ORIGINAL ARTICLE

Evaluation of antibacterial and antioxidant properties of some traditional medicinal plants from India

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Background: Medicinal plants have been used to prevent and treat various health problems. **Aim:** The present study was conducted to evaluate the antibacterial and antioxidant activities of aqueous and solvent extracts of some selected medicinal plants. **Materials and Methods:** The disc diffusion method was employed for the determination of antimicrobial activity, and antioxidant activity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl radical scavenging, hydrogen peroxide reducing and β-carotene/linoleic acid bleaching inhibition assays. Folin-Ciocalteu reagent method was employed for the determination of total phenolic contents. **Results:** Aqueous and solvent extracts of *Acacia catechu*, *A. ferruginea*, *Adenanthera pavonina*, *Albizia odoratissima*, *Anogeissus latifolia*, *Breynia vitis-idaea*, *Salacia oblonga*, *Senna spectabilis* and *Solanum indicum* showed significant antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus faecalis,* and promising antioxidant properties. The antioxidant activities were positively correlated with total phenolic contents. **Discussion and Conclusion:** The promising antibacterial and antioxidant activities of these plants validated their traditional use in various herbal preparations to treat various ailments associated with pathogenic microbes and oxidative stress. Further investigations such as isolation of active principles and toxicological studies to ascertain the safety, and *in vivo* experimentations on suitable models are required to explore the therapeutic usage of these plants on humans.

Key words: Antimicrobial activity, antioxidant activity, medicinal plants, total phenolic content

INTRODUCTION

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day.[1] In recent years, drug resistant human pathogenic bacteria have been commonly reported from all over the world.[2-4] According to centers for disease control and prevention statement, more than 2 million people get antibiotic-resistant infections every year.[5] These drug resistant bacteria have further complicated the treatment of infectious diseases in immuno-compromised persons particularly AIDS and cancer patients.[6-8] As a result, society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to many and in some case all, effective antibiotics.[9]

The free radicals such as singlet oxygen ($^1O_2$), superoxide anion (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) are highly reactive unstable molecules, which are generated naturally as unwanted products during oxidation-reduction reaction in the human body.[10] The overproduction of these reactive oxygen species causes many oxidative damage associated degenerative diseases such as atherosclerosis, coronary heart diseases, cancer, diabetes mellitus, arthritis, inflammation and neurodegenerative diseases.[11-13] Synthetic antioxidants play an important role in prevention or delaying the onset of major oxidative stress related diseases.[14,15] However, the setback and limitation with many of the synthetic antioxidants are that they cause innumerable side-effects in human.[16] Hence, there is an increasing interest in finding natural antioxidants.[17]

Antimicrobial and antioxidant properties of higher plants are being reported from all over the world.[18,19] Approximately, 80% of the world population relies on traditional medicine for their primary health care.[20] Even though, the WHO is encouraging, promoting, and facilitating the effective use of herbal medicine, only a small percentage (5–15%) of the estimated 400,000–500,000 plant species have been scientifically and systematically evaluated for their pharmacological activities. Considering the vast potentiality of plants as a source of new pharmacological agents, a detailed investigation was conducted to test the antibacterial and

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antioxidant efficacies of some selected Indian medicinal plants with the ultimate aim to develop plant based drugs for the management of diseases caused by pathogenic bacteria and free radicals.

MATERIALS AND METHODS

Chemicals and Culture Media

The Mueller-Hinton agar (MHA), dimethyl sulfoxide (DMSO), β-carotene, H_2O_2, linoleic acid, butylated hydroxytoluene (BHT), ascorbic acid, all solvents and synthetic antibiotics were purchased from Hi-media, Mumbai (India). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma, Germany. All chemicals and solvents used were of analytical grade.

Plant Materials

Fresh disease free leaves of 42 different plants [Table 1], with reported medicinal property in traditional literature and generally available in the southern part of Karnataka (India) were selected for the study. The plant samples were identified by Dr. Seetharam, Professor, Department of Biological Sciences, Bangalore University, Bangalore (India). The authenticated voucher specimens of these plants were deposited in the Department of Microbiology and Biotechnology, Bangalore University, Bangalore along with proper voucher numbers (Voucher numbers: BUB/MB-BT/DCM/JU10/01 to BUB/MB-BT/DCM/JU10/42). The collected fresh plant materials were washed separately with tap water (2–3 times) and once with sterile distilled water, shade dried, powdered and used for extraction.

