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BIOACTIVITY STUDIES ON *LANTANA CAMARA* LINN.

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ABSTRACT

Diphenyl-2-picryl-hydrazyl (DPPH) radical, reducing power and Nitric oxide radical scavenging assays were carried out to evaluate the antioxidant potential of the Methanol, Chloroform, Hot water and Diethyl ether extracts of the roots of *Lantana camara*. The methanolic extract of the roots of *L.camara* was found to be the most effective. The total phenolic content of the roots were 39.32µg pyrocatechol equivalents of phenols respectively. GC-MS analysis of the methanolic extracts from roots revealed the presence of β-caryophyllene (21.22%), Limonene (13.27%), Isocaryophyllene (12.73%), oleanolic acid (9.98%) γ-terpinene (8.38%) and α-humulene (8.17%) as the main components. The results of the present study give credence to the innumerable therapeutic claims of the plant in the traditional system. Antibacterial activity was tested against four different bacteria using agar well diffusion method. Zones of inhibition were measured with the different concentrations of the root extracts. Methanol extract was effective against all the bacteria tested.

KEY WORDS:*Lantana camara*; antioxidant activity; DPPH; GC-MS; antibacterial activity

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INTRODUCTION

The practice of using medicinal herbs for treating diseases is very well known from ancient time. Plants are potent biochemical factories and have been components of phytomedicine since times immemorial. Antioxidants are radical scavengers, which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias [1]. This has attracted a great deal of research interest in natural antioxidants. *Lantana camara*, Linn. (Verbenacaea) is an aromatic shrub, native to tropical America and was introduced in India as an ornamental and hedge plant. Since a very long time *L.camara* has been reported to be used in traditional medicine system for the treatment of itches, cuts, ulcers, swellings, bilious fever, cataract, eczema and rheumatism. Various parts of the plants are used in the treatment of cold, headache, uterine haemorrhage, chicken pox, eye injuries, whooping cough, asthma[2], bronchitis and arterial hypertension[3, 4]. The fruits are useful in fistula, pustules, tumors and rheumatism. An infusion of the leaves is good for bilious fever, vitiated condition of vata and kapha, eczema and eruptions[5]. The root of this plant is used for the treatment of malaria, rheumatism and skin rashes [6.] *L.camara* oil is sometimes used for the treatment of skin itches, as an antiseptic for wounds, and externally for leprosy and scabies[7]. Previously, *L. camara* has been extensively investigated for the phytochemical compositions. Several tri-terpenoids, napthaquinones, flavonoids, alkaloids and glycosides isolated from this plant are known to exert diverse biological activities including cytotoxic and anticancer properties [8]. Antibiotic resistance has become a global concern[9]. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientists to search for new antimicrobial substances from various sources like the medicinal plants. Search for new antibacterial agents should be continued by screening many plant families. Recent work revealed the potential of several herbs as sources of drugs[10]. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes[11]. Numerous studies have identified compounds within herbal plants that are effective antibiotics[12]. Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics[13]. Some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria[14]. The results of these indicate the need for further research into traditional health systems[15]. It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity[16]. With this background and abundant source of unique active components harboured in this plant, the present study was taken up on the screening of the antioxidant activity and antibacterial activity of the roots of *L. camara.*

MATERIALS AND METHODS

A. Plant material

Roots of the selected species viz. *L. camara* were collected from Navi Mumbai, Maharashtra, India. Dr. R. Ananthan, Scientist, National Institute of Nutrition and Health, Hyderabad identified the plant materials. A voucher specimen of the same has been deposited in the institutional herbarium. The plant material was washed under running tap water, dried under the shade and was ground into a uniform powder using a mixer and stored in polythene bag at room temperature for further uses.

B. Solvent extraction

10 gm of powdered plant material was dissolved in 100 ml of methanol and kept on a magnetic stirrer for 24 hrs. Thereafter, it was filtered and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated out to dryness. The obtained material was stored at 4 °C in airtight bottles for further studies. The same procedure was repeated for obtaining chloroform, Hot water and Diethyl ether extracts.

