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Phytochemical Screening, Antibacterial Activity and Identification of Bioactive Compound(s) in the Leaves of Bell Weed (*Dipteracanthus prostratus*) for Medicinal Purpose

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Abstract: Bell weed (*Dipteracanthus prostratus)* is a medicinal herb, traditionally used in the treatment of wounds, anti-cancer, hypoglycemic, anti-inflammatory, anti-ulcer and anti-oxidant activities. Based on the above information, the present study is planned to know the presence of various phytochemicals in the leaves of bell weed. Bio chemicals like tannin, phenol, terpenoids, flavonoids, amino acid, protein, carbohydrate, phylobatannins, volatile oils, hydrolysable tannins and glycosides are present in aqueous and ethanol extracts, whereas steroids are absent in both extracts. Gas chromatography-mass spectrometry (GC-MS) analysis showed 36 compounds viz among those dotriacontane, 2-hexadecen-1-ol,37,11,15-tetramethyl-(R-(R*, Spinacen, 2,6,10-trimethyl-14-ethylene-14-pentadecane, 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl) and stigmasterol were highly present in leaves extract. The antimicrobial activity of *Dipteracanthus prostratus* leaves extract against *Escherichia coli, Entrobacter aerogenes, Shigella flexneri* and *Vibrio cholera* in ethanol and aqueous extracts. The ethanolic extracts of *Dipteracanthus prostrates* showed the maximum level of zone of inhibition towards the pathogenic bacteria *Vibrio cholera* and *Entrobacter aerogenes* when compared to aqueous extracts, standard and control and the aqueous extract showed the maximum level of zone of inhibition against pathogenic bacteria like *Entrobacter aerogenes* and *Shigella flexneri* when compared to ethanol extract. The results suggest that these phytochemicals and compounds may be used against pathogenic bacteria, probably it is used to delivering new drug for cure many infectious diseases.

Key words: Phytochemicals · *Dipteracanthus prostratus* · Pathogenic bacteria · GC-MS

INTRODUCTION

The plants are valuable source of new medicinal agents [1]. Medicinal plants have various effects on living systems [2]. Medicinal plants contents are used for the development of new drug compounds that are used in the treatment of many infectious diseases [3]. *Dipteracanthus prostratus* (Acanthaceace) is an important medicinal plant and popularly known as black weed [4]. *Dipteracanthus prostratus* belongs to Acanthaceace family and very important indigenous medicinal plant, which present in moist shady places throughout India. It is used as a remedy for ear disease and believed to be anti-cancer against the nasopharynx region, slightly hypoglycemic, anti-inflammatory and anti-microbial and also the leaves are eaten as vegetable [5].

The flowers are normally sessile, axillary's solitary or few together. Pale blue to light violet and occasionally white in colour, braceoles like leaves but smaller. Calyx are par title and hairy. Corollas are infundibulate from with narrow tube. Capsules are many seeded [6-8]. Herbal medicine has been practiced worldwide and is now recognized by WHO as an essential building block for primary healthcare. Though the traditional Indian system of medicine has a long history of use, they lacked adequate scientific documentation, particularly in the light of modern scientific knowledge [9]. The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind [10].

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In recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants in various traditional, complementary and alternative systems of treatment of human diseases [11]. The antibacterial activity of *S. nigrum, S. torvum, S. trilobatum, S. surattense* and *S. melongena* are important medicinal plants against some common human pathogenic bacteria [12].

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties [13]. Medicinal herbs have been used in one form or another under indigenous systems of medicine [14]. Hence, the present study analyze the phytochemicals and identify the active compounds in the bell weed, *Dipteracanthus prostratus* leaves extract against some pathogenic bacteria like *Escherichia coli, Enterobacter aerogenes, Shigella flexneri* and *Vibrio cholera* to cure many infectious diseases.

MATERIALS AND METHODS

Collection of Plant Material: The fresh leaves of *Dipteracanthus prostratus* plants were collected from Rajah Serfoji Govt. College campus, Thanjavur, Tamilnadu, India.

Preparation of Leaves Extract: The collected plant leaves were washed thoroughly using water and transferred to the laboratory. The plant material was shade dried for two to three days, after drying, plant material was powdered with the help of mixer grinder. Twenty gram of powdered plant material was mixed with 100 ml of solvents like ethanol and aqueous. The extracts prepared in succession from powdered leaf material by Soxhlet method [15]. The collected extracts were stored in a vial for further studies.

Phytochemical Screening: The aqueous extracts were subjected to phytochemical screening for secondary plant metabolites according to the methods described [16-19].