Preparation of Aqueous Extract

Fifty grams each of powdered plant material was macerated separately with 250 mL of sterile distilled water. The macerate was first filtered through double-layered muslin cloth and then centrifuged at 4000 g for 30 min. The supernatant was filtered through Whatman No. 1 filter paper and the obtained extracts were considered as mother extracts (100% concentration) and subjected to evaluation of antibacterial and antioxidant properties, and estimation of total phenolic contents.[21]

Preparation of Solvent Extracts

The plants *viz.*, *Acacia catechu*, *A. ferruginea*, *A. pavonina*, *A. odoratissima*, *A. latifolia*, *B. vitis-idaea*, *S. oblonga*, *S. spectabilis* and *S. indicum* showed significant antibacterial activity in aqueous extract, were selected for successive solvent extraction following the procedure of Amoo *et al.*[22] Briefly, 50 g powder of each plant material was filled in the thimble and extracted successively with 200 mL of petroleum ether, toluene, chloroform, methanol and ethanol using a Soxhlet extractor until colourless extract was obtained on

Table 1: Antibacterial activity of aqueous extract of some traditional plant species against *E. coli* and *S. aureus*

Name of the plants	Family	Antibacterial activity (ZOI in mm)	
		S. aureus	*E. coli*
Acacia catechu (L.f.) Willd.	Fabaceae	12.1±0.6	07.2±0.6
Acacia chundra (Rottler) Willd.	Fabaceae	0.0±0.0	0.0±0.0
Acacia ferruginea DC.	Mimosaceae	08.5±0.8	06.0±0.6
Adenanthera pavonina L.	Mimosaceae	08.2±0.7	06.5±0.6
Albizia odoratissima (L.f.) Benth.	Fabaceae	09.5±0.7	06.5±0.5
Anogeissus latifolia (*Roxb. ex DC.*) *Wall. ex Bedd.*	Combretaceae	09.5±0.7	06.5±0.8
Abrus precatorius L.	Fabaceae	0.0±0.0	0.0±0.0
Argemone mexicana L.	Papaveraceae	0.0±0.0	0.0±0.0
Artabotrys odoratissimus Blume	Annonaceae	0.0±0.0	0.0±0.0
Asparagus racemosus Willd.	Liliaceae	0.0±0.0	0.0±0.0
Bauhinia acuminata L.	Caesalpiniaceae	06.7±0.4	0.0±0.0
Breynia vitis-idaea (Burm.f.) C.E.C.Fisch.	Phyllanthaceae	08.5±0.7	06.5±0.5
Calotropis gigantea (L.) Dryand.	Apocyanceae	0.0±0.0	0.0±0.0
Carissa carandas L.	Apocyanceae	0.0±0.0	0.0±0.0
Cassia alata L.	Fabaceae	07.5±0.4	0.0±0.0
Cassia siamea Lam.	Fabaceae	0.0±0.0	0.0±0.0
Cassia tora L.	Fabaceae	07.8±0.5	0.0±0.0
Coleus amboinicus Lour.	Lamiaceae	06.5±0.5	0.0±0.0
Couroupita guianensis Aubl.	Lecythidaceae	0.0±0.0	0.0±0.0
Delonix regia (Hook.) Raf.	Fabaceae	0.0±0.0	0.0±0.0
Dodonaea viscosa Jacq.	Sapindaceae	07.5±0.7	06.2±0.5
Ficus benghalensis L.	Moraceae	0.0±0.0	0.0±0.0
Ficus religiosa L.	Moraceae	0.0±0.0	0.0±0.0
Gliricidia sepium (Jacq.) Walp.	Fabaceae	07.6±0.8	0.0±0.0
Holoptelea integrifolia Planch.	Ulmaceae	06.5±0.5	0.0±0.0
Lagerstroemia speciosa (L.) Pers.	Lythraceae	07.8±0.6	0.0±0.0
Millingtonia hortensis L.f.	Bignoniaceae	0.0±0.0	0.0±0.0
Phyllanthus amarus Sch. and Thonn.	Phyllanthaceae	06.5±0.8	0.0±0.0
Peltophorum pterocarpum K .Heyne	Fabaceae	0.0±0.0	0.0±0.0
Ricinus communis L.	Euphorbiaceae	06.5±0.4	0.0±0.0
Saccharum spontaneum L.	Poaceae	0.0±0.0	0.0±0.0
Salacia oblonga Wall.	Celastraceae	08.0±0.5	06.1±0.6
Senna spectabilis (DC.) H.S.Irwin and Barneby	Fabaceae	12.5±0.8	07.5±0.7
Sesbania grandiflora (L.) Pers.	Fabaceae	06.5±0.4	0.0±0.0
Solanum indicum L.	Solanaceae	08.2±0.6	06.5±0.5
Spathodea campanulata P. Beauv.	Bignoniaceae	0.0±0.0	0.0±0.0
Spilanthes paniculata Wall. ex DC.	Asteraceae	06.1±0.4	0.0±0.0
Tabebuia aurea Benth. and Hook. f. ex S. Moore	Bignoniaceae	0.0±0.0	0.0±0.0
Thespesia populnea (L.) Sol. ex Correa	Malvaceae	07.0±0.6	0.0±0.0
Tylophora indica (Burm. f.) Merr.	Asclepiadaceae	06.2±0.4	0.0±0.0
Vitex negundo L.	Lamiaceae	0.0±0.0	0.0±0.0
Ziziphus mucronata Willd.	Rhamnaceae	06.8±0.4	0.0±0.0
F	-	86.19	104.50

