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 of 3-O-Methyl-d-glucose (73%), followed by Benzofuran, 2,3-dihydro (9.84%) and $n$-Hexadecanoic acid (4.70%) 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).

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 FeCl3, 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 MayorÖs reagents/WagnerÖs reagent/Dragnetoff 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 + FeCl3 + conc. H2SO4); 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. H2SO4. 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 μl was employed (split ratio of 10:1) injector temperature 290 °C; ion-source temperature 200°C. The oven temperature was programmed from 50°C, with an increase of 87 °C/min, to 220°C hold for 5min, then 8°C /min to 280°C 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, ovate, 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, viscosic 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,
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 identification and quantification 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 | Mayor’s test | Wagner’s test | 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              | 2.68           | 566902    | 0.1447     |
|       | Formula: C4H8O3                                |                |           |            |
|       | MW: 104                                        |                |           |            |
| 2.    | Name: Acetic acid, 1-methylethyl ester         | 3.33           | 191896    | 0.0490     |
|       | Formula: C5H10O2                               |                |           |            |
|       | MW: 102                                        |                |           |            |
| 3.    | Name: 5-Hexen-2-one                            | 5.61           | 313432    | 0.0800     |
|       | Formula: C6H10O                                |                |           |            |
|       | MW: 98                                         |                |           |            |
| 4.    | Name: 2-Cyclopenten-1-one, 2-hydroxy-8-one    | 6.99           | 368597    | 0.0941     |
|       | Formula: C5H6O2                                |                |           |            |
|       | MW: 98                                         |                |           |            |
| 5.    | Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one | 7.90      | 100411    | 0.0256     |
|       | Formula: C6H8O4                                |                |           |            |
|       | MW: 144                                        |                |           |            |
| 6.    | Name: Alpha-monopropionin                      | 8.83           | 392761    | 0.1003     |
|       | Formula: C6H12O4                               |                |           |            |
|       | MW: 148                                        |                |           |            |
| 7.    | Name: 5-Hexen-3-ol, 2,2,4-trimethyl-10-one     | 10.06          | 2105867   | 0.5375     |
| No. | Name                                                                 | Formula               | MW  | Purity  | CAS Number | Density |
|-----|----------------------------------------------------------------------|-----------------------|-----|---------|------------|---------|
| 8   | 3-Penten-2-one, 3-ethyl-4-methyl-                                      | C₈H₁₄O               | 142 | 10.67   | 200057     | 0.0511  |
| 9   | Cyclohexanol, 4-[(trimethylsilyl)oxy], cis-                           | C₉H₂₀O₂Si            | 126 | 11.74   | 496708     | 0.1268  |
| 10  | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-                   | C₆H₉O₄              | 188 | 11.93   | 880701     | 0.2248  |
| 11  | cis-á-Terpineol                                                      | C₁₀H₁₈O              | 144 | 12.41   | 336950     | 0.0860  |
| 12  | 2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-                              | C₁₀H₁₆               | 136 | 12.75   | 413609     | 0.1056  |
| 13  | Benzofuran, 2,3-dihydro-                                            | C₈H₈O               | 120 | 13.81   | 38569260   | 9.8447  |
| 14  | 2-Methoxy-4-vinylphenol                                             | C₉H₁₀O₂              | 150 | 15.32   | 12808473   | 3.2693  |
| 15  | Hydroquinone                                                        | C₆H₆O₂               | 110 | 15.76   | 3461378    | 0.8835  |
| 16  | Phenol, 2,6-dimethoxy-                                              | C₈H₁₀O₃              | 154 | 16.25   | 3979017    | 1.0156  |
| 17  | Benzaldehyde, 3-hydroxy-4-methoxy-                                   | C₈H₈O₃              | 152 | 18.03   | 117271     | 0.0299  |
| 18  | Caryophyllene                                                       | C₁₅H₂₄               | 204 | 18.75   | 130861     | 0.0334  |
| 19  | 2-Butenoic acid, 4,4-dimethoxy-, methyl ester                       | C₇H₁₂O₄              | 160 | 21.29   | 228778     | 0.0584  |
| 20  | 3',5'-Dimethoxyacetophenone                                         | C₁₀H₁₂O₃             | 180 | 22.82   | 2371573    | 0.6053  |
| 21  | Benzoic acid, 2-(1-oxopropyl)-                                      | C₁₀H₁₀O₃             | 178 | 23.26   | 1320971    | 0.3372  |
| 22  | Megastigmatrienone                                                  | C₁₃H₁₈O              | 190 | 24.20   | 581219     | 0.1484  |
| 23  | 3-O-Methyl-d-glucose                                                | C₇H₁₄O₆              | 194 | 26.14   | 288638752  | 73.6740 |
| 24  | Myo-Inositol, 4-C-methyl-                                            | C₇H₁₄O₆              | 194 | 26.71   | 1695484    | 0.4328  |
| 25  | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol                               | C₂₀H₄₀O              | 27.67| 27.67   | 4107243    | 1.0484  |
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 content 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 roots were the last. Ben-Hammouda et al. (2001) also investigated 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 parts in *H. vulgare* plant parts.

Rice (1984) has classified these allelochemicals into 14 categories based on their diversiform chemical structures. Allelochemicals, 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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