Test Microorganisms: Disease causing infectious bacteria in animals and human such as *Escherichia coli*, *Entrobacter aerogenes, Shigella flexneri* and *Vibrio cholera* were used in the present study. They were collected from the Microbial Type Culture Collection (MTCC) at Chandigarh, India.

Antibacterial Activity of Plant Extract: Antibacterial assay was carried out by agar diffusion method. The sterile Muller-Hinton agar plates were prepared. The test organisms were spread over the Muller-Hinton agar plates by using separate sterile cotton swabs. The prepared sterile disc was placed on the surface of the medium at equal distances and then the plates were incubated at 37°C for 24 hours to determine the antibacterial activity of the respective solvent extracts. Antibiotic (Ciprofloxacin) discs (15mg/disc) were used as positive control. Each extract was treated in triplicate for calculation of mean value.

GC-MS Analysis of Samples: The GC-MS analysis was performed in a gas chromatograph (Perkin-Elmer, Auto system XL) linked to a mass spectrometer (Turbo mass) available at the Jawaharlal Nehru University New Delhi, India. An aliquot of 2 Ml of extract was injected into the PE-5MS column of 20mm0.18 mm internal diameter 0.18 mm film thickness glass capillary column using the following temperature programme: initial oven temperature of 40°C for 5 minutes, increasing to 100°C at a rate of 7°C for 6 minutes and then to 250°C at a rate of 7°C for 9 minutes. The injector temperature was maintained at 260°C. The interface temperature was 250°C. Helium was used as a mobile phase at a flow rate of 1.1 mL/min. Mass spectral detection was carried out in electron ionization mode by scanning at 20 to 650 (m/z). Finally, unknown compounds were identified by comparing the spectra with that of the National Institute of Standard and Technology library. The total time required for analysing a single sample was 45 minutes. A blank was run after every five samples.

Statistical Analysis: Mean and standard deviation were calculated to facilitate the comparison of the data. The obtained data were computed by ANOVA test followed by the pos hoc Duncan's test. All the data analyses were significant at $P< 0.05$ [20].

RESULTS

Screening of Phytochemicals: The phytochemicals of *Dipteracanthus prostratus* leaves extract were qualitatively analyzed and showed tannins, phenols, terpenoids, flavonoids, amino acid and protein, carbohydrates, phylobatannins, volatile oils, hydrolysable tannins and glycosides were present and saponin is absent in both (aqueous and ethanol) extracts. The steroids are absent in aqueous extract only (Table 1).

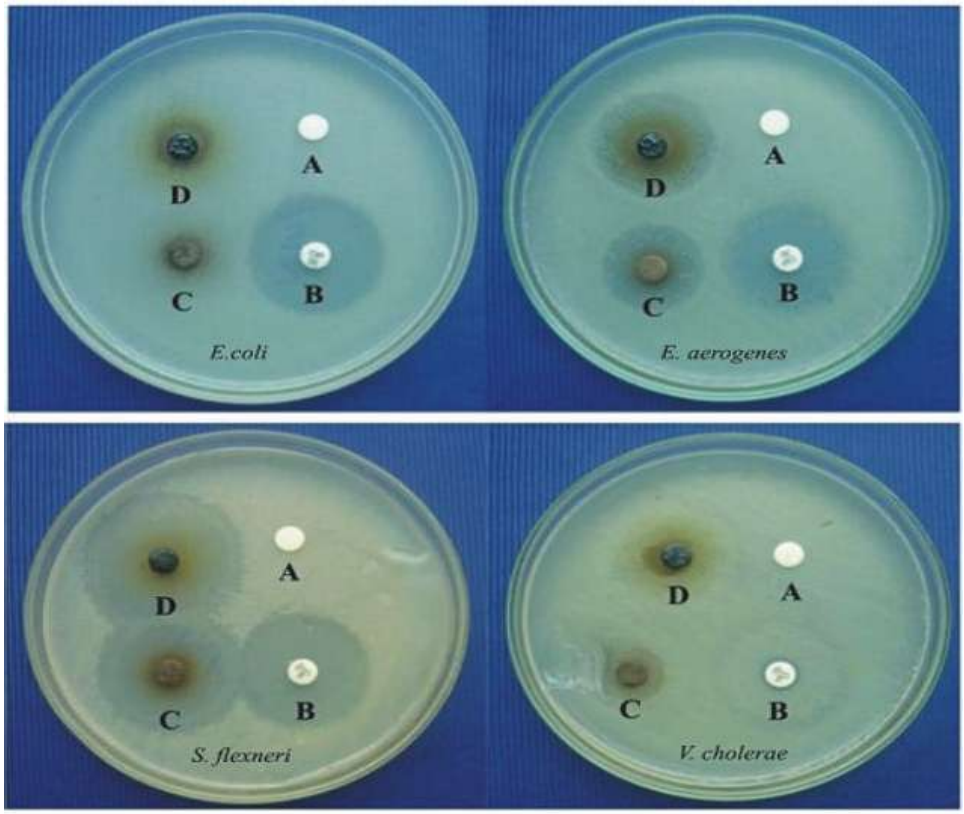

Fig. 1: Antimicrobial activity of *Dipteracanthus prostratus* leaves against pathogenic bacteria

