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RESEARCH ARTICLE

LEAF PHYTOCHEMISTRY OF INVASIVE ALIEN SPECIES 'BILLY GOAT WEED' (*AGERATUM CONYZOIDES* L.)

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ABSTRACT

The present investigation was aimed to investigate the phytochemical profile of invasive weed species 'Billy goat weed' (*Ageratum conyzoides* L.) leaves using GC-MS techniques. Results of preliminary analysis of phytochemical screening showed that the presence of alkaloids, flavonoids, terpenoids, glycosides, steroids, tannins, volatile oils and,saponin in methanolic leaf extracts. In pet.ether, alkaloids, flavonoids and volatile oils were noticed. But in chloroform extract, alkaloids alone was found. The quinone was not found in all the five solvents i.e.pet. ether, methanolic, chloroform, ethanolic and aqueous extracts. The GC-MS study revealed the presence of thirty four compounds in the leaves of *A. conyzoides*. Among the identified compounds, the principal compounds are Glycerin (33.23%), Benzofuran, 2,3-dihydro (12.06%), n-Hexadecanoic acid (10.62%),2H-1-Benzopyran-2-one (8.35%) and 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (7.611%).

INTRODUCTION

Allelopathy in natural and agricultural ecosystems is receiving increasing attention because allelochemicals significantly reduce the growth of other plants (Inderjit and Duke, 2003). Allelochemicals are secondary plant products or waste products generated by the plant's main metabolic pathways which are released into the environment (Putnam, 1988). These chemicals are present in many plants and in many organs, including leaves, flowers, fruits and buds (Putman and Tang, 1986). Under certain conditions these compounds are released into the environment, either as exudates from living tissue or by decomposition of plant residues in sufficient quantities to affect neighboring or successional plants (Einhellig, 1986). Allelochemicals include a wide range of phenolic acids such as benzoic and cinnamic acids, alkaloids, terpenoids and others (Rice, 1984). Evidence of allelopathy has accumulated in the literature over many years and many kinds of allelochemicals have been isolated and characterized from various plants (Duke, 1986; Seigler, 1996). 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, that there are still many natural chemicals unknown to us (Yoshiharu Fujii, 2009). The allelochemicals will become an important impetus for eco-agriculturaldevelopment. On the other hand, studies on allelopathycan help to explain the invasiveness of plant species in the particular environment.There are some general and well-studied traits shared by successful invasive plants such as high seed production, rapid dispersal, high germinability, rapid growth rate, early maturity, phenotypic flexibility (Williamson and Fitter, 1996; Maillet and Lopez-Garcia, 2000; Leps*et al*., 2002). It poses one of the most serious threats to biodiversity, causing major changes in vegetation at a global level (Vitousek *et al*., 1996; Mack *et al*., 2000). The Invasive Alien species *A.conyzoides* is a native of tropical America. It has spreads worldwide in the tropical and subtropical areas (Wagner *et al*., 1999). In India, it was introduced in 1860 as an ornamental plant (National Focal Point for APFISN, India, 2005). Later it escaped as a weed in various habitats throughout India since their successful invasive potential. Hence, the present investigation was aimed to investigate the phytochemical profile of invasive weed species 'Billy goat weed' (*Ageratum conyzoides* L.) leaves using GC-MS techniques.

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MATERIALS AND METHODS

The leaves of invasive alien weed species *A.conyzoides* were collected from the crop fields of Adur-Agaram village, near Kurinjipadi,Cuddalore District, Tamil Nadu and qualitative phytochemical analysis (Jigna and Sumitra, 2007) of the crude leaf powder of *A.conyzoides* 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 Mayor's reagents/Wagner's 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 colour 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 mlfiltrate + conc. HCl + magnesium ribbon pink-tomato red colour indicated the presence of flavonoids (Ogunyemi, 1979).

GC-MS analysis

GC-MS analysis was carried out at CARISM 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 8 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.

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.

RESULTS AND DISCUSSION

A. conyzoides L. is an erect, annual, branched, slender, hairy and aromatic herb, which grows to approximately 1 m in height (Fig.1). The stems and leaves are covered with fine white hairs, the leaves are stalked, ovate, 4-10 cm long and 1-5 cm wide, with tip and base somewhat pointed and with round-toothed margins long. The flowers are purple to white, less than 6 mm across and arranged in close terminal inflorescences. The fruit is black and are easily dispersed while the seeds are photoblastic and often lost within 12 months (Marks and Nwachuku, 1986). The plant has a purplish blue coloured inflorescence which produces an enormous number of seeds (Cypsela; more than 40,000 from a single plant). These seeds easily dispersed into wide areas in the hilly tracts by wind and water, which will help in their establishment in a wide range of climatic conditions (Kohli *et al*., 2006). The plant grows commonly in waste and on ruined sites. It has a peculiar odour likened in Australia to that of a male goat and hence its name 'goat weed' or 'billy goat weed'. Results on the qualitative phytochemical analysis on the leaves of *A. conyzoides* showed (Table-1) that the presence of alkaloids, flavonoids, terpenoids, glycosides, steroids, tannins, volatile oils and, saponinin in methanolic leaf extracts.

