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Short Communication

Antimicrobial activity of the essential oil of *Cestrum diurnum* (L.) (Solanales: Solanaceae)

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Cestrum diurnum **(Solanaceae: Solanales) is a single or multistemmed shrub that is also known as Day Jasmine. The essential oil of the mature leaves of** ***C. diurnum*** **was analyzed by GLC and GLC-MS and altogether 14 components were detected. The main constituents were palmitic acid (27.62%), stearic acid (4.62%) and oleic acid (3.06%). The essential oil of mature leaves of** ***C. diurnum*** **were evaluated for antimicrobial activity against pathogenic strains of Gram positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram negative (*Escherichia Coli, Pseudomonas aeruginosa*) bacteria. The oil showed strong *in vitro* activity against *P. aeruginosa* and *S. aureus*.**

Key words: *Cestrum diurnum*, antimicrobial activity, essential oil, Gram positive bacteria, Gram negative bacteria.

INTRODUCTION

The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. The gram positive bacterium such as *Staphylococcus aureus* is mainly responsible for post operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Mylotte, 1987). *Bacillus subtilis* are rod shaped aerobic bacteria and are reported to have some pathogenic role (Gorden et al., 1973). The gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicemia (Levine, 1987; Singh et al., 2000). *Pseudomonas* is an aerobic, nonfermentative, oxidase positive bacillus which mainly causes urinary tract infection, wound or burn infection, chronic otitis media, septicemia etc. in human (Bodey, 1983) and also causes several diseases in fishes (Bullock et al., 1965).

The genus *Cestrum* (Solanaceae), with more than 300 species, is globally distributed. *Cestrum diurnum* (Solanaceae: Solanales) is a single or multistemmed shrub that is also known as Day Jasmine. There are several application of the plant that have been well documented in several literatures and the toxicity of the species to humans and livestock has been frequently reported (Stone, 1970; Little et al., 1974). The leaves contain a calcinogenic glycoside called 1,25-dihydroxycholocalciferol that leads to a vitamin D toxicity and elevated serum Ca^{2+} and deposition of calcium in soft tissues (Mello, 2003).

The effects of herbal compounds and phytochemical on pathogenic and economically important bacteria have been well studied (Sato et al., 1996; Khalid et al., 1997; Binutu, 1997; Sampietro et al., 1997). The aim of the present study was to examine the effects of essential oil of *C. diurnum* on *S. aureus*, *B. subtilis*, *E. coli* and *P.s aeruginosa* by zone inhibition assay (Paech and Tracy, 1995). The effect of the *C. diurnum* oil was also compared to standard concentration of antibiotic ampicillin.

METHODOLOGY

Plant material: Fresh mature (4-8 weeks old) leaves of *C. diurnum* were harvested randomly during spring season (mid March to mid April 2003) from plants growing at the outskirts of Burdwan

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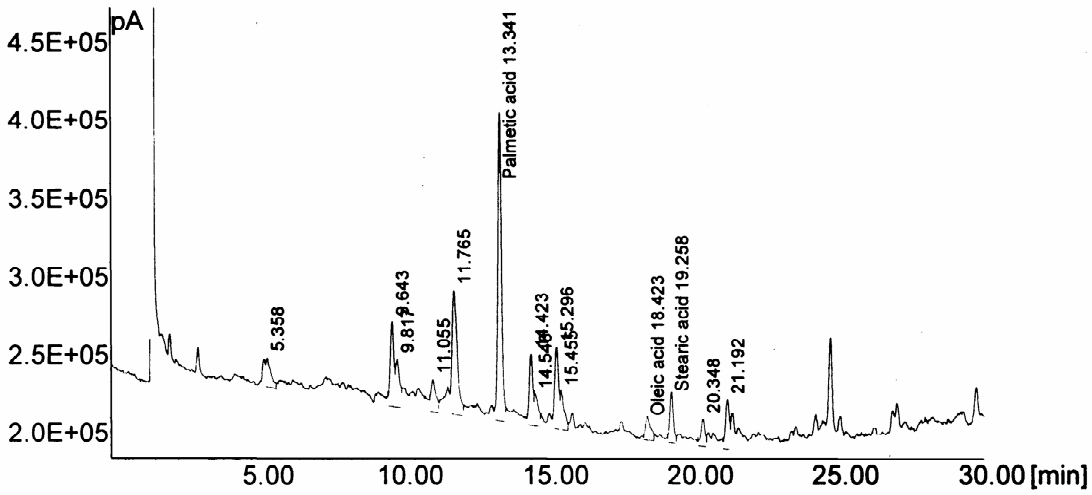

Figure 1. HP 6890 Plus GLC and HP3398A GC Chemisation.

(23°16′N, 87°54′E). Leaves were initially rinsed with distilled water and dried on paper towel.

Isolation of essential oil: The essential oil from 100 g leaves of *C. diurnum* was collected by hydro-distillation for 3 h in a Clevenger type apparatus. A total of 1.07 ml essential oil was obtained (0.587% w/w). The essential oil was dried on anhydrous sodium sulfate.