Table 1: Preliminary phytochemical studies on various extracts of *Dipteracanthus prostratus* leaves

S.No	Phytochemical tests	Aqueous	Ethanol
1	Saponin	-	+
2	Tannins	+	+
3	Phenol	+	+
4	Steroids	-	-
5	Terpenoids	+	+
6	Flavonoids	+	+
7	Amino acid and proteins	+	+
8	Carbohydrate	+	+
9	Phylobatannis	+	+
10	Volatile Oil	+	+
11	Hydrolysable tannis	+	+
12	Glycosides	+	+

'+'- Present, '-' Absent

Antimicrobial Activity of *Dipteracanthus prostratus*: The antimicrobial activities of *Dipteracanthus prostratus* leaves extract were analyzed against *Escherichia coli*, *Entrobacter aerogenes, Shigella flexneri* and *Vibrio cholera* in ethanol and aqueous extract. In the present study, the ethanolic extracts of *Dipteracanthus prostrates* leaves showed the maximum level of zone of inhibition against pathogenic bacteria such as *Vibrio cholera* and *Entrobacter aerogenes* when compared to aqueous extracts, standard and control. In aqueous extracts, the maximum level of zone of inhibition showed in the pathogenic bacteria like *Enterobacter aerogenes* and *Shigella flexneri* when compared to ethanol extracts (Fig. 1, Table 2).

Compound Identification: The analysis and extraction of plant material plays an important role in the development, modernization and quality control of herbal formulations. Hence, the present study is aimed to find out the bioactive compounds present in the ethanol extracts of *Dipteracanthus prostratus* by using Gas chromatography and Mass spectroscopy. The active compounds with their peak number, molecular formula, molecular weight and

