activity was determined by measuring the diameter of the inhibition zone including the diameter of the bore (5 mm). The antibacterial assay for each extracts against all microorganisms tested was performed in triplicates.

Determination of Relative Percentage Inhibition (RPI) - The relative percentage inhibition of the test extract with respect to positive control were calculated by using the following formula[17].

$$\frac{100 \times (x-y)}{(z-y)}$$

Where,

x: total area of inhibition of the test extract

y: total area of inhibition of the solvent

z: total area of inhibition of the standard drug

(The total area of the inhibition was calculated by using area = πr^2; where, r = radius of zone of inhibition)

Determination of Minimum Inhibitory Concentration (MIC) - MIC of the leaves extract was performed by modified agar well diffusion method. Two fold serial dilution of the stock solution was prepared in sterilized distilled water to obtain various concentrations of the stock i.e. 6.25 12.50, 25, 50 & 100 mg/ml and were assayed against the test organisms. The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial growth[18].

Determination of Minimum Bactericidal Concentration (MBC) - To determine the MBC, equal volume of various concentrations of extract and Muller-Hinton broth were mixed in micro tube to make up 1 ml of solution. 1 ml of McFarland standard of the organism suspension was added to each tube. The tubes were incubated aerobically at 37°C for 24 hours. Two control tubes were maintained for each test batch. These include tube-containing extract without inoculum and the tube containing the growth medium and inoculums without extract. A loop full of broth was collected from those tubes and inoculated on sterile Muller-Hinton agar by streaking. The plates were inoculated at 37°C for 18-24 h. The highest dilution that yielded no colony fraction on a solid medium was considered as MBC[19].

Evaluation of Antioxidant Activity

DPPH (1,1-diphenyl-2-picryl-hydrazy) radical scavenging activity - The ability of the extract to scavenge DPPH radical was determined according to the method described by Nickavar *et al*. [20]. Sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 10, 20, 40, 60, 80 and 100 µg/ml in methanol. 1 ml of a 0.3 mM DPPH methanol solution was added to 2.5 ml solution of the extract or standard and allowed to react in dark at room temperature for 30 min. The absorbance of the resulting mixture was measured at 517 nm. Decreasing the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity and the percentage inhibition activity was calculated from [(A0–A1)/A0]×100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/standard.

The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations**.** Solution of ascorbic acid served as positive control.

Scavenging of Hydrogen Peroxide - A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4), different concentrations of plant extract and standard ascorbic acid solution viz. 10, 20, 40, 60, 80 and 100 µg/ml in methanol (1ml) added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for back ground subtraction. The percentage inhibition activity was calculated from [(A0–A1)/A0]×100, where A0 is the absorbance of the control and A1 is the absorbance of extract/standard.

The antioxidant activity of the extract was expressed as IC_{50}. All the tests were performed in triplicate and the graph was plotted with the average of three observations[21]. Solution of ascorbic acid served as positive control.

Total Reductive Potential - This was determined according to the method of Oyaizu[22]. Different concentration (10, 20, 40, 60, 80 and 100 µg/ ml) of leaves extract (1 ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1% w/v). The mixture was incubated at 50^{0}C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10% w/v) was added to the mixture, which was then centrifuged for 10 min at 1000 g. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) with $Fecl_3$ (0.5 ml, 0.1% w/v), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reductive potential. Mean value of three or more experiments were taken.

Statistical Analysis

The results of the experiment are expressed as mean ± SEM of three replicates in each test. Linear regression analysis was used to calculate the IC_{50} value. Data was considered statistically significant only when p value < 0.05.

RESULTS

Preliminary Phytochemistry of the Plant Extract- Preliminary phytochemicals analysis revealed that the plant possessed phytoconstituents were tannins, phenolic compounds, terpenoids and flavonoids.

Antibacterial Activity- The antimicrobial activity of hydro-methanolic extract of *Emblica officinalis* leaves was resulted to a growth inhibition pattern against the tested microorganism. Antibacterial activity of the extracts was recorded when the zone of inhibition was greater than 6mm. The results of the antimicrobial activity were given in the table 1.

