ORIGINAL ARTICLE

Phytochemical screening, free radical scavenging, antimicrobial activity of ethanolic extract of *Leptadenia pyrotechnica*

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Abstract

Background: *Leptadenia pyrotechnica* (LP) is a well-known traditional shrub used by the tribes of Africa and Asia. Locally, it is called as Khimp and recognized as therapeutic uses as well as animal fodder. **Aims:** The present study investigates the preliminary phytochemical contents, free radical scavenging, and antimicrobial activity against the bacterial and fungal strains of ethanolic extract and its fraction of LP (Decne.). **Materials and Methods:** Ethanolic extract and fractions of LP were used to different phytochemical qualitative screening methods to identify the constituents presented in it. Total flavonoids and total phenolic contents were determined to justify its antimicrobial activity. The antibacterial and antifungal activity of the extracts was measured by disc diffusion method, minimum inhibitory concentration, minimum bactericidal concentration, minimum fungicidal concentration, and zone of inhibition against the bacterial and fungal strains. 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging activity had used to evaluate the *in vitro* antioxidant activity of extract and fractions. **Results:** Preliminary phytochemical screening of extract and fractions showed the presence of alkaloids, terpenoids, phenols, flavonoids, glycosides, saponins, tannins, and steroids. Reducing efficiency revealed dose-dependent inflation in concentration (6.25-200 µg/µL) with respect to quercetin and gallic acid. The presence of phenolic compounds, terpenoids, and flavonoids contribute to potent antimicrobial activity against bacterial and fungal strains. **Conclusion:** The present research work concluded the effectiveness of different extract and fractions at various concentrations against the bacterial and fungal strains. Free radical scavenging activity embarks its contribution as antimicrobial along with the presence of terpenoids, flavonoids, and phenolic residues.

Key words: Antimicrobial, flavonoids, *Leptadenia pyrotechnica*, phenolic, phytochemical

INTRODUCTION

Herbal medicines play a pivotal role in treating various ailments in India, and their phytochemicals were isolated for establishing the mechanistic pathways for curing the diseases.[1] Increasing global trends in medicinal plant research led to the fact that maximum plants have antibacterial and antioxidative property.[2] Their low price, fewer side effects, and easy availability made them famous among clinicians.

Microbial resistance toward antimicrobial agents is increasing day by day due to the inappropriate and regular use of existing antimicrobials. Reported studies indicated that around 70-80% of pathogens found in hospitals were resistant for minimal one antibiotic leading to increased mortality due to nosocomial infections.[3] These conditions lead to discovering a new novel molecule to treat microbial infections. This phenomenon of increasing resistance to antimicrobials, many researchers focused on indispensable phytoconstituents isolated from these medicinal plants. It is pivotal to develop new antimicrobial drugs of herbal origin in the pipeline to substitute the older ones.[4]

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Leptadenia pyrotechnica Forsk. (LP) locally known as kheep or khimp belongs to family Asclepiadaceae traditionally used for different diseases. It is ascending shrub, up to 2.5-m height, deciduous, bushy branches with watery sap. Leaves were rarely found; flower and fruits grow in the month of September to January.[5] It was the native of desert areas such as Arab, Sudan, Iran, Egypt, and India (especially Rajasthan, namely, Bikaner, Palod, Jodhpur, Kota and some part of Western Uttar Pradesh, India).[6] Tribal peoples used its branch infusion as diuretic and antiurolithatic.[7,8] LP fibers were used as antihistaminic and expectorant.[9,10] Some literature also referred its applications in gout and rheumatism.[11] In Bikaner region, it has been considered good for constipation, whereas its infusion mixed with buttermilk is used in uterine prolapsed. Plant sap has been used to treat eczema and other skin microbial infections.

Therefore, the present study evaluates the antimicrobial activity of ethanolic extract and fractions in different bacterial and fungal strains along with a screening of phytochemicals of LP and their free radical scavenging activity.

