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Antimicrobial Potential and Phytochemical Investigation of Fixed Oil of Plant *Chenopodium ambrosioides* Linn.

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Chenopodium ambrosioides is widely used in the traditional medicine system, due to anthelmintic, anticancerous ant antiparasitic activities in Asia, America and Africa. To investigate the fixed oil composition of the plant and evaluate its biological activities, we have selected this medicinal plant for further studies. Twenty four different methyl esters were found in the oil having palmitic acid, linoleic acid, oleic acid, stearic acid, linolenic acid, behenic acid, myristic acid and pentadecanoic acid. The oil also had antimicrobial activity against selected strains of Gram-positive bacteria, Gram-negative bacteria and some fungi species. The results confirm the biological activity of the fixed oil and proved its importance as natural medicinal source.

Key Words: *Chenopodium ambrosioides*, Fixed oil, Methyl esters, Palmitic acid, Stearic acid, Antimicrobial activity.

INTRODUCTION

Globally infectious diseases are among the factors underlying deaths and are associated with approximately one-half of all mortalities in tropical countries. New, more effective and yet safe therapeutic agents are critical issues to manage challenging infectious diseases[1,2]. Poor communities especially belonging to small isolated villages and native people in developing countries mainly use folk medicine for treatment of infectious diseases[2]. Medicinal plants are consumed as decoctions, teas and juice preparations to treat respiratory infections or as a poultice and applied directly on the infected wounds or burns[1,2]. So far extensive work has been done and still going on for the search of bioactive compounds to treat microbial infections as more effective and safer therapeutic agents.

Chenopodium ambrosioides is an important medicinal plant widely used in the traditional medicine system in Europe, Asia, North and South America. It is a member of family, *Chenopodiaceae*. This plant is widely distributed in Baluchistan, Peshawar, Dir, Swat, Rawalpindi, Kohala of Pakistan and Kashmir[3]. It is a member of an important plant family, *Chenopodiaceae*, which have an elevated importance for phytochemical investigation and medicinal evaluation. Family *Chenopodiaceae* is consisting of 102 genera and 1400 species[4]. A vast variety of compounds have been reported from the genus *Chenopodium*. Phytochemically chenopods were reported to contain minerals, primary metabolites-carbohydrates, amino acids, proteins, aromatic cytokinins, hormones, secondary metabolites- such as organic acids, phenol derivatives, lignans, coumarines, flavones, flavonols and their glycosides, flavanones and isoflavones, catechins, sterols, monoterpenes, sesquiterpenoids, triterpenes, carotenoid terpenoides, saponins, alkaloids, amides and amines and also vitamins have been reported from this genus[5]. Oxalic acid has been reported from *Chenopodium album* in a range of 360-2000 mg/100 g[6] along with malic acid and succinic acid from *Chenopodium ambrosioides*[7]. A coumarin scopoletin have been isolated from the aerial parts of *Chenopodium murale*[8]. Dihydrowogonin, a flavanone as well as the isoflavones irilin A and irilin B were isolated from the aerial parts of *C. procerum*[9]. Saponins isolated from *C. anthelminticum* were tested against influenza type A infections in mouse tissue and were found the strongest antiviral agents[10]. Essential oil from *C. botrys* was examined for their antibacterial

activities and represent significant action against gram positive and gram negative bacteria[11]. However in literature we could not find any studies on the fixed oil of this important medicinal plant. In our current study we have investigated phytochemical constituents and antimicrobial potential of this plant.

EXPERIMENTAL

Plant was collected from Peshawar in June and was shade dried. Plant specimen was identified by Dr. Abdur Rashid, Department of Botany, University of Peshawar. Methanolic crude extract of the plant (10 kg) was further fractionated in *n*-hexane, ethyl acetate, dichloromethane, *n*-butanol and aqueous fractions. Four samples of fixed oil, one from *n*-hexane fraction [at 10 % ethyl acetate and *n*-hexane solvent system by column chromatography (CC)] and three from ethyl acetate fraction (at 1, 3 and 5 % ethyl acetate and *n*-hexane solvent systems by column chromatography) were obtained. Four samples were analyzed by GC-MS for their chemical composition and were tested for antibacterial and antifungal activities.