Table 1: Zone of Inhibition of *Emblica officinalis* leaves extracts

Extract Concentration	Zone of Inhibition (mm)					
	Staphylococcus aureus	***Bacillus subtilis***	***Salmonella typhi***	***Escherichia coli***	***Pseudomonas Aeruginosa***	***Klebsiella pneumonia***
100 mg/ml	8.87 ± 0.12	09.52 ± 1.90	09.33 ± 0.12	8.10 ± 0.65	09.90 ± 0.72	11.61 ± 0.46
200 mg/ml	12.37 ± 0.*98*	12.05 ± 0.14	11.82 ± 0.37	13.12± 0.93	11.25 ± 1.14	15.67± 0.31
300 mg/ml	14.29 ± 0.12	13.87 ± 0.*11*	12.75 ± 0.14	17.98± 1.44	15.89 ± 0.78	18.25 ±*1.*95
400 mg/ml	18.9±1.07	16.00 ± 0.20	13.81 ± 0.11	23.37 ± 0.12	19.51 ± 1.94	22.09 ± 1.40
Gentamycin (10µg/ml)	25.6±1.52	19.75 ± 1.65	22.6±1.15	25.6±2.08	24.6±1.52	23.3±0.57
Gatifloxcin (10µg/ml)	26.61±1.45	24.52±0.98	23.82±1.02	27.73±1.52	30.91±1.00	27.56±1.23

Values are expressed as mean ± SEM of the three replicates

Diameter of the well is not included in Zone of inhibition.

These data revealed that the extract showed significant antimicrobial activity against all bacterial strains (3 Gram-positive and 3 Gram-negative bacteria) at different concentrations but dose dependent. It is particularly effective against gram-negative (*Escherichia coli & klebsiella pneumonia)* at concentration 400 mg/ml when compared with gentamycin and gatifloxcin which was used as a positive control against all bacterial strains assayed, produced a zone of inhibition between 19.75 ± 1.65 to 30.91±1.00 mm while no inhibitory effect could be observed for DMSO used as negative control. But the maximum antibacterial activity (23.37 ± 0.12 mm *Zone of inhibition*) of extract at 400 mg/ml was exhibited against *Escherichia coli.*

The results of MIC and MBC of 6 bacteria strain are shown in table 2. The minimum inhibitory concentration (MIC) of the hydro-methanolic extract of *Emblica officinalis* leaves for different organisms ranged between 12.50 and 50 mg/ml. Also the MIC of gatifloxcin control ranged between 1 and 2 µg/ml (table 2). The minimum bactericidal activity (MBC) of the extract for different bacteria ranged between 200 to 300 mg/ml (table 2). This may be attributed to the presence of soluble phenolic, flavonoids and terpenoids compounds[23]. The result of this study showed that the hydro-methanolic extract of *Emblica officinalis* leaves exhibited varied range of antimicrobial activity against the tested organism including gram positive and gram negative bacteria, which is comparable to standard antibiotic effect.

The results of antimicrobial activity of crude extract at various doses were compared with the positive control (Standard drugs) for evaluating their relative percentage inhibition (table 3). The 400 mg extract exhibits maximum relative percentage inhibition against *E. coli* (70.53%), followed by *Klebsiella pneumonia (*64.18 *%), Staphylococcus aureus* (50.48%), *Bacillus subtilis* (42.57%), *Pseudomonas aeruginosa* (39.83%) and *Salmonella typhi* (33.58%) respectively.

