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CHEMICAL INFORMATION FROM GC-MS STUDIES OF METHANOLIC LEAF EXTRACT OF *ANDROGRAPHIS PANICULATA* AND *DATURA METEL* AND THEIR ANTIBACTERIAL ACTIVITY AGAINST ISOLATED *PSEUDOMONAS AERUGINOSA* (PB112) STRAIN

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ABSTRACT

The present study was carried out to determine the phytochemical profile and antibacterial activity of *Andrographis paniculata* and *Datura metel* extracts. For the present investigation, extracts of *A. paniculata* and *D.metel* were prepared by extraction in methanol, chloroform, acetone and benzene and were compared for their antibacterial activity. The antibacterial activities were assessed by measuring the diameter of the inhibition zones by Disc diffusion method. In comparison to this different extracts, the methanol extract showed better antibacterial activity against the isolated *P.aeruginosa* bacterial strain tested. The chemical composition of methanol extracts of these two plants were investigated by GC-MS, while the mass spectra of the compounds found in the extract was matched with the NIST and WILEY library.

KEY WORDS: ***Andrographis paniculata, Datura metel, P.aeruginosa,*** **antibacterial activity, GC-MS analysis.**

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INTRODUCTION

Herbal medicine has wide range in the treatment of several kinds of diseases. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body (Edeoga et al., 2005). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect different diseases. Many plants, herbs and spices used today have been valued for their antimicrobial activity and medicinal properties (Ceylon et al.,2004; De 2004; Davidson et al.,2005).In India, above five hundred medicinal plants are used to control pathogenic bacteria. In recent years, research has been started to evaluate the feasibility of using herbal medicines for controlling diseases specially bacterial disease (Bhubaneswari et al., 2006; Abutbul et al., 2005, Bhattacharjee et al., 2010 and Chatterjee et al., 2011). The present scenario of emergence multiple drug resistance activity has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Service, 1995). In addition to this problem, antibiotics are sometimes associated with adverse effects on the host. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Cordell, 2000). There are several reports on the antimicrobial activity of different plant extracts in different regions of the world (De Boer et al, 2005). In the present study, some plants have been chosen which are used in producing herbal medicine to determine their antibacterial activity. Plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive antimicrobial activities, because their structures are different from those of the more studied microbial sources, and therefore, their mode of action may too very differ (Fabricant and Fansworth 2001). There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity (Prachayasittikul et al., 2008; Nogueira et al., 2008; Costa et al., 2008; Al-Bayati and Al-Mola, 2008; Chen et al., 2008; Pesewu et al., 2008; Turker and Usta, 2008). The present study was carried out to determine the possible chemical components of methanolic leaf extract of *D. metel* and *A. paniculata* using GC-MS and antibacterial activity of leaf extracts of these plants in different solvent system against isolated *Pseudomonas aeruginosa* (PB112) strain. *Andrographis paniculata* (commonly known as Kalmegh) is a herbaceous plant belonging to the family *Acanthaceae*, native to India and Sri Lanka. Mostly the leaves and roots were used for medicinal purposes. It is an annual herb extremely bitter in taste in all parts of the plant body. The genus *Andrographis* Wall. consists of 28 species in tropical Asia. Only a few species are medicinal of which *A. paniculata* is most popular.

Datura metel (Linn) belong to the family Solanaceae is a medicinal plant widely used in phytomedicine to cure diseases. It is a shrub like perennial herb. It is popular all over the world for its medicinal uses.

MATERIALS AND METHODS

a) Plant collection

Mature and fresh leaves of *Andrographis paniculata* and *Datura metel* were collected from the University campus, Kalyani, West Bengal. The leaves were washed thoroughly two times with water and once with distilled water. The plant materials were shed dried and powdered. The powdered samples were kept in separate polythene bags.

b) Plant sample extraction

10 gm powdered leaves of each plant were soaked successively with 50 ml of methanol, chloroform, acetone and benzene in forty eight hours and then filtered through whatman filter paper No.1 along with 2 gm of sodium sulfate to remove the sediments and traces of warter in the filtrate. The filtrates were evaporated to a thick residue at 40°C. The mother extracts were reconstituted according to the solvents that were used to extract them with the concentration of 100 mg/ml to assay the antibacterial activity. For GC-MS study the methanolic extracts of these two leaf samples were then concentrated and reduced to the volume of 2ml. The extracts contain both polar and non-polar phytocomponents of the plant materials. The final extracts were used for GC-MS analysis.

