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QUALITATIVE AND GC-MS ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS OF TICK WEED (*CLEOME VISCOSA* L.)

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ABSTRACT

The potential of an allelopathic plant to exert direct and indirect effects depends in large part on the chemistry of the plant and whether putative allelochemicals reach meaningful levels in the environment surrounding the plant. *Cleome viscosa* L. (Capparidaceae) (synonym: *C. icosandra* L.) is a weed distributed throughout the tropics of the world and the plains of India Results on the qualitative analysis on the root, stem and leaves of *C. viscosa* showed that the presence of saponins and flavonoids in all their three organs. The presence of alkaloids was noticed only in Wagner's test not in the Mayor's and Dragendorff's test. GC-MS results of whole plant of *C.viscosa* showed the presence 3-O-Methyl-d-glucose (73%), followed by Benzofuran, 2,3-dihydro (9.844%) and : n-Hexadecanoic acid (4.707%) of the total 32 compounds.

Keywords: *Cleome viscosa*, GC-MS analysis, phytochemical.

1. INTRODUCTION

Allelochemicals are the small molecular weight compounds excreted from plants during the process of secondary metabolism (Rice, 1984). These chemicals usually accumulated in plants, soils, and other surrounding organisms. These compounds also vary in chemical composition, concentration and localization in plant tissues and from plant to-plant with changes in both biotic and abiotic conditions. (Waller and Einhellig, 1999). Allelochemicals produced in the tissues of such plants may enter soils as leaf leachates or root exudates or during tissue decomposition (Inderjit and Duke, 2003).There is even evidence for air borne allelopathy mediated by volatile allelochemicals (Matsuyama *et al.,* 2000).Impacts of putative allelochemicals produced by plants on other organisms can be direct, mediated through their acute or chronic toxicity to physiological processes in target organisms (Bais *et al*., 2003). Impacts can also be indirect, where putative allelochemicals modify the environment for other organisms in some way, such as through alterations in soil microbial communities, nutrient availability, or pH (Blum *et al.,* 1993).

The potential of an allelopathic plant to exert direct and indirect effects depends in large part on the chemistry of the plant and whether putative allelochemicals reach meaningful levels in the environment surrounding the plant. The suite of compounds possessed by each species has the potential to have both direct and indirect allelopathic effects, assuming that they can reach bioactive levels in the environment outside of the plant. For example, phenolic acids are noted for their direct toxicity to some organisms (Chon and Kim, 2002) and are also capable of interacting with nutrients in soils (Blum *et al.,* 1993), altering their availability to target organisms. The importance of such indirect effects probably varies across environmental gradients in the field.

Recently, allelopathy is getting more and more important. One reason is that this concept helps in the organic or natural farming without or less use of synthetic agrochemicals (herbicide, insecticide, fungicides, etc.). Other reason is the understanding of allelopathy in natural ecosystems. Allelochemicals belong to "Secondary metabolites". Secondary metabolites mean not indispensable constituents in plants and exist only in plant kingdom. In the past, the meaning of these chemicals in plants seemed to be a pool of energy or reducing agents, or simple wastes. But recently, the Allelopathy hypothesis describes the real meaning of these secondary metabolites as a tool of immobile plants to protect themselves from surrounding plants or other life that might attack them, or a tool to communicate each other or to communicate with other life for their survival. It has been commonly assumed that there are more than 500,000 plant species and more than 30,000 secondary natural chemicals in this world. However, we are sure that there are still many natural chemicals unknown to us. Then the third importance of allelochemicals is their use as a source of new agrochemicals (Yoshiharu Fujii, 2009). Chemical components known to exert pharmaceutical effects may also be effective in allelopathy against other plants. On the other hand, chemical screening for allelopathy may lead us to the discovery of new biologically functional compound. “Interdisciplinary information sharing” must, thus, be desired for a broader aspect. Hence the present work has been aimed to investigate the phytochemical analysis of Tick weed (*Cleome viscosa* L.) using GC-MS techniques.