MATERIALS AND METHODS

Chemicals and Reagents

Folin–Ciocalteu reagent, gallic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and quercetin were purchased from Sigma-Aldrich (GmbH, Sternheim, Germany). Anhydrous sodium carbonate, sodium nitrite solution, sodium hydroxide, and aluminum chloride hexahydrate solution were purchased from SD Fine-Chem Limited, Mumbai. The solvent used in extraction, fractionation, and other analytical procedure was purchased from Merck, Germany.

Plant Material

The raw materials of LP were collected in the month of August to October from surroundings of Jodhpur, Rajasthan, India. Botanist Prof. N. K. Dubey, Department of Botany, Faculty of Science, Banaras Hindu University, Varanasi, India, authenticated the plant. The authentication letter kept in the department for the future references.

Preparation of Extract and Fractions

The air-dried and powdered plant material (1.8 Kg) was macerated at room temperature with ethanol for 72 h affording 160 g of ethanolic extract after evaporation of the solvent under vacuum. A part of this extract (100 g) was diluted with distilled water (3 L) and subjected to solvent–solvent fractionation using petroleum ether, chloroform, ethyl acetate, and methanol. The resulting soluble fractions were concentrated to dryness under reduced pressure to give the petroleum ether fraction (15 g), chloroform fraction (10 g), ethyl acetate fraction (20 g), and methanol fraction (40 g). These extract and fractions were kept at 4°C for further studies.

Extractive value = (Weight of dry extract/weight of raw material taken) ×100

Preliminary Phytochemical Screening

The extract and fractions were analyzed for the phytochemicals such as flavonoids, phenolic compounds, alkaloids, tannins, terpenoids, steroids, glycosides, and proteins.[12,13]

Test for alkaloids (Mayer's test) - chloroform was added to the extract and fractions of LP. It was mixed well and then Mayer's reagent was added to it, creamy precipitate confirmed the presence of alkaloids.

Test for flavonoids (alkaline reagent test) - stock solution of extract and fractions were mixed with drops of dilute sodium hydroxide. Yellow color appears which disappeared after the addition of few drops of dilute hydrochloric acid. This confirmed the presence of flavonoids in LP.

Test for Saponins (Froth test) - to the stock solution, 10 mL dilute water was added and shaken for 15 min. Formation of froth confirms the presence of saponins.

Test for steroid - To the stock solution of extract and fractions, chloroform and concentrated sulphuric acid in equal volume was added. The lower layer indicates yellow with green fluorescence and upper layer showed red color, confirming the presence of steroids.

Test for terpenoids (Salkowski's test) - to crude extract, 2 mL chloroform and 1 mL acetic anhydride were added. 1 mL concentrated sulfuric acid was added carefully. Formation of reddish violet color indicates the presence of terpenoids.

Test for glycosides (Keller–Kiliani's test) - in a test tube, extract and fractions were treated with 2 mL of glacial acetic acid followed by 5% ferric chloride and 1 mL of concentrated sulfuric acid. The presence of brown ring at junction and violet and green color beneath brown ring confirmed the presence of glycosides.

Test for Tannins - to the stock solution, water was mixed, heated, filtered and ferric chloride was added. Dark green color formation confirms the presence of tannins.

Test for protein (Biuret test) - extract and fraction were diluted with distilled water and biuret reagent was added. The presence of pink color indicated the existence of protein in LP.

Total phenolic content (TPC) estimation - TPC was measured by employing the x.[14] A stock solution of extract and fractions of concentration 1 mg/mL was prepared. 0.125 mL

of the aliquot was mixed with 0.5 mL of distilled water and 0.125 mL of Folin–Ciocalteu reagent was added. After 5 min of incubation, add 1.25 mL of 7% sodium carbonate solution and 1 mL distilled water. After incubation for 90 min at 25°C, the absorbance of the mixture was measured at 760 nm by ultraviolet (UV)-visible spectrophotometer. A calibration curve was drawn using gallic acid as reference solution in the range of 50-1000 µg/mL ($R^2 = 0.99$). TPC was represented as mg of gallic acid equivalents (GAE) per gram of dry weight.