Data given are the mean of four replicates±standard error, ANOVA df 41 at $P<0.001$. The antibacterial activity was evaluated at 50 µg/well concentration. No activity was observed in DMSO impregnated disc. ZOI – Zone of inhibition; *E. coli* – *Escherichia coli*; *S. aureus* – *Staphylococcus aureus*; ANOVA – Analysis of variance; DMSO – Dimethyl sulfoxide

the top of the extractor. Each of the solvent extracts was concentrated separately under reduced pressure using rotary flash evaporator (Superfit, R/150/22, India). The dried solvent extracts were weighed, re-suspended in DMSO and subjected to evaluation of antibacterial and antioxidant and estimation of total phenolic contents.[21]

Evaluation of Antibacterial Activity

Test Bacteria

Seven human pathogenic bacteria *viz.*, *Escherichia coli* (National Centre of Industrial Microorganisms [NCIM] 2065), *Klebsiella pneumoniae* (NCIM 2957), *Proteus vulgaris* (NCIM 2027), *Pseudomonas aeruginosa* (NCIM 5031), *Salmonella typhi* (NCIM 2501), *Staphylococcus aureus* (NCIM 2079) and *Streptococcus faecalis* (NCIM 5025) were procured from NCIM, National Chemical Laboratory, Pune (India), and subcultured on nutrient agar and stored at 4°C. Twenty four hours old cultures were used as test bacteria for antibacterial activity assay.

Antibacterial Activity of Aqueous Extracts

Antibacterial activity of aqueous extracts of all 42 plants were determined by cup diffusion method on the MHA medium following the procedure of National Committee for Clinical Laboratory Standards (NCCLS).[23] Briefly, five wells of 5 mm diameter each were made on MHA plate, and 100 µL of bacterial inoculum (10^8 CFU/mL) of *E. coli* and *S. aureus* were spread on the MHA plates separately using sterile moistened swab. Then, 50 mL of aqueous extract (20 mg/mL) of all 42 plants were placed in the wells separately and incubated at 37°C for 24 h. The same amount of sterile distilled water served as a control and the synthetic antibiotics *viz.*, augmentin (30 µg/disc), bacitracin (10 U/disc), cephotaxime (30 µg/disc), chloramphenicol (30 µg/disc), co-trimoxazole (25 µg/disc), erythromycin (10 µg/disc), gentamycin (10 µg/disc), neomycin (30 µg/disc), oflaxacin (5 µg/disc), penicillin-G (10 µg/disc) and polymyxin B (300 µg/disc) served as positive control. The diameter of the zone of inhibition (ZOI) around the wells was measured in millimeter (mm). For each treatment, four replicates were maintained. The plants which showed promising antibacterial activity against *S. aureus* with ZOI > 8.0 mm and *E. coli* with ZOI > 6.5 mm were selected for evaluation of antibacterial activity against remaining test bacteria as mentioned above, and further subjected to successive solvent extraction.

Antibacterial Activity of Solvent Extracts

The successive solvent extracts of activity guided nine plants [Table 2] were subjected to antibacterial activity assay by disc diffusion method following the procedure of NCCLS.[23] Briefly, four layers of 6 mm sterilized filter paper discs (Whatman No. 1) were individually impregnated with 20 µL (50 mg/mL concentration) of each solvent extract and allowed to air dry. The MHA medium was prepared and inoculated with 100 µL of bacterial inoculum (10^8 CFU/mL) of seven human pathogenic bacteria, separately. The discs impregnated with extracts were placed on the surface of preinoculated MHA medium and kept at 4°C for 1 h for diffusion of extracts, thereafter the plates were incubated at 37°C for 24 h. The disc devoid of extract and presence of DMSO served as control. In each treatment four, replicates were maintained. After incubation, the diameter of ZOI around the disc was measured in millimetre. The toluene extract of *A. pavonina*, chloroform extracts of *B. vitis-idaea*, *S. spectabilis* and *S. indicum*, and methanol extracts of *A. catechu*, *A. ferruginea*, *A. odoratissima*, *A. latifolia* and *S. oblonga* showed highest antibacterial activity against one or more organisms, were selected for evaluation of total phenolic contents and antioxidant activities.

Determination of Total Phenolic Contents

The aqueous and antibacterial activity guided solvent extracts of all nine plants were subjected to estimation of

Table 2: Antibacterial activity of activity guided solvent extracts of some selected plants against some human pathogenic bacteria