c) Test microorganism

The isolated pure strain of *Pseudomonas aeruginosa* (PB112) was identified by 16S rRNA sequencing and FAME analysis and deposited in the GenBank under accession no. JN996498. The bacterial isolate was cultured in nutrient broth (Himedia) for twenty four hours at 37˚C. The culture was sub-cultured and maintained on nutrient agar slants and stored in refrigerator at 4^0C.

d) Antibacterial activity assay

Minimum *I*nhibitory Concentration (MIC) of the plant extracts obtained by different solvents was determined by Disc diffusion method adopted from Taylor et al. (1995). Bacteria inoculums were spread on the agar plates. The 5 mm diameter sterile Whatman No.1 filter paper discs were soaked and saturated in extracts. Prepared discs were placed on the inoculated agar plate. Levofloxacin (1mg/ml) was used as positive control. The antibacterial assay plates were incubated at 37ºC for 24 hrs. The diameters of the inhibition zones were measured in mm.

e) GC-MS analysis

GC-MS technique was used in this study to identify the phytocomponents present in the extracts. This technique was carried out at the Indian Institute of Chemical Biology, Kolkata, West Bengal. This analysis was performed using GC SHIMADZU – QP5050A system and gas chromatograph interfaced to a mass spectrometer equipped with Elite-I fused silica capillary column. The constituents were identified after comparison with those available in the computer library (NIST and WILEY) attached to the GC-MS instrument and documented.

f) Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database using the NIST and WILEY library having more patterns. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST107 and WILEY229 library. The Name, Molecular Weight, Molecular formula and Structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Sixteen compounds were identified in methanolic fraction of *Andrographis paniculata* leaf extract and twelve compounds were identified in methanolic fraction of *Datura metel* leaf extract by GC-MS analysis. The chromatogram obtained by methanol fraction of *Andrographis paniculata* and *Datura metel* leaf were shown in Fig 1 and Fig 2 respectively. The active Principle, area of the peak, Concentration (%), Retention Time (RT), Molecular formula and Molecular weight were presented in Table 1 and Table 2. Among the sixteen compound of *Andrographis paniculata* the Cyclopentadecanone, 2-Hydroxy (12.84%), Naphthalene (9.14%) and among the twelve compounds of *Datura metel*

the Phytol (13.37%), 9,12,15-Octadecatrien-1-ol (13.33%) were represented in high percentage. The in vitro potency of the crude extracts of the plant as antibacterial were assessed against *Pseudomonas aeruginosa* strain PB112 by measuring the diameter of the clear zone around the discs placed on the petriplates. The extracts of same plant prepared using different solvents showed different inhibitory effect. The inhibitory zone around the antibiotic discs indicated absence of bacterial growth and it was reported as positive and absence of zone as negative. The diameters of the zones were measured using diameter measurement scale. Respective solvents were used as negative control. Acetone and Benzene solvent extract were non-effective against this bacterial pathogen but methanol and chloroform solvent extract were effective. Among them leaf methanolic extracts show good antibacterial activity. The antibiotic levofloxacin (100µg/ml) has been used as positive control which produces highly significant result (35mm) (Table 3). Plants are the important source of for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Tona et al., 1998). The present study shows the potential antibacterial effect of these locally available plant extracts against a bacterial pathogen of aquaculture. Previous results on inhibitory role of methanol leaf extract of these plants are available against *Pseudomonas* strain. The results of present investigation clearly indicate that the antibacterial activity varies with the species of the plant and the solvent used for extraction. Therefore, these plant materials should be further screened for commercial use as antibiotics against the bacterial diseases causing great economic loss in the fish industry.