2. MATERIALS AND METHODS

2.1. Qualitative phytochemical analysis

Qualitative phytochemical analysis (Jigna and Sumitra, 2007) of the crude powder of Tick weed (Cleome viscosa L.) was as follows:). Tannins (200 mg plant material in 10 ml distilled water, filtered); a 2 ml filtrate + 2 ml $FeCl_3$, blue-black precipitate indicated the presence of Tannins. Alkaloids (200 mg plant material in 10 ml methanol, filtered); a 2 ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of MayorÕs reagents/WagnerÕs reagent/Dragendroff reagent, creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids. Saponins (frothing test: 0.5 ml filtrate + 5 ml distilled water); frothing persistence indicated presence of saponins. Cardiac glycosides (Keller-Kiliani test: 2 ml filtrate + 1 ml glacial acetic acid + $FeCl_3$ + conc. H_2SO_4); green-blue color indicated the presence of cardiac glycosides. Steroids (Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered); a 2 ml filtrate + 2 ml acetic anhydride + conc. H_2SO_4. blue-green ring indicated the presence of terpenoids. Flavonoids (200 mg plant material in 10 ml ethanol, filtered); a 2 ml filtrate + conc. HCl + magnesium ribbon pink-tomato red color indicated the presence of flavonoids (Oguyemi ,1979).

2.2. GC-MS analysis

GC-MS analysis was carried out in SASTRA University, Thanjavur, Tamil Nadu. GC Clarus 500 Perkin Elmer system interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-5ms fused silica capillary column (30 x 0.25 mm ID x 0.25μm film thickness, composed of 5% phenyl 95% Dimethyl polysiloxane), operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 1.0 μI was employed (split ratio of 10:1) injector temperature 290 ºC; ion-source temperature 200ºC. The oven temperature was programmed from 50ºC, with an increase of 87 ºC/min, to 220ºC hold for 5min, then 8ºC /min to 280ºC hold for 10 min. Mass spectra were taken at 70eV; a scan interval of 0.2 seconds and fragments from 40 to 600 Da.

2.3. Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the separated components was compared with the spectrum of NIST library database. The identity of the spectra above 95% was needed for the identification of components.

3. RESULTS AND DISCUSSION

Cleome viscosa L. (Capparidaceae) (synonym: *C. icosandra* L.) is a weed distributed throughout the tropics of the world and the plains of India (Nadkarni, 1982). The plant is an annual, sticky herb with a strong penetrating odour, and is clothed with glandular and simple hairs. It grows about 30–90 cm high and is branched. The leaves are 3–5 foliate, obovate, and obtuse, gradually becoming shorter upward. The flowers are yellow, axillary, growing out into a lax raceme. The fruits are capsules, compressed and hairy throughout, while the seeds are finely transversely striate, sub globose, and become brownish-black when ripe (Vaidyaratnam, 1994). *C.viscosa* is known by various names such as wild mustard, dog mustard, and sticky cleome. In India, the plant is known by various vernacular names such as Hul-Hul, Pashugandha, Pivala tilvana, Kanphuti, Talwani,Naikkadugu etc.

Results on the qualitative analysis on the root, stem and leaves of *C.viscosa* showed (Table-1) that the presence of saponins and flavonoids in all their three organs. The presence of alkaloids was noticed only in Wagner's test not in the Mayor's and Dragendorff's test. GC-MS results of whole plant of *C.viscosa* showed (Fig-1 and Table-2) the presence 3-O-Methyl-d-glucose (73%), followed by Benzofuran, 2,3-dihydro (9.844%) and : n-Hexadecanoic acid (4.707%) of the total 32 compounds.

The seeds of *C. viscosa* are reported to have nutritive value, and have been found safe as edible material for human beings. The seeds are reported to contain 18.3% oil, a mixture of five fatty acids, seven amino acids, and sugar sucrose (Rukmini and Deosthale, 1979). The oil obtained from the seeds is rich in linoleic acid and other fatty acids such as palmitic, stearic, oleic, and linolinic (Rukmini, 1978; Afaq *et al.,* 1984; Deora *et al*., 2003). Gupta and Dutt (1938) reported two chemical constituents, viscocic and viscosin (a monomethoxy trihydroxyfavone), from the seeds. A novel umbelliferone derivative, designated as cleosandrin, has been isolated from the ethanol extract of the seeds (Ramchandran, 1979). The seeds are also reported to contain cleomiscosin- A, a coumarinolignoid (Ray *et al.,* 1980); cleomiscosin B (Ray *et al*., 1982); and cleomiscosin- C (Ray *et al*., 1985) and its regioisomer cleomiscosin D, a minor coumarino-lignan (Kumar *et al.,* 1988). Chattopadhyay *et al.* (2007) have developed a simple, accurate, and reproducible reverse-phase high performance liquid chromatography (HPLC) method for identifcation and quantifcation of two isomeric coumarino-lignoids, cleomiscosin A and B, in different extracts of the seeds using photodiode array detection at 326 nm.

Table 1. Qualitative phytochemical analysis of *C. viscosa*.