Plants name	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*P. aeruginosa*	*S. typhi*	*S. aureus*	*S. faecalis*
A. catechu (M)	10.1±0.7^{c}	10.6±0.6^{c,d}	07.0±0.7^{a,b}	06.8±0.5^{a}	10.2±0.6^{c}	12.6±0.6^{d,e}	15.1±0.3^{e,f}
A. ferruginea (M)	06.5±0.7^{a}	07.0±0.8^{b,c}	06.8±0.7^{b}	06.5±0.4^{a}	09.8±0.5^{d}	10.5±0.6^{d,e}	11.3±0.4^{f}
A. pavonina (T)	10.5±0.8^{c}	11.4±0.4^{d}	07.8±0.3^{b}	06.5±0.6^{a}	07.4±0.7^{b}	11.5±0.5^{d}	12.6±0.5^{e}
A. odoratissima (M)	07.2±0.5^{b}	07.7±0.5^{c}	07.0±0.6^{a,b}	06.8±0.8^{a}	07.5±0.4^{b,c}	10.3±0.5^{d}	12.8±0.7^{e}
A. latifolia (M)	08.6±0.8^{b,c,d}	09.0±0.3^{c,d}	07.9±0.7^{b}	07.0±0.5^{a}	09.1±0.6^{c,d}	10.2±0.7^{d,e}	12.9±0.8^{f}
B. vitis-idaea (C)	07.6±0.4^{c}	07.5±0.5^{c}	06.8±0.4^{b}	0.00±0.0^{a}	07.5±0.7^{c}	10.6±0.7^{d}	10.9±0.5^{d,e}
S. oblonga (M)	06.5±0.7^{b}	07.4±0.7^{b,c}	06.3±0.3^{b}	0.00±0.0^{a}	08.3±0.5^{c}	09.8±0.6^{d}	10.6±0.7^{e}
S. spectabilis (C)	07.8±0.7^{b}	09.2±0.8^{c}	07.0±0.5^{a}	06.7±0.4^{a}	10.5±0.4^{d}	12.7±0.6^{e}	18.9±0.7^{f}
S. indicum (C)	06.5±0.5^{b}	06.6±0.4^{b}	0.00±0.0^{a}	0.00±0.0^{a}	07.5±0.6^{c}	08.5±0.5^{d}	12.0±0.4^{e}
F	19.75	18.65	44.52	70.75	28.28	20.86	70.55

Data given are the mean of four replicates±standard error. The antibacterial activity was evaluated at 1 mg/disc concentration. C – Chloroform extract, M – Methanol extract, T – Toluene extract. ANOVA df 8 at $P<0.001$. The values followed by different superscript letters differ significantly when subjected to Tukey's HSD analysis at 0.5 subset, no activity was observed in DMSO impregnated control plate. ANOVA – Analysis of variance; HSD – Honestly significant difference; *E. coli – Escherichia coli*; *K. pneumoniae – Klebsiella pneumoniae*; *P. vulgaris – Proteus vulgaris*; *P. aeruginosa – Pseudomonas aeruginosa*; *S. typhi – Salmonella typhi*; *S. aureus – Staphylococcus aureus*; *S. faecalis – Streptococcus faecalis*; *A. catechu – Acacia catechu*; *A. ferruginea – Acacia ferruginea*; *A. pavonina – Adenanthera pavonina*; *A. odoratissima – Albizia odoratissima*; *A. latifolia – Anogeissus latifolia*; *B. vitis-idaea – Breynia vitis-idaea*; *S. oblonga – Salacia oblonga*; *S. spectabilis – Senna spectabilis*; *S. indicum – Solanum indicum*; DMSO – Dimethyl sulfoxide, ^{a,b,c,d,e,f}(Superscript letters) represent the least significant difference when subjected to Tukey's HSD analysis (row by row)

total phenolic contents by Folin-Ciocalteu (FC) reagent method following the procedure of Li *et al.*[24] with some modifications. Briefly, 100 µL of mother aqueous extracts (20 mg/mL) and 1 mL of solvent extracts (1 mg/mL in methanol) were separately added to 0.1 mL of FC reagent and incubated for 5 min at room temperature. After incubation, 2 mL of 15% sodium carbonate was added and made up to 10 mL by adding distilled water, then incubated at room temperature for 90 min in dark condition. Absorbance was measured at 750 nm using the double beam ultraviolet-visible (UV-VIS) spectrophotometer (Shimadzu, UV-1800, USA). A standard calibration curve was plotted using the gallic acid (0.0–1.0 mg/mL). Total phenolic content (TPC) was expressed as gallic acid equivalent (GAE) (mg of gallic acid/mg of dry weight of extract) based on the calibration curve.

Evaluation of Antioxidant Activity

2, 2-diphenyl-1-picrylhydrazyl Radical Scavenging Assay

The DPPH radical scavenging activity of aqueous and antibacterial activity guided solvent extracts of nine plants were evaluated following the procedure of Ebrahimabadi *et al.*[13] with slight modifications. Desired concentrations of aqueous and solvent extracts were mixed separately with 3 mL of freshly prepared DPPH solution (40 mg/L in methanol) and incubated for 30 min in the dark at room temperature. After incubation, absorbance of the solutions was recorded using UV-VIS spectrophotometer at 517 nm. The same concentration of ascorbic acid and BHT were used as positive controls, and methanol solution of DPPH served as a negative control. The same amount of plants extracts without DPPH served as blank for corresponding plant extracts. Percent inhibition of DPPH radical was calculated using the following formula:

$$I\% = \frac{(A_{Control} - A_{Sample})}{(A_{Control})} \times 100$$

Where, $A_{control}$ is the absorbance of the control reaction, and A_{sample} is the absorbance of the test sample.