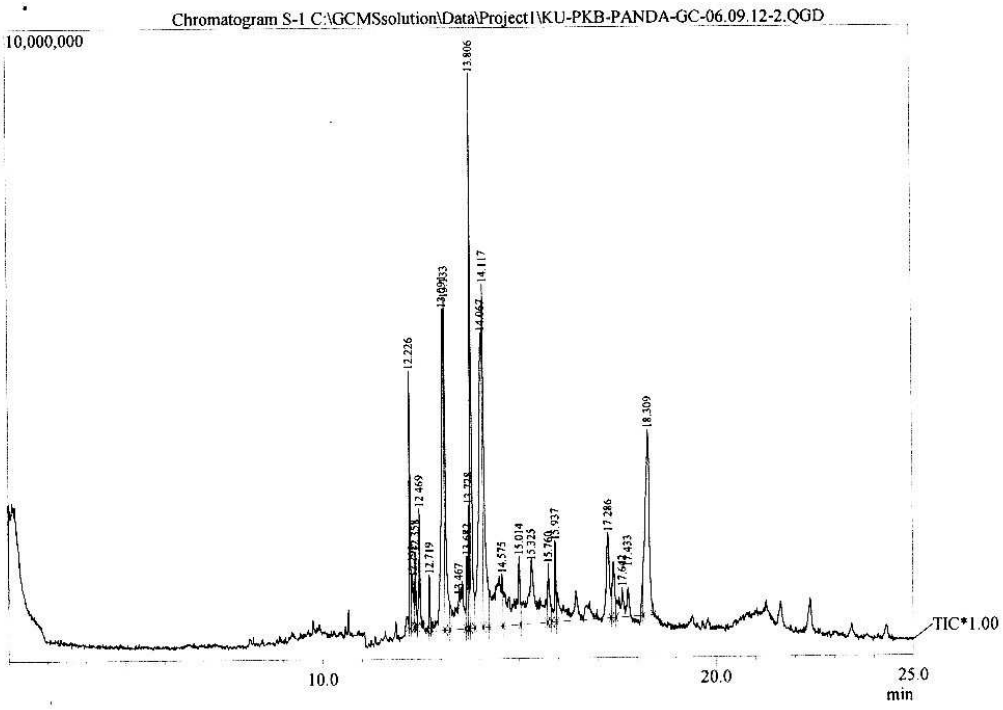

Figure 1
Chromatogram of A. paniculata

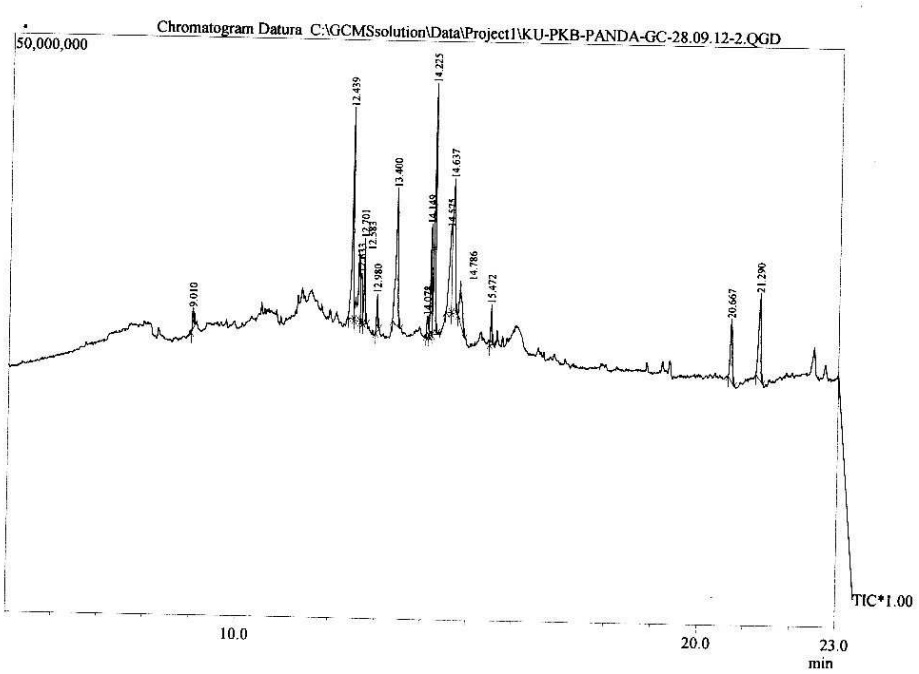

Figure 2
Chromatogram of D metel

Table 1
Characterization of peaks obtained from GC-MS spectrum of methanolic leaf extract of Andrographis paniculata