Table 2: MIC and MBC (mg /ml) of *Emblica officinalis* leaves extracts

Tests	Concentration	***Staphylococcus aureus***	***Bacillus subtilis***	***Salmonella typhi***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Klebsiella pneumonia***
MIC	HMLEO mg /mL	50	25	25	50	12.50	12.50
	Gatifloxcin µg/ml	2	1	1	2	1	1
MBC	HMLEO mg /mL	300	200	200	300	200	200

Table 3: Relative Percentage inhibition (RPI) of *Emblica officinalis* leaves extracts (400 mg/ml)

Test organisms	Relative percentage inhibition (%)
Staphylococcus aureus	50.48
Bacillus subtilis	42.57
Salmonella typhi	33.58
Escherichia coli	70.53
Pseudomonas aeruginosa	39.83
Klebsiella pneumonia	64.18

For RPI, Gatifloxcin is the standard drug

Antioxidant Activity

DPPH Radical Scavenging Activity - A freshly prepared DPPH solution exhibited a deep purple color with a maximum absorption at 517 nm. This purple color disappears when an antioxidant is present in the medium. Fig. 1 illustrates a significant (p < 0.05) decrease in the concentration of DPPH radicals due to the

scavenging ability of *Emblica officinalis* leaves extract. This activity was dose dependent. Maximum scavenging activity (79.35 %) was observed at 100 µg/ml concentration and the IC50 value of *Emblica officinalis* leaves extract and ascorbic acid were found to be 45.38 µg/ml and 40.24 µg/ml respectively (table. 4).

Scavenging of Hydrogen Peroxide - Fig. 2 reveals that a significant (p < 0.05) dose dependent response was found in the hydrogen peroxide scavenging activity in *Emblica officinalis* leaves extract. Maximum scavenging activity (84.15%) was observed at 100 µg/ml concentration and the IC50 value of *Emblica officinalis* leaves extract and ascorbic acid were found to be 42.87 µg/ml and 34.51 µg/ml respectively (table. 4).

Table 4: Antioxidant activity of hydro-methanolic extract of *Emblica officinalis* leaves

Concentration (µg/ml)	DPPH Radical Scavenging Activity (% inhibition)		Hydrogen Peroxide Scavenging Activity (% inhibition)	
	Ascorbic Acid	HMLEO	Ascorbic Acid	HMLEO
10	29.43	25.13	26.22	21.12
20	39.78	35.62	38.73	32.25
40	49.09	47.28	53.71	45.36
60	63.46	59.65	68.34	60.71
80	76.3	70.59	82.75	72.54
100	85.93	79.35	97.23	84.15
IC_{50}	40.24 µg/ml	45.38 µg/ml	34.51 µg/ml	42.87 µg/ml

