Vol.1.Issue.2., 2013

Bulletin of Pharmaceutical and Medical Sciences (BOPAMS)
A Peer Reviewed International Journal http://www.bopams.com

RESEARCH ARTICLE

INTERNATIONAL STANDARD SERIAL NUMBER
E-ISSN 2341-4103

Phytochemical screening and antimicrobial assessment of *Cyperus Iria* (L) weeds roots

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Article Info:
Received: 20/08/2013
Revised on : 28/08/2013
Accepted on: 02/09/2013

ABSTRACT

Cyperus iria, family Cyperaceae a weed plant commonly occurred in rice fields known as rice flatsedge act as feasible source of potential drugs to increase the health status of public. The plant is a rich source of Phenols and Alkaloids. The n-hexane and ethyl acetate extracts of roots of the plant were screened for phytochemical analysis and antimicrobial activity for *Escherichia coli, Staphylococcus aureus* and *Salmonella typhii*. Phytochemical screening showed the presence of carbohydrates, alkaloids, Phenols, steroids, and flavonoids etc., 17 compounds in n-hexane and 12 compounds in ethyl acetate extracts of the plant root. The present investigation also shows that prepared extracts possess notable antimicrobial activity.

Key words: *Cyperus iria*, Weed roots, phytochemical analysis, antimicrobial activity.

Introduction

Medicinal plants are a source of naturally active compounds used extensively by tribal people worldwide for many ailments. Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently en vogue in parts of the world. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region [1-2]. Drugs derived from natural sources plays a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary health care systems [3]. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure [4]. This therefore underscores the need to try as much solvents as possible in screening plant parts for phytochemicals. Antimicrobial compounds of plant origin may found in plant stems, roots, leaves, bark, flowers, or fruits [5].

Cyperus iria (Figure 1) is most often found as a weed in Indian environments especially in paddy fields, commonly known as rice flatsedge. The height of *C. iria* plants varies from 8 to 60cm. The roots are numerous, short and yellowish-red. The culms are tufted, triangular, glabrous, green and 0.6-3.0 mm thick. The leaves are linear-lanceolate, usually all shorter than the culm, 1-8 mm wide, flat, and scabrid on the margin and major ribs. The plant often grows in rice paddies, where it is considered to be a weed[11].

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Monocotyledonae
Order: Cyperales
Family: Cyperaceae
Genus: *Cyperus*
Species: *Cyperus iria*

←Figure 1: *Cyperus iria* plant (inset : root powder) (right side: botanical classification)
Source: http://www.cabi.org/isc/datasheet/17501

The present study focuses on phytochemical screening and antimicrobial activity of two different solvent extract of *Cyperus iria* roots.

Experimental

MATERIALS AND METHODS

***Chemicals*:** All Chemicals used in the entire study were AR grade obtained from SD fine, Merck chemicals, India, Pvt Ltd.,

***Plant Material*:** Fresh roots of *Cyperus iria* were collected in the month of September 2013 from paddy fields of local area of Ongole revenue subdivision, India. Roots were washed and completely dried for one week in sunlight to eliminate surface moisture. Then roots packed into envelop and kept in oven at 55^{o}C temperature for further dryness. Dried roots were grinded separately in a mortar obtained fine powder and sieved; which was then kept in plastic bags for further use.

Preparation of plant extract: The dry root material of *C. iria* passed through sieve (100). The coarse powdered drug (200grams) was extracted in Soxhlet apparatus for 48 h with n-hexane (60-75°C, 2L) and ethyl acetate (76-78°C, 2L extract obtained was concentrated under reduced pressure in rotatory evaporator below 60°C temperature to get semisolid sticky residue (15gm)

***GC-MS Analysis*:** GC-MS analysis of each extract sample was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with Elite-I, fused silica capillary column (30mm x 0.25mm 1D x 1 µMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1mL/min and an injection volume of 2µl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da. Total GC running time was 72 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Table 2 and 3 & Figure 2a, b).