Preparation of methyl ester of essential oil: The dried sample was treated with chloroform: perchloric acid mixture (96 :5 v/v) and the solution was placed on a water bath at 50 to 60°C for 10 min. After cooling, the solution was extracted with petroleum ether (50 ml PET at a time) in a separating funnel (repeated thrice). The PET fractions were collected and dried over anhydrous Na_2SO_4 and kept in a rotary evaporator for drying. The concentrated fraction was developed in thin layer chromatography (TLC) and methyl esters were identified in iodine chamber (mobile phase; hexane:ethyl acetate 1:1, v/v). The spots were scrapped, dissolved in dehydrated ethanol and the ethanolic solution was boiled on water bath at 55 to 60°C for 25 min. The ethanol fraction was kept in a 250 ml round bottom flask discarding the silica gel fraction. Above methods were used for the preparation of methyl esters of standard fatty acids such as myristic, palmitic, stearic, oleic and linoleic acids. The methyl esters thus prepared were subjected to GCMS study.

Analytical gas chromatography: The dried sample was eluted with petroleum ether extract. The eluent was fractioned by TLC on silica gel G (sigma, St. Louis. Mo, USA) layers (thickness 0.5 mm), which had been prepared using a Unoplan (Shandon, London) coating apparatus, within benzene:ethyl acetate (1:1) as the mobile phase. The single band was separated and unsaturation was identified.

The purified fatty acid fraction was analyzed directly by GC on a Hewlett Packard (HP; Palo Alto, CA, USA) model HP-6890 PLUS GLC and HP 3398. A GC chemostation instrument fitted with an column HP-5 (capillary column 0.32 mm in diameter, length 30 m). The oven temperature programme was initially 150°C (4 min) and then the sample was subjected to heat at 250°C (5 min).

Identification of components: The identification of each compound was carried out by comparison of RRT (Relative Retention Time) and mass spectral data obtained with standard methyl esters preparation of palmitic, stearic, oleic, linoleic, myristic acids (obtained from Sigma).

Antimicrobial activity assay: All pure cultures of the microorganisms were taken from the microbiology laboratory of Burdwan Medical College. Inocula of *B. subtilis, S. aureus, P. aeruginosa* and *E. coli* were applied to agar plates (Petri plates) for 24 h and each of them was mixed with a concentration of *C. diurnum* essential oil. The activity was measured by the standard disc diffusion method (Ingolfsdottir et al., 1997). Zones of inhibition were determined by Fischer-Lilly zone reader. The concentration of the reference antibiotic ampicillin was 1 mg/ml (the reference antibiotic was assayed separately to avoid mixing with the sample of oil). All measurements were made in triplicate. The values were expressed as mean ± standard error.

RESULTS AND DISCUSSION

Volatile oils of many plants are known to have antimicrobial activity. This activity could act as chemical defence against plant pathogenic diseases. Pathogens can readily penetrate at wound sites caused, for example, by herbivores. Wounding of leaves which are covered with volatile oil glands results in the rupture of glands causing the oil to flow over the wound. The existence, therefore, of antimicrobial activity in the oil, would be of considerable benefit to the plant. Indeed, a good majority of aromatic and medicinal plants do not succumb too many of the commonest diseases. The Gas chromatographic analysis (Figure 1) of the essential oil of *C. diurnum* showed a very diverse composition with 14 constituents reported in Table 1. The oil was dominated by palmitic acid (27.62%), stearic acid (4.62%) and oleic acid (3.06%). The present study indicated the presence

Table 1. Relative amount of identified and unidentified fatty acid.

Name of Fatty Acids	Retention Time (min)	Relative Amount	Area (pA*s)
Palmetic Acid	13.341	27.62	1638291
Oleic Acid	18.423	3.06	181198
Stearic Acid	19.258	4.62	274152
Peak1	5.358	3.44	204103
Peak2	9.643	7.69	456133
Peak3	9.817	4.93	292183
Peak4	11.055	4.09	242473
Peak5	11.765	16.51	978866
Peak6	14.423	6.22	369157
Peak7	14.548	2.92	173331
Peak8	15.296	8.16	484091
Peak9	15.455	3.14	185969
Peak10	20.348	2.76	163610
Peak11	21.192	4.84	287249

Table 2. Antimicrobial activity of the essential oil from leaves of *Cestrum diurnum* (L).

Species of microorganisms	Inhibition Zone (mm)					Ampicillin (1mg/ml)	Control (Dimethyl sulfoxide mg/100ml)
	1000 ppm	900 ppm	800 ppm	700 ppm	600 ppm		
B. subtilis	0	0	0	0	0	32.56±0.56	0
S. aureus	21±1.527	20±3.055	19±1.154	17±1.00	15±0.577	29.48±0.82	0
P. aeruginosa	24±0.577	20±1.154	18±1.732	16±2.081	14±1.527	31.42± 0.12	0
E. coli	0	0	0	0	0	33.56±0.78	0

of a high percentage of saturated fatty acid in comparison to unsaturated fatty acids. This is probably due to the distribution pattern of *C. diurnum*, as it is a tropical rather than a temperate species.

The result of antimicrobial activity of the essential oil from leaves of *C. diurnum* has been presented in Table 2. Different concentrations of oil showed antibacterial activity against *S. aureus* and *P. aeruginosa*.

Regarding antimicrobial activity, higher sample concentration (1000 ppm) exhibited higher activity against microorganisms used, compared to lower sample concentration (600 ppm). *B. subtilis* and *E. coli* were considered resistant to essential oil since no inhibition zone was observed (Table 2). The reference antibiotic ampicillin showed the highest antimicrobial activity against all tested microorganism.

Because of the appearance of bacterial resistance to antimicrobial agents, more effort is being made to find alternative antimicrobial components. It had been suggested that natural products are preferable to synthetic ones. Present study revealed the role of essential oil of *C. diurnum* as a strong antibacterial agent against *P. aeruginosa* and *S. aureus* in laboratory condition which may considered as a fruitful approach in the search of new drugs.

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