C. Antioxidant activity tests

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity:

The antioxidant activity of the plant extracts and the standard were assessed on the basis of the radical scavenging effect of the stable, Diphenyl-2-picrylhydrazyl (DPPH) - free radical activity using a modified method of Blois, 1958[17]. Different concentrations of the plant extracts and standard compound (ascorbic acid) were taken in 10mg, 20mg, 30mg and 40mg/ml of methanol solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min. Optical density was measured at 517 nm using a UV- VIS spectrophotometer (Shimadzu, Japan). A control solution was prepared containing 1 ml of methanol and 1 ml of 0.002% DPPH. Methanol as blank was used to zero the spectrophotometer. Percentage of inhibition was calculated by using the formula given below.

Percentage (%) of inhibition (DPPH activity) = $\frac{A - B}{A} \times 100$

Where A = optical density of the Control and

B = optical density of the test.

Where, Control is the absorbance of DPPH radical and methanol

Test is the absorbance of DPPH radical + sample extract /standard.

Reducing Power

Reducing capacity of the compound may serve as indicator of its antioxidant activity. The reducing power of the plant extract was determined by the method of Oyaizu[18]. Different concentrations of plant extract and standard compound (Sodium metabisulphite) were taken in10mg, 20mg, 30mg and 40mg per ml of distilled water and phosphate buffer (2.5ml, 0.2M,pH 6.6) along with Potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of Trichloroacetic acid (10%) was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%). The absorbance was measured at 700 nm.

Inhibition of Nitric oxide radical

Nitric oxide generated from Sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction[19]. Different concentrations 10mg, 20mg, 30mg and 40mg of the plant extracts and the reference compound (Sodium metabisulphite) were taken in the reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS)[7.4 PH, 1X]. The solutions were incubated at 25°C for 150 min. At intervals of 30 min, 0.5 ml of the incubated sample was removed and 0.5 ml of the Griess reagent (1% Sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2%H3PO4) was added. The absorbance of the pink chromophore formed was measured at 540nm. The increase in % of inhibition was calculated using the formula given below:

Percentage (%) of inhibition = $\frac{A - B}{A} \times 100$

Where A = optical density of the Control and

B = optical density of the test (Standard and sample extract)

All the experiments were repeated thrice.

Amount of total phenolic compounds

Total soluble phenolic compounds present in the methanol extract of the roots of *L. camara* were determined with the Folin-Ciocalteu reagent, according to the method

suggested by Slinkard and Singleton[20]. To 0.1 ml of root extract (1 mg/ml in distilled water) in Erlenmeyer flask, 1ml Folin-Ciocalteu reagent was added. After three minutes, 3 ml 2% Na_2CO_3 was added. Subsequently, the mixture was shaken for 2 hrs at room temperature and absorbance was measured using a spectrophotometer at 760 nm. The concentration of total phenolic compounds in samples was determined as µg pyrocatechol equivalents, using the following equation obtained from a standard pyrocatechol graph:

Absorbance = 0.001 x pyrocatechol (µg) + 0.0033

Gas chromatography-Mass spectral analysis

The methanolic extracts from the roots of *L. camara* was subjected to GC-MS analysis on a Fissions 8000 series Gas chromatograph with Fissions 800 series Mass selective detector, and NIST database and Wiley Library. The column used was DB-5 MS and the flow rate was maintained at 1.2 ml/min with helium as the carrier gas. The temperature of the injector and detector was 275 °C. The oven temperature was programmed from 35° to 200° C at a rate of 4° C /min. Identification of oil components was based on their retention indices, which were determined with references to a series of standards, and by comparison of their mass spectral fragmentation pattern with NIST database (John Wiley Library 229119)[21].

d. Antibacterial activity studies:
Well Diffusion assay:

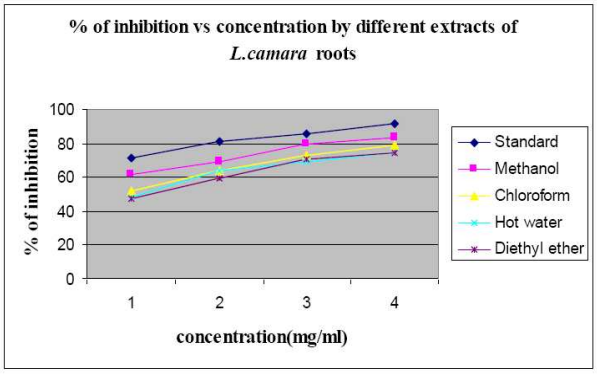
A modification of the Well Diffusion assay protocol[22] was used to screen the extract for antimicrobial activity against *Salmonella typhi* (MTCC 734*), Salmonella paratyphi A* (MTCC 3220)*, Vibrio cholerae* (MTCC 3904) and *Klebsiella pneumoniae* (MTCC 109) . Nutrient agar plates were swabbed with the respective broth culture of the organisms and kept for 15 min for absorption to take place. Wells were made in agar plates using the broad end of a sterile Pasteur pipette (5mm diameter).10 µl of the crude extract in methanol, Diethyl ether, chloroform and hot water , at concentrations 2mg/mL, 3mg/mL, 4 mg/mL and 5 mg/mL were added to each well. A mixture of penicillin and streptomycin (1:1) was used as positive control. The various solvents, in which dilutions were made, were used as negative controls. Plates were incubated at 37°C for 24 h and the diameters of the inhibition zones were measured in millimeter after the incubation period.

RESULTS

The scavenging effects of plant extracts and ascorbic acid on the DPPH radical are illustrated and compared in Graph 1. The DPPH radical scavenging activity of the plant extracts increased in a concentration dependent manner. The scavenging effect of methanolic extract from roots was considerably higher than those of the other extracts, followed by the chloroform, Hot Water and then Diethyl ether.

Graph 1

DPPH radical scavenging activity by different extracts of L. camara roots



The reducing power of plant extracts along with that of Sodium metabisulphite is shown in Table 1.The reducing power of methanolic extract was found to be considerably higher than those of the other extracts.

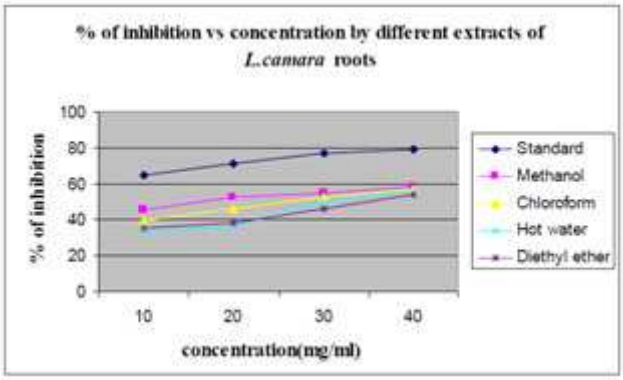
Table 1
Reducing Power of Root Extracts of L.camara

Concentration (mg)	O. D. At 700 nm				
	Standard	Methanol extract	Chloroform extract	Hot water extract	Diethyl ether extract
10	2.59±0.35	2.55± 0.45	1.14± 0.72	0.92± 0.57	0.32± 0.66
20	2.69± 0.43	2.57± 0.35	1.15± 0.57	1.03± 0.68	0.34± 0.43
30	2.78± 0.47	2.66± 0.33	1.16± 0.32	1.06± 0.58	0.37± 0.45
40	2.99± 0.36	2.70± 0.38	--	1.09± 0.51	0.49± 0.35

Values are mean± SE; n=3

The inhibition of Nitric oxide was best with the methanolic extracts from roots (Graph 2).

Graph 2
Inhibition of Nitric oxide by the different extracts of roots L. camara roots



The roots contain 39.32µg pyrocatechol equivalents of phenols respectively. Thirty compounds were identified by GC-MS, (Table 2). β -caryophyllene (21.22%) was the most abundant compound.