Weed Parts	Alkaloids: Mayor's test	Alkaloids: Wagner's test	Alkaloids: Dragendorff's test	Saponins	Flavonoids	Steroids	Cardiac glycosides
Root	-	+	-	+	+	-	-
Stem	-	+	-	+	+	-	-
Leaf	-	++	-	++	++	-	-

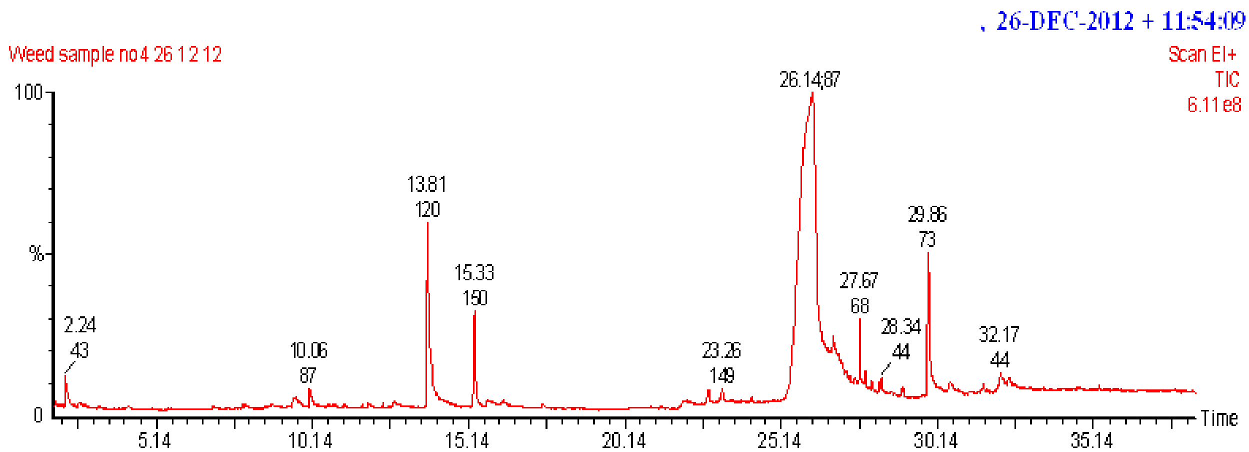

Fig.1. GC-MS spectrum of *C. viscosa.*

Table 2. Results of Phytochemical constituents of *C. viscosa* using GC-MS.

S.No.	Peak Name	Retention time	Peak area	%Peak area
1.	Name: 1,2-Ethanediol, monoacetate Formula: $C_4H_8O_3$ MW: 104	2.68	566902	0.1447
2.	Name: Acetic acid, 1-methylethyl ester Formula: $C_5H_{10}O_2$ MW: 102	3.33	191896	0.0490
3.	Name: 5-Hexen-2-one Formula: $C_6H_{10}O$ MW: 98	5.61	313432	0.0800
4.	Name: 2-Cyclopenten-1-one, 2-hydroxy- Formula: $C_5H_6O_2$ MW: 98	6.99	368597	0.0941
5.	Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: $C_6H_8O_4$ MW: 144	7.90	100411	0.0256
6.	Name: Alpha-monopropionin Formula: $C_6H_{12}O_4$ MW: 148	8.83	392761	0.1003
7.	Name: 5-Hexen-3-ol, 2,2,4-trimethyl- Formula: $C_9H_{18}O$	10.06	2105867	0.5375