Total flavonoids content (TFC) estimation - TFC was calculated according to Gajula *et al*. 2009. A stock solution of extract and fraction of concentration 5 mg/mL was prepared. An aliquot of 0.25 mL was mixed with 0.075 mL of 5% sodium nitrate solution and then incubated for 6 min. After that add 0.15 mL of freshly prepared 10% aluminum chloride solution and incubated for 5 min at room temperature. In this mixture, 0.5 mL of 1M sodium hydroxide solution was added and then volume was maintained up to 2.5 mL with distilled water. The absorbance of this mixture was estimated at 510 nm using UV-visible spectrophotometer. A calibration curve was drawn using quercetin as reference solution in the range of 50 µg/mL. TFC were measured as µg of quercetin equivalents per gram of dry weight.[14]

Screening of Antibacterial and Antifungal Activity

Media used

Muller-Hinton agar (MHA) and broth (Hi-media, Mumbai, India) and Sabouraud dextrose agar maintained at pH 7.3 ± 0.2 were used for evaluating the antibacterial and antifungal activity, respectively.

Test organisms

Totally, four bacterial strains and four fungal strains were used for the study. All bacterial and fungal strains were obtained from the American Type Culture Collection (ATCC), Microbial Type Culture Collection (MTCC), and clinical strains from the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Among bacterial strains, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27893, *Salmonella typhi* MTCC 3216, and *Staphylococcus aureus* ATCC 25323 were selected for this study. Fungal strains, namely, *Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Candida tropicalis* ATCC 750, and *Candida parapsilosis* ATCC 22019 were used to check the efficacy of extract and fractions.

Preparation of Sample Extract for Microbiological Assay

A volume of 1 g of ethanolic extract and fractions was mixed in 10 mL (100 mg/mL) of peptone to get a stock solution. The extract and fractions were prepared of dilution 1:10 and 1:5 equivalent to obtain 100 mg/mL and 50 mg/mL, respectively. From this dilution, 5 µL was taken to sterile disc of Whatman's filter paper No.1 of 6 mm diameter to check the susceptibility of extract and fractions.

Antimicrobial Susceptibility Evaluation

The disc diffusion method was routinely used to evaluate the antibacterial and antifungal activity.[15,16] MHA plates were prepared by pouring 15 mL of molten media into a sterile Petri dish. The test microbes (bacteria) were dissolved in sterile normal saline to obtain the concentration of 10^7 CFU/mL and later dispersed on the surface of MHA plates. The Petri dish was incubated for 5 min and different concentration of extract and fractions were prepared. The different concentrations of extract and fractions were placed on the sterile disc of Whatman's filter paper No.1 having 6-mm diameter. This disc was placed for the diffusion of compounds for 5-10 min, and then incubated at 37°C for 24 h for bacteria and at 25°C for 48 h for fungal agents. At the ending of prescribed time for incubation, the zone of inhibition was examined around the disc and measured in millimeters. These experiments were repeated in the set of triplicate for the authentication of results.

Determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Minimum Fungicidal Concentration (MFC)

Microdilution method was employed to estimate the MIC of diluted extract and fractions as per the guidelines led by the National Committee for Clinical Laboratory Standards (2000).[17,18] MIC of the extract and fractions were estimated by diluting polyherbal drug in several concentrations. An equivalent volume of every extract, fractions, and the nutrient broth was assorted in wells of a microtiter plate.[19] Precisely, 0.1 mL of uniform inoculums (2×10^7 CFU/mL) was added in all tube. Under the aerobic condition, these plates were incubated at 37°C for the 24 h for bacterial growth and at 25°C for 48 h for fungal growth. The control batch, namely, antibiotic control (containing extract/fractions and growth media without inoculum) and organism control (consisting of growth media, saline, and inoculum). The highest dilution of extract or fractions (lowest concentration of extract/fraction) which inhibit the bacterial growth in the culture with no visible turbidity compared to the control one is designated as MIC. MBC and MFC were defined as the concentration required to inhibit the bacterial and fungal growth by subculturing the test drug on drug-free media on further incubation, respectively.[19]