In pet.ether, alkaloids, Flavonoids andvolatile oils were noticed. But in chloroform extract, alkaloids alone found. The quinone was not found in all the five solvents i.e., pet ether, methanolic, chloroform, ethanolic and aqueous extracts. The GC-MS study revealed the presence of thirty four compounds were identified in methanol extract of leaves of *A. conyzoides* (Table 2 and Fig. 2). Among the identified compounds, the principal compounds are recorded based on their percentage of peak area are Glycerin (33.23%), Benzofuran, 2,3-dihydro (12.06%), n-Hexadecanoic acid (10.62%), 2H-1-Benzopyran-2-one (8.35%) and 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (7.611%). *A.conyzoides* is a strong invader and increased their abundance, cover and density poses a threat to the native species which also include medicinally important species in Shivalik hills of Himachal Pradesh. The invasion of *A. conyzoides* also alters the physico-chemical properties of soils in the invaded areas. The soils in the invaded areas become nutrient rich which generally help in the growth of invasive species (Dogra *et al*., 2009).

The allelochemicals besides imparting the plant allelopathic property also regulate the biotic communities of soil and alter the physical and chemical properties of soil (Nardi *et al*., 2000). Many studies suggest that allelopathy maycontribute to the ability of particular alien species tobecome dominant in the native plant communities (Abdul Wahab and Rice, 1967; Vaughn and Berhow, 1999; Ridenour and Callaway, 2001). Several aggressiveweeds exhibit the phenomenon of allelopathy as amechanism of interference which provides them competitive advantage over other plants. Abundant evidences support the idea that higher resource availability increases the susceptibility to invasion of plant communities (Burke and Grim, 1996; Maron and Connor, 1996). Further, Dietz *et al*. (1996) concluded that factors other than allelopathy might be operating in nature that favours rapid establishment and persistence of dense stands of alien species.

Invasive species are capable of altering ecosystem services by affecting populations, community interactions, ecosystem processes, and abiotic variables. Virtually all ecosystem services can be negatively impacted by invasive species, although positive impacts do exist. Many invasive species cause cascading effects in communities and/or affect both biotic and abiotic components of ecosystems (Charles and Dukes, 2007). Invasive plants may decrease the suitability of soil for other species by secreting salts (e.g., Tamarisk, Zavaleta, 2000; the ice plant *Mesembryanthemum crystallinum*, Vivrette and Muller, 1977) by acidifying the soil, or by releasing novel chemical compounds, as in allelopathy (Callaway and Ridenour 2004). Hence, the allelochemicals

Table 1. Preliminary phytochemical screening of *A. conyzoides* leaves

Phytochemicals	Test	Pet. Ether Extract	Methanol Extract	Chloroform Extract	Ethanol	Aqueous
Alkaloids	Mayer's reagent	+	+	+	+	+
Flavonoids	Ferric chloride test	+	+	-	+	+
Terpenoids	Noller's test	-	+	-	+	+
Glycosides	Raymond's Test	-	+	-	+	-
Steroids	Libermann-Burchard test	-	+	-	-	+
Tannins	Lead acetate test	-	+	-	-	-
Volatile Oils	Lead acetate test	+	+	-	-	-
Saponin	Foam test	-	+	-	-	+
Quinone	Sodium hydroxide test	-	-	-	-	-