β-carotene/Linoleic Acid Bleaching Inhibition Assay

The β-carotene/linoleic acid bleaching inhibition potency of aqueous and antibacterial activity guided solvent extracts of nine plants were determined following the procedure of Ebrahimabadi *et al.*[13] Briefly, desired concentrations of aqueous and solvent extracts were added to 2.5 mL of β-carotene-linoleic acid emulsion mixture separately, mixed thoroughly and incubated at 50°C for 2 h in the water bath. After incubation, the absorbance was measured at 470 nm using UV-VIS spectrophotometer. The corresponding solvent served as a negative control, and BHT and ascorbic acid were used as positive controls. The same amount of plant extracts without β-carotene-linoleic acid emulsion served as blank for corresponding plant extracts. The antioxidant activity (inhibition percentage, *I*%) was calculated following the formula:

$$I\% = \frac{A_{\beta-carotene\ after\ 2h}}{A_{initial\ \beta-carotene}} \times 100$$

where, $A_{\beta\text{-carotene after 2 h}}$ is the absorbance of β-carotene after 2 h and $A_{initial\ \beta\text{-carotene}}$ is the absorbance of β-carotene at the beginning.

Hydrogen Peroxide Reducing Power Assay

Hydrogen peroxide reducing power of aqueous and antibacterial activity guided solvent extracts of nine plants were analyzed following the procedure of Khan *et al.*[25] with some modifications. Two millilitres of 10 mM H_2O_2 (pH 7.4) was mixed with desired concentrations of aqueous and solvent extracts, separately. The absorbance was measured at 230 nm at intervals of 0 min and after 10 min incubation. One milliliter of 50 mM phosphate buffer without H_2O_2 served as blank. BHT and ascorbic acid were used as a positive control, and same reaction mixture without plant extracts served as a negative control. The same amount of plants extracts without H_2O_2 served as blank for corresponding plant extracts. The percent H_2O_2 reducing ability was calculated by the following formula:

$$\%H_2O_2\ scavenging = \frac{(A_{Control} - A_{Sample})}{(A_{Control})} \times 100$$

where, $A_{control}$ is the absorbance of control set at 230 nm and $A_{treated}$ is the absorbance of treated set at 230 nm.

Statistical Analysis

Values were expressed as mean ± standard error. Analysis of variance was performed, and the differences between values were tested for significance by Tukey's multiple comparison tests employing the SPSS 20 IBM Corporation (IBM, USA) program. Differences at $P \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The antibacterial activity of aqueous extracts of 42 plants was evaluated against Gram-positive *S. aureus* and Gram-negative *E. coli* by cup diffusion method. The diameter of ZOI around the well was measured in millimeter, and the obtained results are presented in Table 1. Among the extracts tested, aqueous extract of 24 plants *viz.*, *A. catechu*, *A. ferruginea*, *A. pavonina*, *A. odoratissima*, *A. latifolia*, *B. acuminata*, *B. vitis-idaea*, *C. amboinicus*, *D. viscosa*, *G. sepium*, *H. integrifolia*, *L. speciosa*, *P. amarus*, *R. communis*, *S. oblonga*, *S. alata*, *S. tora*, *S. spectabilis*, *S. grandiflora*, *S. indicum*, *S. paniculata*, *T. populnea*, *T. indica* and *Z. mucronata* showed antibacterial activity against *S. aureus* with ZOI that ranged from 6.1 to 12.5 mm depending upon plant species screened

at 50 µL concentration. Whereas, 11 plants *viz.*, *A. catechu*, *A. ferruginea*, *A. pavonina*, *A. odoratissima*, *A. latifolia*, *B. vitis-idaea*, *D. viscosa*, *S. oblonga*, *S. spectabilis*, *S. indicum* and *Z. mucronata* showed antibacterial activities against *E. coli* with ZOI that ranged from 6.0 to 7.5 mm depending upon plant species tested at 50 µL concentration. Among which, nine plants *viz.*, *A. catechu*, *A. ferruginea*, *A. pavonina*, *A. odoratissima*, *A. latifolia*, *B. vitis-idaea*, *S. oblonga*, *S. spectabilis* and *S. indicum*, showed promising antibacterial activities against both *S. aureus* and *E. coli* with ZOI that ranged from 8.0 to 12.5 mm and 6.1 to7.5 mm, respectively, which were selected for further antibacterial evaluation against remaining bacteria. The antibacterial activities of aqueous extracts of nine plants against seven human pathogenic bacteria were evaluated and the obtained results are presented in Table 3. Results indicate that, the aqueous extracts of *A. catechu* and *S. spectabilis* showed highest antibacterial activity against all the tested bacteria with ZOI that ranged from 6.0 to 18.0 mm. Whereas aqueous extracts of *A. ferruginea*, *A. pavonina*, *A. odoratissima*, *A. latifolia*, *B. vitis-idaea*, *S. oblonga* and *S. indicum* showed antibacterial activity against one or more bacteria tested, with ZOI that ranged from 0.0 to 11.5 mm. Among the bacteria tested, *S. faecalis* was highly susceptible bacteria followed by *S. aureus*, whereas *P. vulgaris* was resistant organism.

The antibacterial activity of successive solvent extracts of nine plants was evaluated against seven human pathogenic bacteria. Among the five successive solvent extracts tested, toluene extract of *A. pavonina*, chloroform extract of *B. vitis-idaea*, *S. spectabilis* and *S. indicum*, and methanol extract of *A. catechu*, *A. ferruginea*, *A. odoratissima*, *A. latifolia* and *S. oblonga* showed highest antibacterial activity with diameter of ZOI ranged from 0.0 to 18.9 mm at 1 mg/disc concentration [Table 2]. Tukey's multiple comparisons tests revealed that the methanol extract of *A. catechu* showed the highest activity, whereas least activity was observed in *S. oblonga*. Among the bacteria tested, *S. faecalis* was most susceptible bacteria followed by *S. aureus*, whereas *P. vulgaris* and *P. aeruginosa* were found to be most resistant bacteria. The present study clearly indicates that Gram-positive bacteria were more susceptible than Gram-negative bacteria.