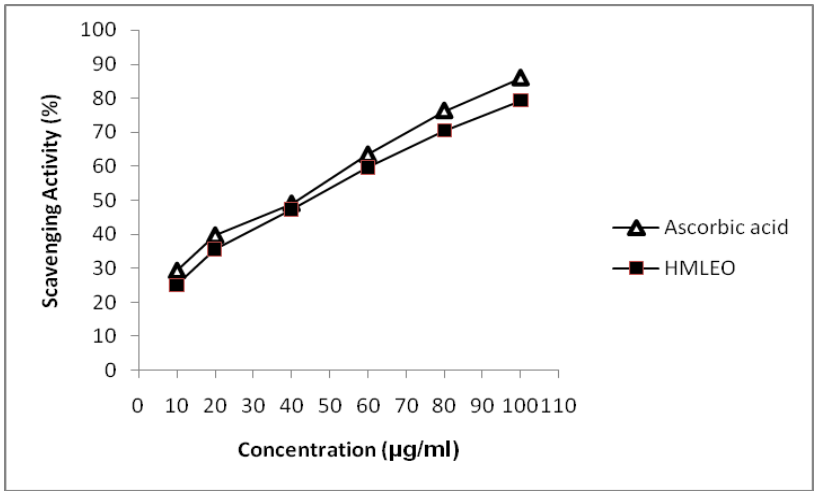

Fig. 1: DPPH radical scavenging activity of *Emblica officinalis* leaves extract

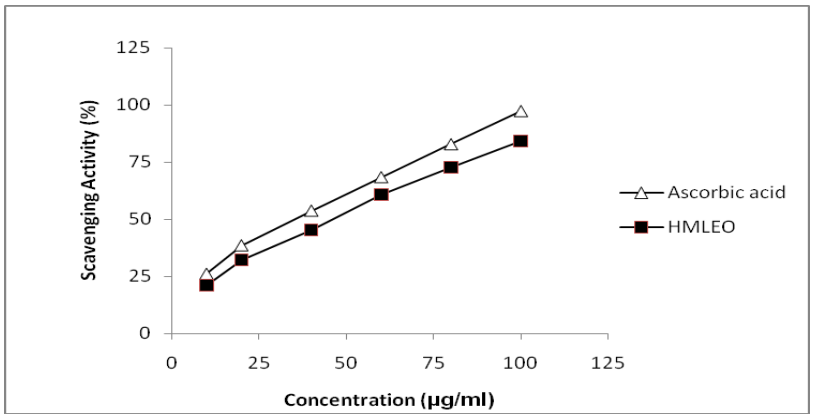

Fig. 2: Hydrogen peroxide radical scavenging activity of *Emblica officinalis* leaves extract.

Total Reductive Potential - The total reducing power measurements of *Emblica officinalis* leaves extracts are shown in table 5. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of different concentration. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom[24-25]. The hydro-methanolic extract at dose 100 µg/ml had maximum reductive potential as measured by ferric ion reduction.

Table 5: Absorbance of standard and HMLEO at various concentrations (µg/ml) in ferric reducing power determination model

Concentration (µg/ml)	Absorbance	
	Standard	HMLEO
10	0.065±0.003	0.049±0.003
20	0.088±0.002	0.071±0.002
40	0.117±0.003	0.098±0.002
60	0.150±0.003	0.131±0.003
80	0.189±0.002	0.167±0.002
100	0.230±0.002	0.201±0.001

*Each Value represents Mean ± SEM (n=3)

CONCLUSIONS

The antimicrobial activity of various plants has been reported by many researchers[26]. As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids, tannins, saponins and tri-terpenoids are producing a better opportunity for testing wide range of microorganism. The results obtained from this work revealed that the plants contained bioactive agents which are connected with antimicrobial properties in plants.

Antioxidants protect cells against damage caused by molecules known as free radicals the antioxidant effects of plant extracts are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes[27]. Phenolic are the largest group of phytochemicals and have been touted as accounting for most of the antioxidant activity of plants or plant products. The present work also reveals that the extract from the leaves of *Emblica officinalis* possesses good antioxidant potential presumably because of its phytochemical constituents. The DPPH scavenging activities of *Emblica officinalis* leaves extract showed a good correlation with its reductive potentials.

Based on the result of this study it can be said that *Emblica officinalis* leaves is an effective antimicrobial and antioxidant agent that can be used for folk medicine and will be a good source to treat and control many diseases. These findings could also be of commercial interest to both pharmaceutical companies and research institutes in the production of new drugs.