Table 2
Constituents of Methanolic Extracts from the roots of L.camara

Compound No.	Name	RI (DB-5)	%
1	α-pinene	940	0.22
2	Camphene	954	0.76
3	Myrcene	991	0.30
4	α-phellandrene	1003	1.89
5	Limonene	1030	13.27
6	γ-Terpinene	1033	8.38
7	Cis-β-Ocimene	1040	1.36
8	Trans-β-Ocimene	1051	1.45
9	α-Terpinene	1072	0.59

10	Linalool	1104	0.26
11	Cis- Sabinene hydrate	1120	1.23
12	α-Terpinolene	1183	0.71
13	β-Cyclocitral	1218	0.62
14	Iso boronyl formate	1227	0.47
15	Boronyl acetate	1291	0.20
16	4-Isopropyl-2-cyclohexen-1-one	1320	3.09
17	α-Terpenylacetate	1352	2.83
18	α-Copaene	1370	1.25
19	β-Elemene	1389	0.28
20	Cyperene	1391	3.20
21	Isocaryophyllene	1399	12.73
22	Bornyl formate	1409	1.66
23	β-Caryophyllene	1417	21.22
24	α-Humulene	1447	8.17
25	Oleanolic acid	1455	9.98
26	Curcumene	1477	1.49
27	Bicyclogermacrene	1490	0.23
28	Zingiberine	1503	0.84
29	Selina-3,7(11)-diene	1532	0.99
30	Caryophyllene oxide	1565	0.33

We also examined the inhibitory effect of the solvent extract of *L.camara roots* against four different bacterial strains. Among all solvent extracts the methanol extract showed the best inhibitory activity. The effects of different extracts on the bacteria tested are shown in table 3 & 4. The maximum zone of inhibition was obtained against *Salmonella paratyphi A and Vibrio chloreae* with 5 mg/ml of methanol extract. The zone of inhibition in all the cases increased with an increase in concentration.

Table 3
Antibacterial activity of the root extracts of L. camara

Concentration (mg/mL)	**Zone of inhibition (mm) observed against the pathogenic bacteria under study**					**Antibiotic**	**Solvent**
	Organisms	Methanol extract	Chloroform extract	Diethyl ether extract	Hot water extract		
5	*S. typhi*	20 ± 0.22	16± 0.14	14± 0.11	10± 0.25	24± 0.12	0
	S. paratyphi A	25± 0.18	21± 0.23	18± 0.28	14± 0.13	21± 0.15	0
	K. pneumonia	18± 0.23	12± 0.21	10± 0.26	-	24± 0.24	0
	V. cholerae	24± 0.15	19± 0.10	16± 0.17	13± 0.10	22± 0.22	0
4	*S. typhi*	17± 0.16	15± 0.18	11± 0.12	09± 0.17	24± 0.16	0
	S. paratyphi A	22± 0.25	18± 0.26	15± 0.24	13± 0.17	21± 0.23	0
	K. pneumonia	16± 0.22	10± 0.20	08± 0.26	-	24±	0

						0.27	
	V. cholerae	20± 0.21	17± 0.18	14± 0.21	11± 0.18	22± 0.21	0
3	*S. typhi*	15± 0.13	12± 0.14	10± 0.10	-	24± 0.17	0
	S. paratyphi A	18± 0.20	17± 0.24	12± 0.11	09± 0.21	21± 0.13	0
	K. pneumonia	14± 0.24	08± 0.28	-	-	24± 0.20	0
	V. cholerae	17± 0.11	15± 0.16	12± 0.13	09± 24	22± 0.11	0
2	*S. typhi*	12± 0.10	10± 0.12	09± 0.30	-	24± 0.18	0
	S. paratyphi A	15± 0.24	12± 0.25	10± 0.21	-	21± 0.15	0
	K. pneumonia	11± 0.12	-	-	-	24± 0.11	0
	V. cholerae	17± 0.17	11± 0.27	09± 0.22	-	22± 0.19	0