The antibacterial activities of synthetic antibiotics *viz.*, augmentin, bacitracin, cephotaxime, chloramphenicol, co-trimoxazole, erythromycin, gentamycin, neomycin, oflaxacin, penicillin-G and polymyxin B were evaluated and the obtained results are presented in Table 4. The results revealed that all the bacteria were susceptible to the antibiotics cephotaxime, chloramphenicol, co-trimoxazole, erythromycin, gentamycin, neomycin and oflaxacin with ZOI ranged from 13.1 to 40.0 mm. Whereas, bacitracin did not show antibacterial activity against any of the Gram-negative bacteria tested, but it showed activity against Gram-positive *S. aureus* and *S. faecalis*. Augmentin and penicillin-G was not effective against *K. pneumoniae* and *P. vulgaris*. Polymyxin B was not effective against *P. vulgaris*. These results confirm that *E. coli, K. pneumonia* and *P. vulgaris* were resistant to one or the other antibiotics tested. Even though some of the extracts showed activity against these resistant bacteria, none of the extracts showed comparable activity with synthetic antibiotics tested on a comparative evaluation.

The TPCs of aqueous and antibacterial activity guided solvent extracts of nine plants were quantified spectrophotometrically based on the reduction of FC reagent, and the amount of TPC was expressed in mg of GAE. The TPC ranged between 1.03 and 532.5 mg GAE/100 µL in case of aqueous extracts and 2.38–989.0 mg GAE/g of dry extract in case of activity guided solvent extracts [Table 5]. Among the plants tested, the aqueous extract of *A. odoratissima* showed highest TPC (532.5 mg GAE/100 µL) followed by

Table 3: Antibacterial activity of aqueous extracts of some activity guided selected plants against some human pathogenic bacteria

Plants name	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*P. aeruginosa*	*S. typhi*	*S. aureus*	*S. faecalis*
A. catechu	07.2±0.6^{a,b}	07.5±0.7^{b}	06.5±0.5^{a}	06.0±0.6^{a}	07.4±0.6^{b}	12.1±0.6^{c,d}	14.0±0.5^{d}
A. ferruginea	06.0±0.6^{b}	06.6±0.7^{b,c}	0.0±0.0^{a}	0.0±0.0^{a}	06.0±0.4^{b}	08.5±0.8^{d}	10.2±0.5^{e}
A. pavonina	06.1±0.6^{b}	07.3±0.5^{c}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	07.5±0.7^{c}	09.8±0.6^{d}
A. odoratissima	06.5±0.5^{b}	07.5±0.7^{c}	06.0±0.4^{b}	0.0±0.0^{a}	06.9±0.6^{b,c}	09.5±0.7^{d}	12.5±0.8^{e}
A. latifolia	06.5±0.8^{b}	07.4±0.6^{c}	06.0±0.5^{b}	0.0±0.0^{a}	08.2±0.7^{c,d}	09.5±0.7^{e}	10.5±0.8^{f}
B. vitis-idaea	06.1±0.5^{b}	06.5±0.7^{b}	0.0±0.0^{a}	0.0±0.0^{a}	07.1±0.8^{b,c}	08.5±0.7^{d}	09.8±0.6^{e}
S. oblonga	06.1±0.6^{b}	07.2±0.6^{b,c}	0.0±0.0^{a}	0.0±0.0^{a}	07.5±0.8^{b,c}	08.0±0.5^{d}	09.4±0.6^{e}
S. spectabilis	07.5±0.7^{b}	08.2±0.8^{b,c}	06.8±0.6^{a}	06.4±0.4^{a}	10.2±0.6^{c,d}	12.5±0.8^{d}	18.0±0.5^{e}
S. indicum	06.1±0.5^{b}	06.3±0.7^{b}	0.0±0.0^{a}	0.0±0.0^{a}	06.5±0.7^{b}	08.2±0.6^{c}	10.5±0.5^{d}
F	20.86	20.71	57.24	86.57	26.14	15.65	66.20

Data given are the mean of four replicates±standard error, ANOVA df 8 at $P<0.001$. The values followed by different superscript letters differ significantly when subjected to Tukey's HSD analysis at 0.5 subset; leaves were used as test material; no activity was observed in DMSO impregnated disc. ANOVA – Analysis of variance; *E. coli* – *Escherichia coli*; *K. pneumoniae* – *Klebsiella pneumoniae*; *P. vulgaris* – *Proteus vulgaris*; *P. aeruginosa* – *Pseudomonas aeruginosa*; *S. typhi* – *Salmonella typhi*; *S. aureus* – *Staphylococcus aureus*; *S. faecalis* – *Streptococcus faecalis*; *A. catechu* – *Acacia catechu*; *A. ferruginea* – *Acacia ferruginea*; *A. pavonina* – *Adenanthera pavonina*; *A. odoratissima* – *Albizia odoratissima*; *A. latifolia* – *Anogeissus latifolia*; *B. vitis-idaea* – *Breynia vitis-idaea*; *S. oblonga* – *Salacia oblonga*; *S. spectabilis* – *Senna spectabilis*; *S. indicum* – *Solanum indicum*; DMSO – Dimethyl sulfoxide; HSD – Honestly significant difference, ^{a,b,c,d,e,f}(Superscript letters) represent the least significant difference when subjected to Tukey's HSD analysis (row by row)