Determination of Antioxidant Activity by DPPH Free Radical Scavenging Assay

The DPPH was vastly used for the estimation of free radical scavenging activity of natural products. Free radical scavenging activity of extract and fractions was estimated by

the standard procedure with little modifications.[20,21] Different concentration (5, 10, 20, 40, 80, and 100 µg/mL) of extract and fractions were prepared in methanol. The 2, 2-diphenyl-1-picrylhydrazyl was prepared for the antioxidant assay. 2 mL of each extract and fractions were mixed with 1 mL of methanol containing DPPH (0.8 mmol/L). The prepared mixture was shaken vigorously and incubated for 30 min in the dark. Later on, the absorbance of the mixture was measured at 517 nm using UV/visible spectrophotometer against a blank sample. Quercetin and gallic acid were used as a reference standard in free radical scavenging activity[22] was calculated as:

$$\text{Scavenging activity } (\%) = \frac{\text{Abs}_{517} \text{ of control} - \text{Abs}_{517} \text{ of sample}}{\text{Abs}_{517} \text{ of control}} \times 100$$

Statistical Analysis

All experimental results were expressed as mean ± SEM. The results with $P < 0.05$, $P < 0.01$ were regarded to be statistically significant.

RESULTS

Leptadenia species such as *Litsea reticulata* has been reported to exert various therapeutic effects and is the major part of Ayurvedic formulations. In this context, we searched and found that another species of Leptadenia (LP) was frequently used by the folklore of deserts as decoction or infusion for their ailments such as kidney stones and skin diseases treatment.

Phytochemical Screening

The preliminary phytochemical screening of ethanolic extract and its fractions showed the presence of various phytoconstituents enlisted in Table 1 that confirms the presence of alkaloids, glycosides, terpenoids, flavonoids, phytosterols, phenolic compounds, tannins, saponins, carbohydrate, and proteins.

TPC and TFC estimation

In addition to the preliminary phytochemical screening percentage yield which was also regarded as extractive value, calculated along with an estimation of total flavonoids and phenolic contents in the extract and fractions [Table 2]. Phenolic compounds have the history of the antioxidant property and their ability to quench free radical using hydroxyl group present in them.[22] Flavonoids are secondary metabolites of plants, which possess *in vitro* and *in vivo* antioxidant property due to the presence of free hydroxyl group.[23,24]

Antibacterial and Antifungal Activity of LP Extract and Fractions

The antimicrobial properties of ethanolic extract and fractions of the whole plant of LP *in vitro* are represented in Tables 3 and 4. Table 3 illustrated the MIC against bacterial and fungal strain with special attention to minimum bacterial inhibitory concentration and minimum fungal inhibitory concentration. The extract and fractions had antimicrobial activity against both Gram-positive and Gram-negative bacteria and fungal strain.

In vitro antimicrobial test results presented in Table 4 shows the susceptibility test against Gram-positive and Gram-negative bacteria with the strong antifungal activity of LP extract and fractions. The LP extract exhibited a considerable level of inhibition against the entire test organism compared to the standard drug.

ANTIOXIDANT ACTIVITY OF LP EXTRACT AND FRACTIONS

Natural products are rich in secondary metabolites such as phenolic compounds, flavones, condensed tannin, flavonoids,

Table 1: Preliminary phytochemical screening of LP extract and fractions

Constituents	Petroleum ether fraction	Chloroform fraction	Ethyl acetate fraction	Methanolic fraction	Ethanolic extract
Alkaloids	-	-	+	+	+
Flavonoids	-	+	+	+	+
Glycosides	-	+	+	+	+
Steroids	-	+	+	+	+
Terpenes	+	-	+	+	+
Tannin	-	-	+	+	+
Amino acid	-	-	+	+	+
Carbohydrates	+	-	-	+	+
Saponins	-	-	-	+	+
Proteins	+	-	-	+	+

+: Present, -: Absent, LP: *Leptadenia pyrotechnica*

and carotenoids endowed the antioxidant potency due to the presence of hydroxyl group and their tendency to participate in a redox reaction. DPPH is extensively used as a biomarker for free radical scavenging activity. The ethanolic extract and its fractions had good antioxidant property against DPPH radical.

Ethanolic extract possesses good DPPH scavenging activity at 100 µg/mL (62.88 ± 0.15) when compared to standard quercetin (92.10 ± 0.10) and gallic acid (92.94 ± 0.05). Chloroform and ethyl acetate fraction also embarks the free radical scavenging activity.