Gas chromatography-mass spectrometry: All samples were analyzed by GC-MS Model-QP 2010 Plus (Shimadzu). Column Specifications: Length; 30 mm. id; 0.35mm, thickness; 0.25 μM, treated with poly ethylene glycol (TRB-FFAP; Technochroma). Injector temperature was 240 ºC. Ion source temperature (EI) was 250 ºC. The interface temperature was 240 ºC. Pressure was maintained at 70 kPa. The split ratio was 1:50. Injection volume is 1 μL and carrier gas is helium. Identification of the compounds was based on comparison with Wiley and NIST database.

Antibacterial activity

Agar well diffusion assay: Antibacterial activity was studied *via* agar well diffusion method. In this method one loop full of 24 h old culture containing *ca.* 10^4-10^6 CFU was spread on the surface of Mueller-Hinton Agar plates. Wells were dug in the medium with the help of sterile metallic cork borer. Stock solution of the test samples in the concentration of 22 mg/mL were prepared in the dimethyl sulphoxide (DMSO) and 150 μL dilutions were added in their respective wells. The antibacterial activity of samples was compared with standard drug, streptomycin. The standard drug streptomycin and DMSO were used as positive and negative control. The amount of growth in each well was determined visually by comparing with the growth in the controlled wells.

MIC determination by macrodilution method: Extracts (10 mg/mL) were dissolved in DMSO and serially diluted with sterile water in microplates in a laminar flow cabinet. The same volume of an actively growing culture of the test bacteria was added to the different wells and cultures were grown overnight in 100 % relative humidity at 378C. Next morning, tetrazolium violet was added to all the wells and the growth was indicated by a violet colour of the culture. Lowest concentration of the test solution that led to inhibition of growth was taken as MIC. Negative control DMSO had no influence on growth even at the highest concentration used.

Antifungal activity

Antifungal activity was determined by agar well diffusion method. In this method, meconozole was used as the standard drug. Samples were dissolved in DMSO (24 mg/mL). Sterile Sabouraud's dextrose Agar medium (7 mL) was placed in a test tube and inoculated in a sample solution (40 μg/mL) kept in slanting position at room temperature overnight. Fungal culture was then inoculated on the slant. The samples were incubated for 7 days at 30 ºC and growth inhibition was observed. Per cent growth inhibition was calculated with reference to the negative control by applying the formula:

$$(\%)\ \text{Inhibition} = (\text{Linear growth of the negative control} - \text{Linear growth of sample})/100 \times 100$$

TABLE-1
QUANTITATIVE RESULTS OF FATTY ACID METHYL ESTERS OF *n*-HEXANE (10% ETHYL ACETATE AND HEXANE SOLVENT SYSTEM) SAMPLE BY GC-MS