Table 2: Components detected in n-hexane extract of *C.iria* root

S. No.	RT	Compound	% Area	MF	MW	Nature of Compound	Activity≠
1	4.74	2-Cyclopenten-1-one, 2-ydroxy	2.31	$C_5H_6O_2$	98	Diterpene	Antimicrobial, Anti-inflammatory, Anticancer, Dieruretic
2	6.64	Benzofuran, 2,3-dihydro-	10.22	C_8H_8O	120	Coumaran compound	Antimicrobial, Antiinflamatory
3	8.44	2-Methoxy-4-vinylphenol	9.27	$C_9H_{10}O_2$	150	Phenolic	Antimicrobial, Anti-inflamatory Antioxidant
4	9.62	Tetradecanoic acid	7.59	$C_{14}H_{28}O_2$	228	Myristic acid	Cosmetics,Antioxidant, Cancer preventive, Nematicide, Lubricant Hypocholesterolemic
5	12.00	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	2.31	$C_6H_8O_4$	144	Flavanoid	Antimicrobial, Anti-inflammatory
6	27.76	Phytol	12.24	$C_{20}H_{40}O$	296	Diterpene	Antimicrobial, Anti-inflammatory Anticancer, Diuretic
7	14.34	1,4-Dioxaspiro[4.5]decane, 8-(methylthio)-	6.39	$C_9H_{16}O_2S$	188	Thio-compound	Antimicrobial, Anti-inflammatory
8	18.86	3-Hydroxy-4-methoxycinnamic acid	2.18	$C_{10}H_{10}O_4$	194	Phenolic compound	Antimicrobial, Antioxidant Anti-inflammatory
9	14.02	5HCyclopropa[3,4]benz[1,2-e]azulen-5-one, 4,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-,1a,1b,4,4a,7a,7b,8,9,9a-decahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-	2.94	$C_{28}H_{36}O_{11}$	548	Keto compound	Anti microbial, Anti-inflammatory and anti proliferative activity
10	28.82	Caryophyllene	0.94	$C_{15}H_{24}$	204	Sesqui terpenoid	Anti-tumor, Analgesic, Anti -bacterial,Anti-inflammatory Sedative,Fungicide
11	31.00	1,4-Benzenediol, 2,6-bis(1,1-dimethylethyl)-	2.06	$C_{14}H_{22}O_2$	222	Alcoholic compound	Antimicrobial
12	36.42	Squalene	7.28	$C_{30}H_{50}$	410	Triterpene	Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant,
13	38.04	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	1.55	$C_{19}H_{32}O_2$	292	Linolenic acid	Anti-inflammatory,Hypocholester–olemic, Cancer preventive, Hepatoprotective, NematicideInsectifuge

14	44.70	12-Methyl-E,E-2,13-octadecadien-1-ol	3.64	$C_{19}H_{36}O$	280	Unsaturated fatty alcohol	No activity reported
15	44.90	5HCyclopropa[3,4]benz[1,2-e]azulen-5-one, 4,9,9a-tris(acetyloxy)-3-[(acetyloxy)methyl]-,1a,1b,4,4a,7a,7b,8,9,9a-decahydro-4a,7b-dihydroxy-1,1,6,8-tetramethyl-	6.21	$C_{28}H_{36}O_{11}$	548	Keto compound	No activity reported
16	48.80	3-O-Methyl-d-glucose	4.22	$C_7H_{14}O_6$	194	Sugar moiety	Preservative
17	53.62	10-Methyl-E-11-tridecen-1-ol propionate	3.21	$C_{17}H_{32}O_2$	268	Alcoholic compound	Antimicrobial activity