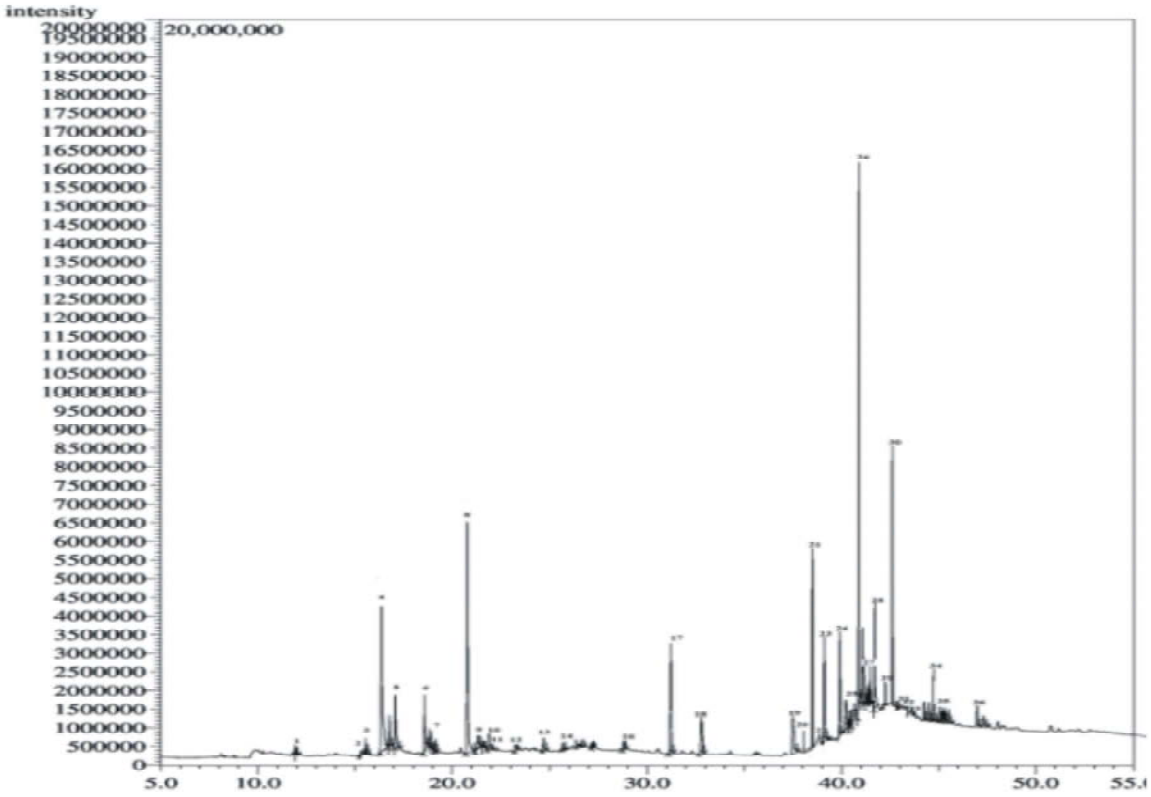

Fig. 2: GC-MS analysis of *Dipteracanthus prostratus* leaves extract

Table 2: Antimicrobial activity of *Dipteracanthus prostratus* leaves against some pathogenic bacteria

		Zone of inhibition (mm in diameter)			
S. No.	Name of the Bacteria	Control	Standard*	Aqueous	Ethanol
1	*Escherichia coli*	-	20.67±1.36^{a}	10.67±1.28^{b}	17.33±1.15^{a}
2	*Enterobacter aerogenes*	-	10.50±1.34^{a}	13±1.06^{a}	11.50±0.99^{a}
3	*Shigella flexneri*	-	21.33±0.88^{a}	19.83±1.04^{a}	20.67±1.22^{a}
4	*Vibrio cholera*	-	27.5±0.76^{b}	29.5±0.99^{ab}	31.83±0.95^{a}

Standard*- Ciprofloxacin (30mg/disc) Ref. Hi Media Standard value

Values are expressed in Mean ± Standard Error and the values in horizontal rows are significantly different at P>0.05% level

Table 3: Compounds identified in ethanolic extract of bell weed plant leaves

S.No	Name of the Compounds	Molecular Formula	M.W	Peak Area %
1	1-Heptadecene	C17H34	238	0.22
2	Tetradecanoic Acid	C14H28O2	228	0.27
3	1-Octadecene	C18H36	252	0.31
4	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	C20H38	278	8.03
5	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl-[R-[R*,	C20H40O	296	10.51
6	Palmitic Acid	C16H32O2	256	2.30
7	1-Heneicosanol	C21H44O	312	0.70
8	4-(3,5-Di-Tert-Butyl-4-Hydroxyphenyl)Butyl Acrylate	C21H32O3	332	0.35
9	Cis,Cis,Cis-7,10,13-Hexadecatrienal	C16H26O	234	0.87
10	9,12,15-Octadecatrienoic Acid, Ethyl Ester	C21H36O4	352	0.34
11	N-Tetracosanol-1	C24H50O	354	0.71
12	Octanoic Acid, 2-Dimethylaminoethyl Ester	C12H25NO2	215	0.31
13	1-Heptacosanol	C27H56O	396	1.52
14	3-Cyclopentylpropionic Acid, 2-Dimethylamino Ethyl	C12H23NO2	213	0.24

Table 3: Continued

15	Hexadecanal	C16H32O	240	0.28
16	Hexatriacontane	C36H74	506	1.09
17	Spinacen	C30H50	410	10.08
18	Tetracontane	C40H82	562	1.84
19	Gamma.-Tocopherol	C28h48O2	416	2.63
20	Stigmast-5-En-3-Ol, Oleat	C47H82O2	678	0.20
21	Dotricontane	C32H66	450	36.69
22	Cholest-5-En-3-Ol (3.Beta.)-	C27H46O	386	0.25
23	2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl)	C35H60O7	592	3.86
24	Ergost-5-En-3-Ol, (3.Beta.,24R)-	C28H48O	400	2.01
25	Octadecanal	C18H36	268	0.35
26	Stigmasterol	C29H48O	412	3.85
27	Stigmast-5-En-3-Ol, (3.Beta.)-	C29H50	414	3.01
28	24-Isopropyl-5,24-Cholestadien-3.Beta.-Ol	C30H50O	426	0.15
29	14B-Octamethyl- Octadecahydro-2H-Picenone	C30H48O	424	0.31
30	Oxirane, Hexadecyl-	C18H36	268	2.24
31	9,19-Cyclolanostan-3-Ol, 24-Methylene-(3.Beta.)	C31H52O	440	0.23
32	Dec-5"-En-1"-Yloxy Tetrahydrofuran	C22H42O2	338	1.43
33	Triacontanoic Acid, Methyl Ester	C31H62	446	0.47
34	2-Heptadecanone	C17H34O	254	0.20
35	E,E,Z-1,3,12-Nonadecatriene-5,14-Diol	C19H34O2	294	0.61
36	Z-2-Octadecen-1-Ol	C18H36O	268	0.68