R.time	Area	Area%	Name	Mol. Formula	Mol.wt	Mass peak	Base peak
12.226	7389167	3.2	Neophytadiene	$C_{20}H_{38}$	278	40	68.15
12.292	2642932	1.14	Androstan-11-amine	$C_{21}H_{37}N$	303	81	124.20
12.469	3929488	1.7	2- Hexadecan-1-ol	$C_{20}H_{40}O$	296	55	81.15
13.133	14139347	6.12	n-Hexadeconoic acid	$C_{16}H_{32}O_2$	256	76	73.10
13.467	9177351	3.97	Cyclodecanol	$C_{10}H_{20}O$	156	176	81.15
13.728	3664754	1.59	9,12,15-Octadecatrienoic acid	$C_{19}H_{32}O_2$	292	68	79.15
13.806	21103468	9.13	Phytol	$C_{20}H_{40}O$	296	33	71.15
14.067	29666172	12.84	Cyclopentadecanone, 2-Hydroxy	$C_{15}H_{28}O_2$	240	96	67.15
14.117	17394697	7.53	8,11,14-Eicosatrienoic acid	$C_{20}H_{34}O_2$	306	98	79.15
14.575	12236087	5.29	E-8-Methyl-9-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	157	55.15
15.014	11272851	4.88	Eicosanoic acid	$C_{20}H_{40}O_2$	312	99	99.15
15.325	15840094	6.85	Stigmast-5-en-3-ol	$C_{29}H_{50}O$	414	214	414
15.760	3031763	1.31	Hexadeconoic acid	$C_{19}H_{38}O_4$	330	146	98.10
15.937	3499129	1.51	Bis(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	390	21	149.15
17.286	17085515	7.39	2 beta-hydroxy-9-oxoverrucosane	$C_{20}H_{32}O_2$	304	162	217.20
18.309	21114364	9.14	Naphthalene	$C_{15}H_{24}$	204	169	107.15

Table 2
Characterization of peaks obtained from GC-MS spectrum of methanolic leaf extract of *Datura metel*

R.time	Area	Area%	Name	Mol. Formula	Mol.wt	Mass peak	Base peak
9.010	3703202	1.11	2-Methoxy-4-vinylphenol	$C_9 H_{10} O_2$	150	62	77.05
12.439	40014118	11.97	1-Octadecyne	$C_{18} H_{34}$	250	67	43.10
12.633	10550820	3.16	Alpha-[5-Ethyl-2-furyl]glycine	$C_8 H_{11} NO_3$	169	75	124.10
12.701	12910931	3.86	1-Eicosyne	$C_{20} H_{38}$	278	70	43.10
13.400	38923213	11.64	1,2-Benzenedicarboxylic acid	$C_{30} H_{50} O_4$	474	84	149.05
14.149	17989490	5.38	9,12,15-Octadecatrienoic acid	$C_{19} H_{32} O_2$	292	94	79.10
14.225	44699915	13.37	Phytol	$C_{20} H_{40} O$	296	54	71.10
14.575	29059496	8.69	Z-10-Pentadecenol	$C_{15} H_{30} O$	226	133	55.10
14.637	44545626	13.33	9,12,15-Octadecatrien-1-ol	$C_{18} H_{32} O$	264	100	79.10
14.786	14012328	4.19	Hyoscyamine	$C_{17} H_{23} NO_3$	289	78	124.10
20.667	16118571	4.82	17-(1,5-Dimethylhexyl)-2,3-dihydroxy-10	$C_{27} H_{44} O_3$	416	135	151.15
21.290	24754546	7.41	Vitamin e	$C_{29} H_{50} O_2$	430	90	165.15

Table 3
Antibacterial activities of these plant extracts (mg/ml) and antibiotic (100 µg /ml) against Pseudomonas aeruginosa strain PB112 tested by disc diffusion assay. "–" indicates absence.

Name of plants	Solvent used	Zone of inhibition (Diameter in mm)			
		100mg/ml	50mg/ml	control	Levofloxacin
Andrographis paniculata	Acetone	-	-	-	35±1.5
	Benzene	-	-	-	
	Chloroform	3±0.05	1±0.05	-	
	Methanol	15±0.15	12±0.5	-	
Datura metel	Acetone	-	-	-	35±1.5
	Benzene	-	-	-	
	Chloroform	2±0.12	-	-	
	Methanol	10±0.5	8±0.5	-	

CONCLUSION

The study reveals that the chemical information of two plants namely *Andrographis paniculata* and *Datura metel* which has been used to control the bacteria. This study suggested that *A.paniculata* and *D.metel* contain many organic and inorganic substances which have been listed in table 1 and 2.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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