DISCUSSION

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. In the present study roots of *L. camara*, traditionally used in India for various disorders were studied for their antioxidant activity like free radical scavenging activity on DPPH, the reducing power and inhibition of nitric oxide. Owing to the complexity of the antioxidant materials and their mechanism of actions, it is obvious that no single testing method is capable of providing a comprehensive picture of the antioxidant profile of a studied sample and a combination of different methods is necessary. Despite such limitations, methods like DPPH free radical scavenging activity , reducing power and inhibition of nitric oxide can be helpful for primary screening and finding of novel antioxidants. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts[23]. In the present study, DPPH test showed the ability of the test compound to act as a free radical scavenger. DPPH has a proton free radical and shows characteristic absorption at 517 nm (purple). When the odd electron of DPPH becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolorized from purple colour[24, 25] to light yellow which, suggests that antioxidant activity of *L. camara* root extract is due to its proton donating ability. Acting as an electron donor reacting with free radicals, it converts them to more stable products and terminates radical chain reactions. This mechanism may explain its use for the treatment of wounds and sores and thus has a medicinal value. The reduction of Fe3+/ferricyanide complex to the ferrous form by the plant extracts was observed using the reducing power assay. This reducing capacity of compounds could serve as an indicator of potential antioxidant properties[26]. The present study indicates that the quantity of phenolic compounds were more in roots. Phytochemicals, especially plant phenolics constitute a major group of compounds that act as primary antioxidants[27]. They can react with active oxygen radicals, such as hydroxyl radicals superoxide anion radicals and lipid peroxy radicals[28], and inhibit lipid oxidation at an early stage[29]. They also inhibit cyclooxygenase and lipoxygenase of platelets and macrophages, thus reducing thrombotic tendencies *in vivo*[30]. Naturally

occurring phenolic compounds have free radical scavenging properties, due to their hydroxyl groups[31]. Further, phenolic compounds are effective hydrogen donors, which make them antioxidant[32]. The antioxidant properties of the extracts of *L. camara* could thus be attributed to the presence of phenolic compounds in them. The GC-MS analysis of the methanolic extracts indicate the presence of β-caryophyllene, Limonene, Isocaryophyllene, Oleanolic acid, γ-terpinene and α-humulene as the major constituents. The presence of compounds like Limonene and γ-terpinene has contributed to the antioxidant potential of essential oils from plants[33, 34]. Also α-humulene and β-caryophyllene have been found as main constituents in many kinds of plant species which exhibit antioxidant activity[35, 36, 37]. In an earlier study, Misra et al, (1997)[38] isolated oleanolic acid from the root of *L. camara, which* showed antiulcer, antimicrobial, hypoglyceamic and anticarcinogenic activity. It could thus be concluded that the higher antioxidant activity shown by the roots could have been due to the presence of the above mentioned phytochemicals and a higher amount of phenolics in them. The result obtained is compatible with one of the uses of *L.camara* preparation in traditional medicine for treatment of rheumatism, since free radicals usually induce cellular damage and play a crucial role in many diseases such as rheumatism, cancer, hepatic disorder and aging disease[39]. Screening of bioactive agents from plant is one of the most intensive areas of natural product research today. The presence of antibacterial substances in the higher plants is well established[40]. Thus, they can be used in the treatment of infectious diseases caused by microbes. Earlier reports suggest that the methanol extract of *L. camara* inhibited the growth of *E.coli* [41]. In the present study we report that, crude methanol extract of *L. camara* showed the best antibacterial activity against *S. paratyphi* A and *V.cholerae.* The reputation of *L. camara* as a remedy for different microbial diseases traditionally including its use as an antiseptic was supported by the antibacterial screening.

CONCLUSIONS

Thus, the potent antioxidant activity validates the innumerable therapeutic claims of the plant in the traditional system and especially its use in rheumatism, wound healing and against tumors. It is our strong conviction that these results will inspire and motivate even more researchers to look for new leads from plants and other natural sources.

ABBREVIATIONS

DPPH- Diphenyl-2-picryl-hydrazyl, GC-MS- Gas chromatography-Mass spectroscopy

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