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REFERENCES

1. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol. 2005; 29: 41-47.
2. Owolabi J, Omogbai EKI, Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigelia africana (Bignoniaceae) stem bark. Afr. J. Biotechnol. 2007; 6 (14): 882-885.
3. Gislene GF, Nascimento, Juliana Locatelli, Paulo C. Freitas, Giuliana Silva, Antibacterial activity of Plant extracts and Phytochemicals on Antibiotic Resistant bacteria. Brazilian Journal of Microbiology. 2000; 31: 247-256.
4. Tachakittirungrod, Siriporn O, Sombat C. Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. Food Chemistry. 2007; 103: 381–388.
5. Shahidi F. Natural antioxidants. An overview: Natural antioxidants, chemistry, health effects and applications. Champaign, IL.1997; AOCS Press: pp. 1–11.
6. Valentao P, Fernandes E, Carvalho F, Andrade PB, Seabra RM, Bastos M. Antioxidative properties of cardoon (Cynara cardunculus L.) infusion against superoxide radical, hydroxyl radical and hypochlorous acid. Journal of Agricultural and Food Chemistry, 2002; 50: 4989–4993.
7. Treadway L. Amla: Traditional food and medicine. The Journal of the American Botanical Council. 1994; 31: 26-42.
8. Nadkarni KM, Nadkarni AK. Indian Materia Medica - with Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home remedies, vol.1. Popular Prakashan Private Ltd., Bombay, India. 1999.
9. Jayaweera DMA. Medicinal Plants used in Ceylon. National Science Council of Sri Lanka. Colombo. 1980 (Part 2)
10. Burkill IH. A Dictionary of the Economic Products of the Malay Peninsula, vol. 1 Ministry of Agriculture and Co-operatives, Kuala Lump. 1966.
11. Drury CH. The useful plants of India; with notices of their chief medicinal value in commerce, medicine and the arts. Higginbotham and Co. Madras. 1873.
12. Anthony CD, Dweck FF, Dweck DDM. Emblica officinalis [Phyllanthus Emblica] or Amla: the Ayurvedic wonder. Chesham Chemicals Ltd . [Cited from : http://www. dweckdata.com/publishedpapers/emblica_officinalis.pdf]
13. Okerulu IO, Ani CJ. The Phytochemical Analysis and Antibacterial screening of extracts of Tetracarpidium Conophorum. J. Chem. Soc. Nig. 2001; 26(1): 223-228.
14. Aneja KR, Experiments in Microbiology, Plant Pathology and Biotechnology, 4th edn. New Age International Ltd., New Delhi, India. 2003; 196-197.
15. National committee for clinical laboratory standards, 5th edn. Methods for dilution, antimicrobial susceptibility tests for bacteria that grow aerobically, 2000.
16. Atata R, Sani A, Ajewole SM. Effect of stem back extracts of *Enantia chloranta* on some clinical isolates. Biokemistri. 2003; 15 (2): 84-92.
17. Gaurav K, Loganathan K, Kokati VBR. Antibacterial Activity of Aqueous Extract of *Calotropis Gigantea* Leaves – An *In Vitro* Study. International Journal of Pharmaceutical Sciences Review and Research. 2010; 4(2): 141-144.
18. Rios JL, Recio MC, Vilar A. Screening methods for natural products with antimicrobial activity: A review of literature. Journal of Ethnopharmacology. 1988; 23: 127-149.
19. Motamedi H, Safary A, Maleki S, Seyyednejad SM. *Ziziphus spina-christi*, A native plant from Khuzestan, Iran, as a potential source for discovery new antimicrobial agents. Asian J. Plant Sci. 2009; 8: 187-190.
20. Nickavar B, Kamalinejad M, Haj-Yahya M and Shafaghi B. Comparison of the free radical scavenging activity of six Iranian Achillea species. Pharm. Biol. 2006; 44: 208-212.
21. Kumaran A, Karunakaran JR. In-vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. Food Science and Technology. 2007; 40 (2), 344- 352.
22. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn. J. Nutr. 1986; 44: 307-310.
23. Kowalski R, Kedzia B. Antibacterial activity of *Silphium perfoliatum* extracts. Pharm.
24. Biol. 2007; 45: 495-500
25. Meir S, Kanner J, Akiri B, Hadas SP. Determination and Involvement of Aqueous Reducing Compounds in Oxidative Defense Systems of Various Senescing Leaves. J. Agr. Food Chem. 1995; 43: 1813-1820.
26. Schimada VL, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry. 1992; 40: 945-948.

27. Dewanjee S, Maiti A, Majumder R, Majumder A. Evaluation of antimicrobial activity of hydroalcohalic extract of *Schma wallichii* bark. Pharmcolologyonline. 2008; 1: 523-528.

28. Polterait O. Antioxidants and free-radical scavengers of Natural Origin. Current Org. Chem. 1997; 1: 415-440.