DISCUSSION

All these LP extract and fractions alone or in combination with other drugs are used in various illnesses. The preliminary phytochemical screening of LP extract of the drug compound is presented in Table 1, showing the presence of alkaloids, phenolic groups, flavonoids, saponins, steroids, tannins, glycosides, and terpenoids, along with amino acid and protein.

Table 2: Extractive yield, TFC, TPC of LP extract

Extractive value (%)	10%
TPC (mg GAE/g DW)[a]	49.47±1.75*
TFC (mg QE/g DW)[b]	34.85±0.29*

[a]mg GAE/g DW, [b]mg QE/g DW, values are mean±SEM of biological triplicate, *P<0.05, GAE: Gallic acid equivalent, DW: Dry weight, QE: Quercetin equivalent, TFC: Total flavonoids content, TPC: Total phenol contents, LP: *Leptadenia pyrotechnica*

Mainly, ethanolic extract, ethyl acetate, chloroform, and amethanolic fraction of LP were fine sources of diverse classes of phytocompounds. These solvents are useful in the isolation of biologically active compounds with respect to their high polarity. Flavonoids were isolated in chloroform, ethyl acetate, methanolic fractions, and ethanolic extracts of the whole plant except petroleum ether fractions. Flavonoids are the polyphenolic compounds and are naturally recognized for health enhancing properties such as antioxidant, anti-inflammatory, antimicrobial, and antitumor properties.[25,26] Phenolic compounds for most of the antioxidant activities in plants. All these extract and fractions of LP except chloroform extract have been detected for the presence of terpenoids, although saponins were altogether absent in chloroform, ethyl acetate, and petroleum ether fractions. Terpenoids have various therapeutic implications such as antibiotics, insecticidal, anthelmintic, and antiseptic.[27,28] Screening of alkaloids indicated their presence in ethyl acetate, methanolic fractions, and ethanolic extract. They have been used as an analgesic, antispasmodic and bactericidal, antimalarial, and anti-inflammatory activities.[29,30] In addition to the

Table 3: Determination of MIC, MBC, MFC values

Organisms	MIC (mg/mL)	MBC (mg/mL)	MFC (mg/mL)
Pseudomonas aeruginosa (ATCC 27893)	16.00	18.00	-
Salmonella typhi	15.00	17.00	-
Escherichia coli (ATCC 25922)	12.50	14.00	-
Staphylococcus aureus (ATCC 25323)	12.50	14.50	-
Candida albicans (ATCC 90028)	12.50	-	14.00
Candida parapsilosis (ATCC 22019)	12.50	-	14.00
Candida tropicalis (ATCC 750)	12.50	-	14.00
Candida krusei (ATCC 6258)	8.00	-	10.00

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, MFC: Minimum fungicidal concentration

Table 4: Antimicrobial activity measured by zone of inhibition (mm) of LP extract

Organisms	Standard drug (mg/mL)	Zone of inhibition (mm) extract concentration (mg/mL)			
		25	50	100	200
Pseudomonas aeruginosa	Tobramycin 28	9.0±0.22	11.00±0.34	21.00±0.50**	19.00±0.12*
Salmonella typhi	Ciprofloxacin 25	10.0±0.11	11.00±0.09	12.00±0.31*	14.00±0.22**
Escherichia coli	Norfloxacin 26	8.0±0.48	7.00±0.1	16.00±0.26*	18.00±0.31**
Staphylococcus aureus	Ampicillin 24	10.00±0.49	11.00±0.51	12.00±0.31*	14.00±0.35*
Candida albicans	Fluconazole 25	12.00±0.48	12.00±0.15	14.00±0.51	15.00±0.42**
Candida tropicalis	Fluconazole 25	10.00±0.9	11.00±0.23	14.00±0.46*	13.00±0.34
Candida krusei	Fluconazole 25	07.00±0.2	09.00±0.27	11.00±0.12*	11.00±0.81
Candida parapsilosis	Fluconazole 25	11.00±0.1	12.00±0.25	14.00±0.57**	13.00±0.34