Table 2. Phytochemical profiles of *A. conyzoides*. leaves by GC-MS

S.No.	Retention Time	Peak Name	Molecular Formula	Molecular Weight	Peak Area	% Peak area
1	8.41	Glycerin	$C_3H_8O_3$	92	165155744	33.23
2	9.91	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	2065563	0.4157
3	10.29	Benzofuran, 2,3-dihydro	C_8H_8O	120	59925552	12.06
4	12.74	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	8708967	1.752
5	13.33	Phenol, 2-methoxy-4-(1-propenyl)	$C_{10}H_{12}O_2$	164	310958	0.0626
6	14.10	2H-1-Benzopyran-2-one, 3,4-dihydro	$C_9H_8O_2$	148	6039262	1.215
7	14.30	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]	$C_{15}H_{24}$	204	2517041	0.5066
8	14.79	2-(2-Naphthyl)-2-propanol	$C_{13}H_{14}O$	186	1082822	0.2179
9	14.90	à-Caryophyllene	$C_{15}H_{24}$	204	257697	0.0519
10	15.06	2H-1-Benzopyran, 7-methoxy-2,2-dimethyl	$C_{12}H_{14}O_2$	190	1958019	0.3941
11	15.19	2H-1-Benzopyran-2-one	$C_9H_6O_2$	146	41266860	8.305
12	15.82	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]	$C_{15}H_{24}$	204	2347362	0.4724
13	16.46	2,2'-Isopropylidenedifuran	$C_{11}H_{12}O_2$	176	528657	0.1064
14	16.63	4-Methoxy-7-methylindan-1-one	$C_{11}H_{12}O_2$	176	5331383	1.072
15	16.75	1,6-Anhydro-á-d-talopyranose	$C_6H_{10}O_5$	162	8875198	1.786
16	16.96	(-)-Spathulenol	$C_{15}H_{24}O$	2201	3414951	0.6873
17	17.63	Acetate, (2,4a,5,8a-tetramethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenyl) ester	$C_{16}H_{26}O_2$	250	1288065	0.2592
18	17.98	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl	$C_{13}H_{16}O_3$	220	1171784	0.2358
19	18.11	1(2H)-Naphthalenone, 7-(1,1-dimethylethyl)-3,4-dihydro	$C_{14}H_{18}O$	202	3805298	0.7658
20	18.28	1H-Inden-1-one, 7-(1,1-dimethylethyl)-2,3-dihydro-3,3-dimethyl	$C_{15}H_{20}O$	216	15626174	3.144
21	18.57	Undecanal	$C_{11}H_{22}O$	170	2463641	0.4958
22	18.68	Ethanone, 1-(7-hydroxy-5-methoxy-2,2-dimethyl-2H-1-benzopyran-6-yl)	$C_{14}H_{16}O_4$	248	20407990	4.107
23	19.34	exo-2-Hydroxycineole	$C_{10}H_{18}O_2$	170	494998	0.0996
24	19.46	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	3097080	0.6233
25	20.09	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	13490652	2.715
26	21.18	Ethanone, 1,1'-(6-hydroxy-2,5-benzofurandiyl) bis	$C_{12}H_{10}O_4$	218	726438	0.1462
27	21.44	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1475790	0.2970
28	22.53	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	52791848	10.62
29	24.63	4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester	$C_{13}H_{15}NO_3$	233	2789064	0.5613
30	25.27	9-Eicosene, (E)	$C_{20}H_{40}$	280	695420	0.1400
31	25.40	Phytol	$C_{20}H_{40}O$	296	20155876	4.056
32	26.45	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	6366395	1.281
33	26.69	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	$C_{18}H_{30}O_2$	278	37820564	7.611
34	27.00	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2437851	0.4906

Fig. 1. ***A. conyzoides***

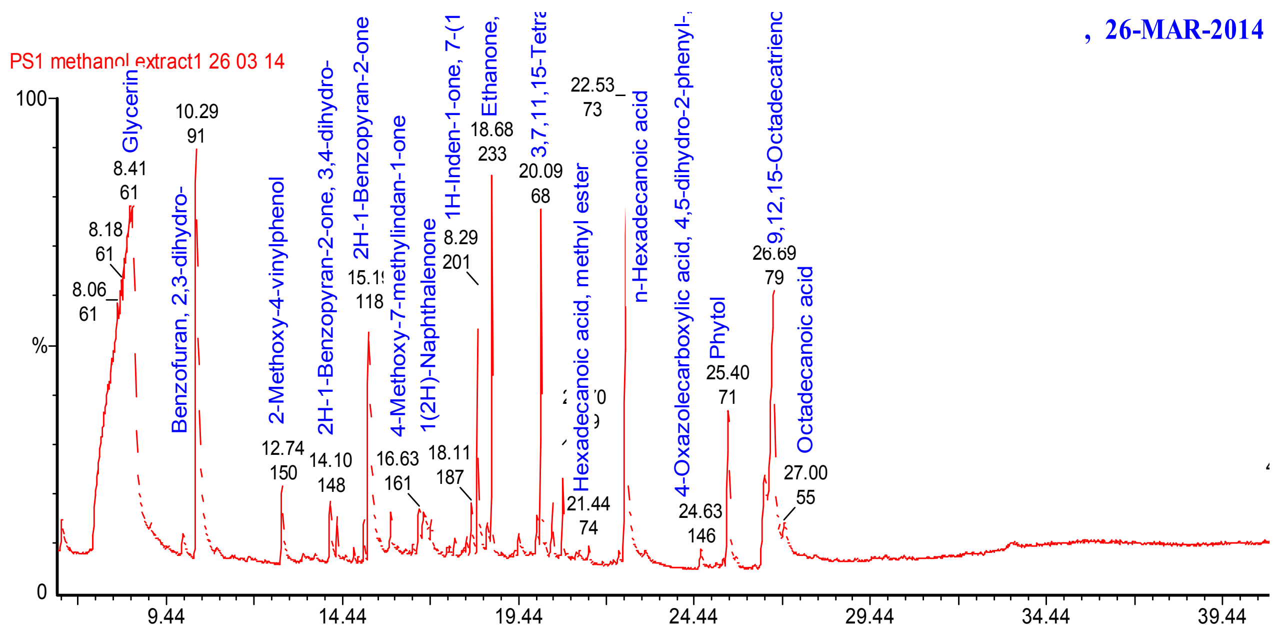

Fig. 2 . GC-MS chromatogram of *A. conyzoides* leaves

present in the leaves of *A.conyzoides* might be as one of the factor for their invasiveness and affecting the survival of native biodiversity through its leachates and decomposing leaf residues. However, the detailed field study is required for understanding the mechanism of allelopathic action of chemical compounds of various organs of *A. conyzoides* against soil health and other plant growth.

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