≠*Source: Dr. Duke's : Phytochemical and Ethnobotanical databases*

Table 3: Components detected in ethyl acetate extract of *C.iria* root

S. No.	**RT**	**Compound**	**% Area**	**MF**	**MW**	**Nature of Compound**	**Activity≠**
1	2.46	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	2.82	$C_{15}H_{24}$	204	Sesquiterpene	Anti-tumor, Analgesic, Anti -bacterial, Anti-inflammatory, Sedative, Fungicide
2	3.27	1,4-Benzenediol, 2,6-bis(1,1-dimethylethyl)-	4.37	$C_{14}H_{22}O_2$	222	Alcoholic compound	Antimicrobial
3	3.99	methyl 12-oxo-octadec-9-enoate -	2.02	$C_{19}H_{34}O_3$	310	Oxo (Keto) Fatty Acid esters	involved in the Krebs citric acid cycle and in glycolysis
4	4.72	2-Methoxy-4-vinylphenol	12.14	$C_9H_{10}O_2$	150	Phenolic	Antimicrobial, Anti-inflamatory Antioxidant
5	6.36	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-	2.54	$C_{15}H_{26}O$	222	Alcoholic compound	Anti-tumor, Analgesic, Anti-bacterial, Anti-inflammatory Sedative, Fungicide
6	7.92	Phytol	4.67	$C_{20}H_{40}O$	296	Diterpene	Antimicrobial, Anti-inflammatory Anticancer, Diuretic
7	10.66	3-Hydroxy-4-methoxycinnamic acid	2.67	$C_{10}H_{10}O_4$	194	Phenolic compound	Antimicrobial, Antioxidant Anti-inflammatory
8	13.35	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	18.32	$C_{19}H_{32}O_2$	292	Linolenic acid	Anti-inflammatory,Hypocholester –olemic, Cancer preventive, Hepatoprotective, NematicideInsectifuge
9	13.62	Z,Z-2,5-	1.88	$C_{15}H_{28}O$	224	Unsaturated	No activity reported

		Pentadecadien-1-ol				alcohol	
10	15.54	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	5.31	$C_6H_8O_4$	144	Flavanoid	Anti-microbial, Anti-inflammatory and anti-proliferative activity
11	18.58	Stigmasterol	5.31	$C_{29}H_{48}O$	412	Steroid	Hypocholesterolemic, sedativeAntiviral, Antioxidant, Antihepatotoxic, Anti-inflammatory Diuretic, Cancer preventive
12	23.02	Ricinoleic acid	3.22	$C_{18}H_{34}O_3$	298	Ricinoleic acid	Antimicrobial Anti-inflammatory