Value expressed as mean±SD, *P<0.05, **P<0.01, SD: Standard deviation, LP: *Leptadenia pyrotechnica*

Table 5: DPPH free-radical scavenging activity of LP extract and fractions

Concentration (µg/mL)	Quercetin	Gallic acid	EELP	PELPF	CLPF	EALPF	MLPF
5	89.25±0.17*	90.25±0.17*	33.05±1.25*	28.24±1.68*	35.65±1.44*	38.20±1.71*	34.25±1.05*
10	89.90±0.15*	91.11±0.12*	38.20±1.02*	29.55±1.71*	39.25±1.02*	40.27±2.08*	37.05±1.89*
20	90.14±0.14**	91.56±0.20**	40.95±2.01*	30.88±2.31*	42.28±1.68*	42.37±1.54*	42.44±2.41*
40	90.72±0.18**	91.88±0.13**	42.08±1.52*	32.01±1.20*	46.24±1.22*	49.24±1.66*	46.05±1.08*
80	91.25±0.13**	92.28±0.07**	49.57±1.05*	35.27±1.45*	49.02±2.01*	55.17±1.06*	49.20±1.45*
100	92.10±0.10**	92.94±0.05**	62.88±0.15**	39.24±1.75*	56.66±1.02*	59.04±1.00*	56.08±1.07*

EELP: Ethanolic extract of LP, PELPF: Petroleum Ether fraction of LP, CLPF: Chloroform fraction of LP, EALPF: Ethyl acetate fraction of LP, MLPF: Methanolic fraction of LP, value expressed as mean±SEM, *P<0.05, **P<0.01, LP: *Leptadenia pyrotechnica*

phytochemical screening, a number of secondary metabolites antimicrobial efficacy was determined [Table 2].

Different classes of phytoconstituents such as alkaloids, saponins, tannins, terpenoids, sterols, and flavonoids have activity against several pathogens and therefore may justify their traditional use for the treatment of various illnesses.[31,32] This is suggestive of the presence of some compounds or groups in the extract with a similar mechanism of action to that of the standard drug used in the bacterial and fungal activity. The highest activity was exhibited by crude extract against *P. aeruginosa* (21.00 ± 0.50 mm) and *C. parapsilosis* (14.00 ± 0.57 mm) and lowest against *S. aureus* (10.00 ± 0.49 mm) suggesting its efficacy in pneumonia, bacteremia, candidiasis, and urinary tract infections. The lowest activity was exhibited by residual portion against *S. typhi* (10.00 ± 0.11 mm). However, it may be suggested that plant extracts exhibiting diameters of zones of inhibition >10 mm considered active.[33] In this line, it may be suggested that the extract and its fractions are better antimicrobial agents for various pathogenic fungus and bacteria. The pharmacological activities of the drug contributed to the presence of secondary metabolites.[34,35] The antihistaminic and anti-allergic properties of *Clerodendrum serratum* are attributed to the presence of saponin. LP extract was tested against the organisms *E. coli* (8.0, 7.0, 16.0, and 18.0 mm) followed by *P. aeruginosa* (9, 11, 21, and 19 mm); *S. aureus* (10, 11, 12, and 14 mm); *C. albicans* (12, 12, 14, and 15 mm); *C. tropicalis* (10, 11, 14, and 13 mm); and *C. krusei* (7, 9, 11, and 11 mm), respectively. These zones of inhibition are against four different concentration (25, 50, 100, and 200 mg/mL) of extract, respectively. The MIC and MBC value of 12.50 and 14.50 mg/mL against *S. aureus* and 12.5 and 14.0 mg/mL followed by *S. typhi* 15.0 and 17.0 mg/mL, *P. aeruginosa* 16.0 and 18.0 mg/mL, and *E. coli* 12.5 and 14 mg/mL, respectively, for remaining organism tested are listed in Table 3.