A. latifolia (532.5 mg GAE/100 µL) and *A. catechu* (400.23 mg GAE/100 µL), while *S. spectabilis* (1.03 mg GAE/100 µL) showed least TPC in 100 µL of aqueous extract. Among the activity guided solvent extracts, methanol extract of *A. latifolia* showed highest TPC (989.0 GAE/g of dry extract) followed by *A. catechu* (756.8 GAE/g of dry extract) and the least amount of TPC was observed in *S. spectabilis* (2.38 GAE/g of dry extract) at 1 mg/mL concentration.

The antioxidant activity of aqueous and antibacterial activity guided solvent extracts of nine plants was evaluated using DPPH radical scavenging, β-carotene/linoleic acid bleaching inhibition and H_2O_2 assays, and the obtained results are presented in Table 5. The percent DPPH scavenging activity of aqueous extract ranged from 32.3 to 91.1%, whereas in case of activity guided solvent extracts ranged between 45.3 and 91.5% at 1 mg/mL. The percent of β-carotene/linoleic acid bleaching inhibition of aqueous extracts ranged from 64.6 to 89.5% at 100 µL concentration, while the activity guided solvent extracts ranged from 66.3 to 90.3% at 1 mg/mL. In H_2O_2 reducing assay, the aqueous and activity guided solvent extracts of all the plants showed significant activity with percent reduction ranged from 69.2 to 83.1% at 100 µL concentration and 66.5–86.5% at 100 µg/mL, respectively. Among the aqueous extracts tested, *A. catechu* showed highest activity followed by *A. latifolia*, whereas the least activity was observed in *S. spectabilis*. Similarly, in case of active solvent extracts, methanol extract of *A. catechu* showed highest percent inhibition, followed by *A. latifolia* and *A. ferruginea*, while *S. spectabilis* showed least percent inhibition. A significant relationship between the antioxidant capacities and TPCs were found, indicating phenolic compounds are the major contributors for the antioxidant actions of these plants.

Table 4: Antibacterial activity of some synthetic antibiotics against some human pathogenic bacteria

Antibiotics	Test bacteria						
	E. coli	*K. pneumoniae*	*P. vulgaris*	*P. aeruginosa*	*S. typhi*	*S. aureus*	*S. faecalis*
Augmentin	08.0±0.2^{b}	06.1±0.4^{a}	06.3±0.3^{a}	27.8±0.7^{f}	16.0±0.8^{c}	20.0±0.5^{d}	22.6±0.8^{e}
Bacitracin	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}	0.00±0.0^{a}	06.3±0.1^{b}	28.0±0.8^{c}	27.8±0.4^{c}
Cephotaxime	36.8±0.7^{d}	40.0±0.8^{e}	34.5±0.5^{c}	31.1±0.8^{b,c}	25.0±0.5^{a}	30.0±0.2^{b}	36.0±0.7^{d}
Chloramphenicol	29.3±0.8^{c}	30.8±0.4^{c,d}	26.0±0.5^{a,b}	30.0±0.8^{c}	24.5±0.7^{a}	31.0±0.8^{c,d}	34.0±0.5^{e}
Co-trimoxazole	26.0±0.5^{c}	26.3±0.8^{c}	20.5±0.7^{a,b}	28.3±0.6^{d}	26.1±0.7^{c}	28.1±0.4^{d}	18.0±0.2^{a}
Erythromycin	14.1±0.7^{a}	27.5±0.7^{d,e}	15.1±0.4^{a,b}	26.0±0.8^{d}	15.0±0.2^{a,b}	22.0±0.5^{c}	38.3±0.6^{f}
Gentamycin	21.6±0.8^{a}	24.1±0.7^{d}	25.1±0.6^{b}	26.5±0.7^{b,c}	23.6±0.6^{c}	23.8±0.4^{c,d}	37.0±0.2^{e}
Neomycin	19.0±0.5^{c}	21.3±0.6^{d}	17.8±0.7^{b}	19.0±0.8^{c}	16.0±0.5^{a}	18.5±0.7^{b,c}	28.1±0.7^{e}
Oflaxacin	31.5±0.7^{c,d}	30.0±0.8^{c}	29.0±0.5^{b,c}	27.1±0.4^{b}	31.6±0.6^{c,d}	21.0±0.5^{a}	20.5±0.5^{a}
Penicillin-G	06.1±0.4^{b}	0.00±0.0^{a}	06.1±0.2^{b}	15.8±0.7^{d}	12.0±0.5^{c}	31.8±0.7^{f}	17.8±0.4^{d,e}
Polymyxin B	15.0±0.2^{b}	16.0±0.5^{b,c}	0.00±0.0^{a}	20.0±0.8^{d,e}	15.0±0.2^{b}	22.1±0.4^{f}	19.1±0.7^{c,d}
F	344.69	427.90	573.69	149.43	159.14	124.80	181.80