concentration (peak area %) are presented. The ethanol extract of *Dipteracanthus prostratus* leaves showed thirty six compounds, among these the maximum level of dotriacontane, 2-hexadecen-1-ol,37,11,15-tetramethyl-,[R-[R*, spinacen, 2,6,10-trimethyl,14-ethylene-14-pentadecane, 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl) and stigma sterol were present (Table 3, Fig. 2).

DISCUSSION

In the present study, the phytochemical screening, identification of active compounds and antimicrobial activity were analysed against some pathogenic bacteria using the bell weed (*Dipteracanthus prostratus*) leaves extract. The phytochemicals such as tannins, phenol, terpenoids, flavonoids, amino acid and protein, carbohydrates, phylobatannins, volatile oils, hydrolysable tannins and glycosides were present in this plant leaves and flowers extracts. Medicinal plants possess a variety of compounds of known therapeutic properties [21-22]. Compared to *Diptheracanthus prostratus*, the *Alternanther atenella* plants have all phytochemicals in more amounts. Saponins, alkaloids, glycosides and tannins have various biological activities including anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial and antiviral activities [23]. Earlier reports show that the alkaloids in all the solvent fractions could be well correlated with the antimicrobial activities [24]. The Flavonoids are super antioxidants which have anti-inflammatory, prevent oxidative cell damage through their water soluble property and also possess anti-cancer activity [25-26]. Flavonoids in intestinal tract lower the risk of heart disease. Triterpenoids shows analgesic, hepatoprotective and anti-inflammatory properties [27]. Tannins may have potential value such as cytotoxic, anti-cancer agents and hasten the healing of wound and inflamed mucous membranes [28-29]. Saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss etc. according to medical field. It is a bioactive antibacterial agent of plants [30-31]. Phenolic compounds have anti-oxidative, anti-diabetic, anti-carcinogenic, anti-mutagenic and anti-inflammatory [32].

The antimicrobial activity of ethanolic and aqueous extract of *Dipteracanthus prostratus* leaves extract showed that the zone of inhibition is significantly higher in ethanolic extract when compared to aqueous extract. Earlier report shows that the ethanol and methanol extracts of *Blepharis* leaf and *Dipteracanthus* stem showed minimum inhibition zone (7-9 mm) against *Bacillus subtilis* [2]. Anti-inflammatory chemical compounds are present in the *Dipteracanthus patulus* plant extracts [33]. The use of medicinal plants produces a variety of compounds of known therapeutic properties [34-36]. These compounds are inhibiting the growth of pathogens or kill them and have no or least toxicity to

host cells is considered for developing new antimicrobial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [37-43].

In the present study, thirty six compounds were identified in *Diptheracanthus prostratus* leaves extract. Among these compounds, the maximum level of Dotriacontane, 37,11,15-Tetramethyl-2-hexadecen-1-ol, Spinacen, 2,6,10-Trimethyl,14-Ethylene-14-Pentadecane, 2,5,7,8-Tetramethyl-2-(4,8,12-Trimethyltridecyl) and stigmasterol are present. The compound 3,7,11,15-Tetramethylhexadec-2-en-1-ol showed good anti-inflammatory, antioxidant, antimicrobial, antituberculosis and insecticidal activity [44]. The compound 2,6,10,-trimethyl,14-ethylen-14-pentadecene identified in the present study is also reported in *Eupatorium triplinerve* leaves which show good anticancer and antiproliferative activities [45]. Similarly, the compound stigmasterol identified in the present study showed good anti-inflammatory, tumor promotion, anti-HIV reverse transcriptase [44]. Hence, the present study clearly indicates that the ethanolic extract of *Dipteracanthus prostratus* leaves may be responsible for better anti microbial activity.

CONCLUSION

The present work concluded that the screening for phytochemicals of *Dipteracanthus prostratus* showed the presence of several bioactive compounds. The compounds present in this plant exhibited good antimicrobial activity against pathogenic bacteria which indicating the potential of this plant as a source of functional ingredient that can be used in pharmaceutical industries so as to develop it as a potent antimicrobial drug. Further molecular and cellular experiments are used for the identification and separation of active compounds is also necessary for delivering new medicines for many infectious diseases.

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