DPPH is one among others free radicals scavenger that is extensively employed for testing free radical scavenging activity of the plant extract. Its working principle based on the capability of DPPH, a stable free radical, to decolorize in the existence of antioxidants.[36] DPPH assay was the *in vitro* method used as a consistent and reproducible factor to investigate the antioxidant activity of plant extracts.[37] In the present study, the percentage of scavenging result on the DPPH radical was concurrently greater than before with the increase in the concentration of extract and fractions from 5 to 100 µg/mL [Table 5].[36]

CONCLUSION

The results of the present study indicate that the ethanolic extract and fractions from LP exhibit good antioxidant properties, expressed by their ability to scavenge DPPH radicals by their reducing power comparing to the standards. Results from the present study showed the ethanolic extract to be the most effective scavenger of reactive oxygen species. The ethanolic extract demonstrated better antioxidant activities than the other samples, which may be attributed to its higher phenolic, and flavonoid content since a linear relation was observed between the TPC and the antioxidant parameters. Given the eventuality of further studies of these phytochemical and antioxidant properties, particular attention should be given to the low toxicity effects of the ethanolic extract which may be useful as a herbal medicine in the alleviation of inflammation mediated diseases, especially in the case of rheumatoid arthritis, gouty arthritis, urolithiasis, diabetes, and ulcerative colitis.

The use of antimicrobial agents exhibiting cooperation is one of the deep-rooted indications for antimicrobial therapy. Thus, evidence of *in vitro* antimicrobial action of plants could be useful in selecting most favorable blends of antimicrobials for the applied therapy of resistant bacterial infections. Our results showed that the practice of using LP extract could be useful in the treatment of microbial infections and useful in eliminating and developing drug resistance, however, *in vivo* experiments are needed to confirm the bacterial protection using this extract/fractions.