Data given are the mean of four replicates±standard error; ANOVA df 10 at $P<0.001$. The values followed by different superscript letters differ significantly when subjected to Tukey's HSD analysis at 0.5 subset. ANOVA – Analysis of variance; HSD – Honestly significant difference; *E. coli – Escherichia coli; K. pneumoniae – Klebsiella pneumoniae; P. vulgaris – Proteus vulgaris; P. aeruginosa – Pseudomonas aeruginosa; S. typhi – Salmonella typhi; S. aureus – Staphylococcus aureus; S. faecalis – Streptococcus faecalis,* ^{a,b,c,d,e,f}(Superscript letters) represent the least significant difference when subjected to Tukey's HSD analysis (row by row)

Table 5: Total phenolic content and antioxidant activity of aqueous and activity guided solvent extracts of some selected traditional plant species

Plants name	Total phenolic content (mg/GAE/100 µL)		% DPPH scavenging activity (1000 µL)		% H_2O_2 reducing power (100 µL)		β-carotene linoleic acid assay (1 mg/mL)	
	Aqueous extract	Solvent extract	Aqueous extract	Solvent extract	Aqueous extract	Solvent extract	Aqueous extract	Solvent extract
*A. catechu**	400.2±11.5	756.8±14.4	91.1±0.9	91.5±1.1	83.1±0.6	86.5±2.5	89.5±1.1	90.2±1.8
*A. ferruginea**	165.2±6.0	445.4±8.6	86.1±0.9	91.2±1.0	82.4±1.4	86.5±1.4	86.5±1.1	87.5±3.0
*A. pavonina***	58.5±3.7	18.3±0.72	74.4±1.1	59.4±0.8	71.1±1.7	71.3±2.7	76.5±2.4	78.0±1.7
*A. odoratissima**	532.5±7.5	525.5±8.6	89.9±1.0	90.1±1.0	78.3±1.7	66.5±2.5	82.6±2.5	83.5±2.0
*A. latifolia**	400.3±7.5	989.0±9.6	89.8±0.9	90.1±0.8	69.2±1.8	69.2±1.4	88.5±2.4	89.5±1.3
*B. vitis-idaea****	153.0±4.9	305.0±5.1	78.5±0.6	84.0±0.5	71.0±3.7	70.3±2.5	74.6±0.8	75.5±3.1
*S. oblonga**	228.2±8.3	170.6±8.6	89.5±0.8	85.0±0.8	80.8±3.4	81.6±2.7	72.6±1.1	74.6±1.4
*S. spectabilis****	1.0±0.5	2.38±0.61	32.3±1.2	45.3±0.7	78.8±2.3	80.7±2.5	64.6±1.1	66.3±1.2
*S. indicum****	58.7±2.3	47.3±2.02	76.6±0.9	80.5±0.8	72.4±1.7	73.0±1.7	73.5±2.0	74.6±1.5
Ascorbic acid	NA		96.8±0.9		87.5±1.0		92.8±0.7	
BHT	NA		94.3±0.4		88.4±1.3		97.3±0.5	
F	777.39	2189.02	283.35	264.87	5.59	8.89	19.50	12.69

Data given are the mean of four replicates±standard error; ANOVA df 10 at $P<0.001$. Denotes for activity guided solvent extracts: *Methanol extract; **Toluene extract and ***Chloroform extract. GAE – Gallic acid equivalent; DPPH – 2; 2-diphenyl-1-picrylhydrazyl; ANOVA – Analysis of variance; *A. catechu – Acacia catechu; A. ferruginea – Acacia ferruginea; A. pavonina – Adenanthera pavonina; A. odoratissima – Albizia odoratissima; A. latifolia – Anogeissus latifolia; B. vitis-idaea – Breynia vitis-idaea; S. oblonga – Salacia oblonga; S. spectabilis – Senna spectabilis; S. indicum – Solanum indicum*; BHT – Butylated hydroxytoluene; NA – Not available

A perusal of the literature revealed that, among the 42 plants screened only few plants were reported for their antimicrobial and antioxidant potencies.[26-40] Further, no reports are available pertaining to the antibacterial and antioxidant activities of solvent extracts of these plants. To the best of our knowledge, this is the first report describing the antibacterial and antioxidant activities of the tested plants.

The emergence of multi-drug resistant human pathogenic bacteria and undesirable side-effects of currently used antibacterial and antioxidant drugs have been pushing researchers to search for new alternative drugs from natural sources including plants. The present study confirms that the plants *viz.*, *A. catechu*, *A. ferruginea*, *A. pavonina A. odoratissima*, *A. latifolia*, *B. vitis-idaea*, *S. oblonga*, *S. spectabilis* and *S. indicum* showed promising antibacterial and antioxidant activities. The study also validates the traditional usage of these medicinal plants for treatment of various ailments.

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