	MW: 142			
8.	Name: 3-Penten-2-one, 3-ethyl-4-methyl- Formula: $C_8H_{14}O$ MW: 126	10.67	200057	0.0511
9.	Name: Cyclohexanol, 4-[(trimethylsilyl)oxy]-, cis- Formula: $C_9H_{20}O_2Si$ MW: 188	11.74	496708	0.1268
10.	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: $C_6H_8O_4$ MW: 144	11.93	880701	0.2248
11.	Name: cis-á-Terpineol Formula: $C_{10}H_{18}O$ MW: 154	12.41	336950	0.0860
12.	Name: 2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)- Formula: $C_{10}H_{16}$ MW: 136	12.75	413609	0.1056
13.	Name: Benzofuran, 2,3-dihydro- Formula: C_8H_8O MW: 120	13.81	38569260	9.8447
14.	Name: 2-Methoxy-4-vinylphenol Formula: $C_9H_{10}O_2$ MW: 150	15.32	12808473	3.2693
15.	Name: Hydroquinone Formula: $C_6H_6O_2$ MW: 110	15.76	3461378	0.8835
16.	Name: Phenol, 2,6-dimethoxy- Formula: $C_8H_{10}O_3$ MW: 154	16.25	3979017	1.0156
17.	Name: Benzaldehyde, 3-hydroxy-4-methoxy- Formula: $C_8H_8O_3$ MW: 152	18.03	117271	0.0299
18.	Name: Caryophyllene Formula: $C_{15}H_{24}$ MW: 204	18.75	130861	0.0334
19.	Name: 2-Butenoic acid, 4,4-dimethoxy-, methyl ester Formula: $C_7H_{12}O_4$ MW: 160	21.29	228778	0.0584
20.	Name: 3',5'-Dimethoxyacetophenone Formula: $C_{10}H_{12}O_3$ MW: 180	22.82	2371573	0.6053
21.	Name: Benzoic acid, 2-(1-oxopropyl)- Formula: $C_{10}H_{10}O_3$ MW: 178	23.26	1320971	0.3372
22.	Name: Megastigmatrienone Formula: $C_{13}H_{18}O$ MW: 190	24.20	581219	0.1484
23.	Name: 3-O-Methyl-d-glucose Formula: $C_7H_{14}O_6$ MW: 194	26.14	288638752	73.6740
24.	Name: Myo-Inositol, 4-C-methyl- Formula: $C_7H_{14}O_6$ MW: 194	26.71	1695484	0.4328
25.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$	27.67	4107243	1.0484

	MW: 296			
26.	Name: 2-Pentadecanone, 6,10,14-trimethyl- Formula: $C_{18}H_{36}O$ MW: 268	27.83	969780	0.2475
27.	Name: Methyl 6-methyl heptanoate Formula: $C_9H_{18}O_2$ MW: 158	29.06	1177290	0.3005
28.	Name: n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$ MW: 256	29.86	18441238	4.7071
29.	Name: 7-Hydroxy-6-methoxy-2H-1-benzopyran-2-one Formula: $C_{10}H_8O_4$ MW: 192	30.55	1856237	0.4738
30.	Name: 13-Octadecenal, (Z)- Formula: $C_{18}H_{34}O$ MW: 266	32.17	3461713	0.8836
31.	Name: Octadecanoic acid Formula: $C_{18}H_{36}O_2$ MW: 284	32.45	1015500	0.2592
32.	Name: 2H-Pyran-2-one, tetrahydro-6-nonyl- Formula: $C_{14}H_{26}O_2$MW: 226	35.30	478497	0.1221

Most tissues of plant, such as leaf, flower, fluid, stem, root and seed, even litter, can release a certain amount of allelochemicals into the surrounding environments. These allelochemicals can be very different as different parts or tissues of plants have different physiological functions. The extracts from the roots and stems were reported (Mo and Fan, 2001) that have autotoxicity and inhibit the root- ing and germination processes of *Braguiera gymnorrhiza,* yet other parts of the plants can stimulate its germination. Wu *et al.* (2001) examined the changes in allelopathic con tent 2,4-dihydroxy-7-methoxy 1,4-benzoxazin 3-one (DIMBOA) in different parts of wheat, and found that DIMBOA level in the root tissues is the highest followed by the stems. Ben-Hammouda *et al.* (2002) studied barley autotoxicity from the roots, stems and leaves extraction of barley, and the result showed that the leaves were the most important source of allelopathic substances, and the rootswere the last. Ben-Hammouda *et al.* (2001) also investi- gated the phytotoxicity of Hordeum vulgare on Triticum durum and *T. aestivum,* and showed that the allelopathic potential increased with physiological maturity, and leaves and roots were the most phytotoxic plant pats in *H. vulgare* plant parts.

Rice (1984) has classified these allelochemicals into 14 categories based on their diversiform chemical structures. Alleolochemicals, which can inhibit the growth of weeds,become the most favorable choice for natural pesticides (Nagabhushana *et al.,* 2001; Reigosa *et al.,* 2001). There have already been many identified allelochemicals that can be used to produce natural weedicides or pesticides (Xuan *et al.,* 2002). Some studies (Olofsdotter *et al.,* 2002) also show how to use allelochemicals as additives. In short, high diversity of allelochemicals means that they can be used in multiple purposes (Eng *et al.,* 2004). Extracting or synthesizing these compounds has great important ecological significance and economic potentials. The allelochemicals will become an important impetus for eco-agricultural development. On the other hand, studies on allelopathy can help explain the inhibitory effects or toxicity in the processes of rotation, intercrop and mulch and such studies can also help avoid wasting billions of dollars in worldwide agricultural practices.

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