≠*Source: Dr. Duke's : Phytochemical and Ethnobotanical databases*

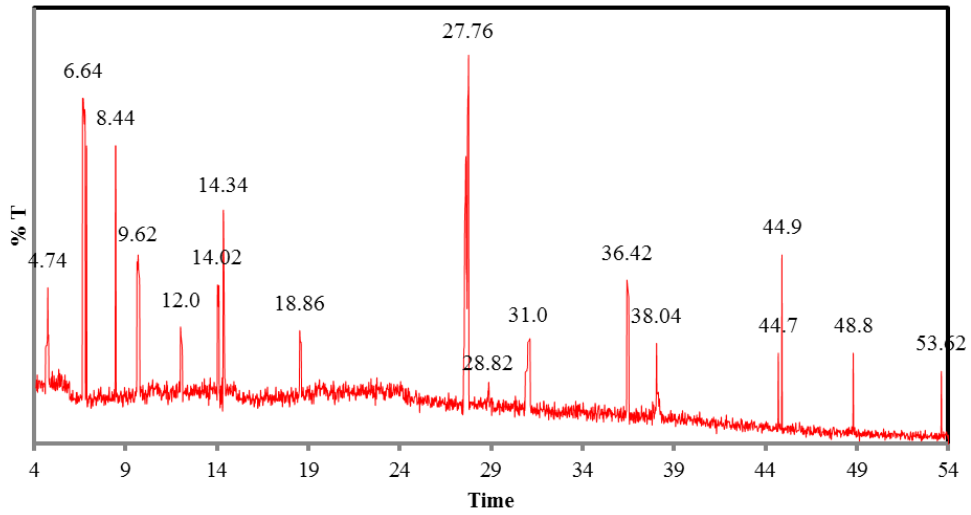

Figure 2a: GC-MS spectra of n-hexane extract

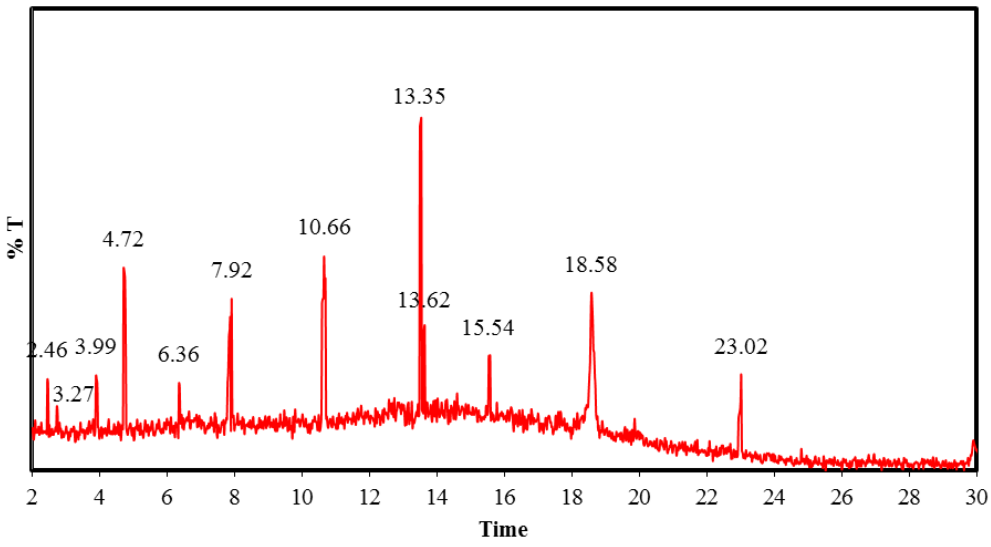

Figure 2b: GC-MS spectra of ethyl acetate extract

Anti-Bacterial Activity by Disc Diffusion Method [10]

Preparation of Inoculum: *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* strains were used. 50ml of nutrient broth was prepared in 100ml conical flask. It was sterilized & then inoculated with inoculum with the help of sterile loop in laminar air flow from preserved slants. They were then kept in incubator at 37^{o}Cfor 24 hours for organism to grow.

Preparation of Media: 200ml of nutrient agar media (NAM) was prepared and the pH was maintained at 7.0 to 7.2.

Pour Plate Method: 1ml of prepared inoculum was poured in sterile Petri dish & then 15 ml of NAM was poured in it & allowed to solidify.

Disc Diffusion Method: After solidification the disc of whatman 42 filter paper imbibed with 20 µl plant extracts were carefully placed with the help of forceps at the centre of the Petri dish and then kept in incubator for 24hrs.

Measurement of Zones: With the help of antibiotic zone scale the zone of inhibition (ZOI) were measured

Results and discussion

The phytochemical constituents of *Cyperus iria* root samples were screened qualitatively and quantitatively using the methods described by Harborne [6] and the results are presented in Table1. Screening involved tests for reducing sugars, alkaloids, cardiac glycosides, saponins, tannins, flavonoids, steroids, and terpenoids etc., the chemical composition of n-hexane and ethyl acetate root extracts of *Cyperus iria* were analysed using GC-MS technique. The identified compounds, their retention time and area percentage along with m/z values are summarized in Tables 2 & 3. It was identified that 17 compounds were identified in hexane extract and 12 compounds with ethyl acetate extract.

In the present study of phytochemical analysis of root extracts of *Cyperus iria*, n-hexane extract was found to have the better stores of phytoactive compounds than the ethyl acetate extract and also reveals that the few bioactive compounds were present are different. According to Tiwari et al., the factors affecting the choice of solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractant [9]. The logic in using different solvents when screening for phytochemicals in plant root material was clearly validated in the present study. For example, the results shows that sterols and terpenoids were exceptionally present in n-hexane extracts but absent in ethyl Acetate extract.

The phenolic compounds are known to be synthesized by plants in response to microbial infection. It is therefore possible that they can act as effective antimicrobial substances against a wide array of microorganisms [8]. However, the antimicrobial activity of plant extracts depends not only on phenolic compounds but also by the presence of different secondary metabolite [7] like hydroxyl groups on the active constituents, because of the ability of these substances to bind to bacterial adhesions and disturb the availability of receptors on the surface. The phenols observed in this study are 2-Methoxy-4-vinylphenol in *C. iria* hexane and ethyl acetate root extract, 3-Hydroxy-4-methoxycinnamic acid in n-hexane extract.