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REFERENCES

1. Cragg GM, Newman DJ. Medicinals for the millennia: The historical record. Ann N Y Acad Sci 2001;953:3-25.
2. Kane JH, Finlay AC, Sobin BA. Antimicrobial agents from natural sources. Ann N Y Acad Sci 1950;53:226-8.
3. Rezai K, Weinstein RA. Antimicrobial resistance-beyond the breakpoint: Reducing antimicrobial-resistant infections in healthcare setting-what works. Issues Infect Dis 2010;6:89-101.
4. Pan SY, Gao SH, Zhou SF, Tang MK, Yu ZL, Ko KM. New perspectives on complementary and alternative medicine: An overview and alternative therapy. Altern Ther Health Med 2012;18:20-36.
5. Shetty BV, Singh V. Flora of India (Series-II). Flora of Rajasthan. Vol. 2. Calcutta: Botanical Survey of India; 1991. p. 453-80.
6. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plant. Lucknow: Central drug research institute. New Delhi: National Institute of Science Communication and Information Resources; 2007. p. 387.
7. Aquino R, Peluso G, De Tommasi N, De Simone F, Pizza C. New polyoxypregnane ester derivatives from *Leptadenia hastata*. J Nat Prod 1996;59:555-64.
8. Manavalan R, Mittal BM. Constituents of the aerialparts of *Leptadenia pyrotechnica*. Planta Med 1980;39:95.
9. Al-Yahiya MA. Triterpenoid from *Leptadenia pyrotechnica*. Fitoterapia 1986;57:179.
10. Panwara J, Tarafdarb JC. Distribution of three endangered medicinal plant species and their colonization with arbuscular mycorrhizal fungi. J Arid Environ 2006;65:337-50.
11. Cioffi G, Sanogo R, Vassallo A, Dal Piaz F, Autore G, Marzocco S, *et al.* Pregnane glycosides from *Leptadenia pyrotechnica*. J Nat Prod 2006;69:625-35.
12. Farnsworth NR. Biological and phytochemical screening of plants. J Pharm Sci 1966;55:225-76.
13. Raman N. Phytochemical techniques. New India Publishing Agency. New Delhi: Pitampura; 2006.
14. Gajula D, Verghese M, Boateng J, Walker LT, Shackelford L, Mentreddy SR, *et al*. Determination of total phenolics, flavonoids, and antioxidant and chemopreventive potential of basil (*Ocimumbasilicum* L. and *Ocimumtenuiflorum* L.). Int J Cancer Res 2009;5:130-43.
15. Metwally AM, Omar AA, Harraz FM, El Sohafy SM. Phytochemical investigation and antimicrobial activity of *Psidium guajava* L. leaves. Pharmacogn Mag 2010;6:212-8.
16. Kumar S, Lincy JL, George M, Bharti V. Antimicrobial activity of methanolic extracts of *Rumex nepalensis* leaves. Int J Pharm Pharm Sci 2011;3:240-2.
17. National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standards. 5th ed. NCCLS document M7-A5. NCCLS. Wayne, PA, USA: NCCLS; 2000.
18. Zulfiker AH, Siddiqui M, Nahar L, Habib MR, Uddin N, Hasan N, *et al*. *In vitro* antibacterial, antifungal and cytotoxic activity of *Scoparia dulcis* L. Int J Pharm Pharm Sci 2011;3:198-203.
19. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc 2008;3:163-75.
20. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1992;40:945-8.
21. Yang B, Wang J, Zhao M, Liu Y, Wang W, Jiang Y. Identification of polysaccharides from pericarp tissues of litchi (*Litchi chinensis* Sonn.) fruit in relation to their antioxidant activities. Carbohydr Res 2006;341:634-8.
22. Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat Res 2005;579:200-13.
23. Geetha S, Sai Ram M, Mongia SS, Singh V, Ilavazhagan G, Sawhney RC. Evaluation of antioxidant activity of leaf extract of sea buckthorn (*Hippophae rhamnoides* L.) on chromium (VI) induced oxidative stress in albino rats. J Ethnopharmacol 2003;87:247-51.
24. Shimoi K, Masuda S, Shen B, Furugori M, Kinae N. Radioprotective effects of antioxidative plant flavonoids in mice. Mutat Res 1996;350:153-61.
25. Aiyelaagbe OO, Osamudiamen PM. Phytochemical screening for active compounds in *Mangifera indica*. Plant Sci Res 2009;2:11-3.
26. Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu Rev Nutr 2001;21:381-406.
27. Duke JA. Handbook of phytochemical constituents of Grass. Herbs and Other Economic Plants. Boca Raton: CRC Press; 1992.
28. Parveen M, Ghalib RM, Khanam Z, Mehdi SH, Ali M. A novel antimicrobial agent from the leaves of *Peltophorum vogelianum* (Benth.). Nat Prod Res 2010;24:1268-73.
29. Okwu DE, Okwu ME. Chemical composition of *Spondias mombia* Linn plant parts. J Sustain Agric Ecosyst Environ 2004;6:140-7.
30. Oomah DB. Isolation, characterization, and assessment of secondary metabolites from plants for use in human health. PBI Bull 2003;1:13-20.
31. Abdullahi SK, Lawal GH. Phytochemical and microbial screening of *Parkinsonia aculeata* L. leaves. Int J Drug Dev Res 2010;2:1-7.
32. Chew AL, Jessica JJ, Sasidharan S. Antioxidant and antibacterial activity of different parts of *Leucas aspera*.

Asian Pac J Trop Biomed 2012;2:176-80.
33. Adeniyi BA, Fong HH, Pezzuto JM, Luyengi L, Odelola HA. Antibacterial activity of diospyrin, isodiospyrin and bisisodiospyrin from the root of *Diospyros piscatoria* (Gurke) (*Ebenaceae*). Phytother Res 2000;14:112-7.
34. Battu GR, Ethadi S, Prayaga MP, Praneeth DV, Rao MT. *In vitro* antibacterial activity and preliminary phytochemical screening of three algae from Visakhapatnam Coast, Andhra Pradesh, India. Int J Pharm Pharm Sci 2011;3:399-401.
35. Panda SK, Padhi LP, Mohanty G. Antibacterial activities and phytochemical analysis of *Cassia fistula* (Linn.) leaf. J Adv Pharm Technol Res 2011;2:62-7.
36. Hasan SM, Hossain M, Raushanara A, Mariam J, Mazumder EH, Shafiqur R. DPPH free radical scavenging activity of some Bangladesh medicinal plants. Full Length Res Pap 2009;3:875-9.
37. Koleva II, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. Phytochem Anal 2002;13:8-17.

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