Among the identified components, Phytol attained the largest peak (27.76%) with the retention time 12.24 followed by Benzofuran, 2,3-dihydro- with the retention time 6.64% having the peak area percentage of 10.22. The third significant peak (8.44%) was attained by 2-Methoxy-4-vinylphenol in the retention time 9.27 in n-hexane extract along with other 14 identified peaks. The compound 10-Methyl-E-11-tridecen-1-ol propionate will be act as antimicrobial agents [12]. Whereas from ethyl extract the largest compound (13.35%) identified with retention time 18.32 was 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, whereas Ricinoleic acid was the last compound which took retention time (23.02) to identify.

Table 1: Display the presence/ absence of different phytochemicals in the root extracts of *C. iria*

Phytoconstituents	**Test**	n-Hexane	Ethyl Acetate
Alkaloids	Wagner's test	+	+
Amino acids	Ninhydrin Test	-	-
Carbohydrates	Molish test	+	+
Cardiac glycosides	Keller-Killani test	-	-
Flavonoids	Shinoda's test	+	+
Phenolics	phenol test	+	+
Polysterols	Salkowski's Test	+	+
Proteins	Biuret test	-	-
Saponins	Frothing test	+	+
Steroids	Libermann-Buchard's test	+	+
Tannins	Ferric chloride test	-	-
Terpenes	Salkwaski's test	+	+

Anti-bacterial Activity

The inhibitory action of identified compounds had been historically recognized and applied as a useful therapeutic agent for preventing wound infections. The antibacterial activities of extracted materials were investigated against three different types of bacterial strains like *Salmonella typhi, Escherichia coli and Staphylococcus aureus.* (Figure 3) showed excellent antibacterial activity against all tested bacterial strains at the volume of 50 µL/well. The zone of inhibition (in mm) ranges identified for *Salmonella typhi,* n-Hexane (25.25 ± 0.11), Ethyl Acetate (11.25± 0.12), *Escherichia coli* n-Hexane (18.25± 0.76), Ethyl Acetate (17.25 ± 0.51) and for *Staphylococcus aureus* n-Hexane (21.25± 0.76), Ethyl Acetate (12.45 ± 0.51). The diameters of the inhibition zones for the all tested pathogens are listed in Table 4. Thus, our results show that root n-hexane extract samples have potential bacterial activity against *S. typhi, S.aureus* compared with *E.coli* in turn, compared with extract of ethyl acetate, n-hexane extract having more antimicrobial ingredients.

Table 4: Zone of Inhibition of selected microbial cultures

Bacteria	Extracts	Zone of inhibition (mm)
Salmonella typhi	n-Hexane	25.25 ± 0.11
	Ethyl Acetate	11.25± 0.12
Escherichia coli	n-Hexane	18.25± 0.76
	Ethyl Acetate	17.25 ± 0.51
Staphylococcus aureus	n-Hexane	21.25± 0.76
	Ethyl Acetate	12.45 ± 0.51

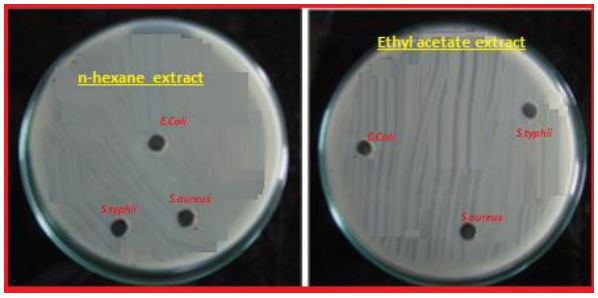

Figure 3: Showing antibacterial activity of n-hexane and ethyl acetate root extract *C.iria* against *Salmonella typhi, Escherichia coli* and *Staphylococcus aureus* microbes

Conclusion

Medicinal plants have curing property of various diseases due to the presence of biologically active compounds which make the plant medicinally important. *Cyperus iria* root phytochemical study revealed the presence of phytochemical compounds such as anthraquinones, flavonoids, steroids, phenols, etc., which have unique medicinal properties and used in the treatment for microbial diseases. The n-hexane extract showed higher antimicrobial activities than the ethyl acetate extract. This implies that n-hexane extracted more active phytoconstituents compared to ethyl acetate and this agrees with the previous works in other plant materials. This is a preliminary work on screening of bioactive compounds in *Cyperus iria* which needs further investigation

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