Peak#	Name	Area	Conc. (%)	Retention time (min)	m/z
2.	C8:0; Caprylic acid, methyl ester	1563	0.01	4.966	87.00
3.	C10:0; Capric acid, methyl ester	3598	0.03	6.802	87.00
4.	C11:0; Undecanoic acid, methyl ester	1332	0.01	7.660	87.00
5.	C12:0; Lauric acid, methyl ester	39792	0.35	8.555	87.00
6	C13:0; Tridecanoic acid, methyl ester	21706	0.20	9.636	87.00
7.	C14:0; Myristic acid, methyl ester	124930	1.09	10.984	87.00
9.	C15:0; Pentadecanoic acid, methyl ester	144081	1.26	12.643	87.00
11.	C16:0; Palmitic acid, methyl ester	5051357	44.22	14.686	87.00
12.	C16:1c; Palmitoleic acid, methyl ester	29100	0.25	15.178	97.00
13.	C17:0; Margaric acid, methyl ester	145469	1.27	16.943	87.00
15.	C18:0; Stearic acid, methyl ester	684728	5.99	19.006	87.00
16.	C18:1c; Oleic acid, methyl ester	1209391	10.61	20.253	97.00
18	C18:1n8T; Octadecenoic acid, methyl ester	100267	0.88	20.451	97.00
19.	C18:2c; Linoleic acid, methyl ester	2513753	22.01	21.888	95.00
20	C18 :2t; Octadecadienoic acid, methyl ester	12412	0.11	22.050	95.00
22	C18:3n3; Linoleic acid, methyl ester	489959	4.29	24.364	95.00
23.	C20:0; Arachidic acid, methyl ester	199210	1.74	27.068	87.00
24	C20:1; Eicosenoic acid, methyl ester	13865	0.12	27.804	97.00
27.	C21:0; Heneicosanoic acid, methyl ester	29418	0.26	30.878	87.00
31.	C22:0; Behenic acid, methyl ester	275888	2.42	34.335	87.00
34.	C23:0; Tricosanoic acid, methyl ester	96202	0.84	37.586	87.00
35.	C24:0; Tetracosanoic acid, methyl ester	230443	2.02	40.675	87.00

RESULTS AND DISCUSSION

Four samples of fixed oil obtained from chenopodium ambrosioides linn were analyzed by GC MS. Results obtained from the GC-MS of fixed oil of this plant along with the GC-MS of 37 components used as standard are given below. Total 22 components (Table-1) were identified from sample 1 by GC-MS. Important components in highest concentration were palmitic acid (44.22 %), linoleic acid (22.01 %), oleic acid (10.61 %), stearic acid (5.99 %), linolenic acid (4.29 %) and behenic acid (2.42 %) while the other components were in the small quantity. In case of compounds in sample 2 (Table-2), major components were palmitic acid (62.41 %), stearic acid (18.31 %), myristic acid (4.33 %), pentadecanoic acid (3.97 %) and behenic acid (3.37 %) apart from minor components. Similarly palmitic acid (66.68 %), stearic acid (8.19 %), oleic acid (8.11 %), tetracosanoic acid (2.56 %) and arachidic acid (2.35 %) are the major components of sample 3 (Table-3) while the others are present in minor quantities. major compounds identified in sample 4 (Table-4) are palmitic acid (61.66 %), stearic acid (11.75 %), pentadecanoic acid (7.92 %), oleic acid (4.92 %) and myristic acid (3.57 %) while the others are present in minor quantities.

Antimicrobial activities: Significant antibacterial activity was found against a set of various human pathogens including *Escherichia coli, Klebsiella pneumoniae* (Gram negative bacteria), *Staphylococcus aureus, Bacilus subtilus* and *Staphylococcus epidermidis* (Gram positive bacteria). The most susceptible tested bacterial strain was *Staphylococcus aureus* against sample 4 followed by sample 2. In all cases oily fraction showed moderate but significant activities (Tables 5 and 6). Similarly good results were obtained against *Escherichia coli* as well. In case of antifungal activities (Table-7), no considerable results were found against tested fungal strains. Only low activity profile was found incase of sample 1 and 2 against various fungi. These inspiring results highlighted the strong potential of fixed to identify chemical constituents that might be involved in antibacterial action followed by mechanistic studies to explore their molecular targets.

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TABLE-2
QUANTITATIVE RESULTS OF FATTY ACID METHYL ESTER OF ETHYL ACETATE FRACTION
(1 % ETHYL ACETATE AND HEXANE SOLVENT SYSTEM) SAMPLE BY GC-MS

Peak#	Name	Area	Conc. (%)	Retention time (min)	m/z
5.	C12:0; Lauric acid, methyl ester	2414	1.19	8.553	87.00
7.	C14:0; Myristic acid, methyl ester	8784	4.33	10.982	87.00
9.	C15:0; Pentadecanoic acid, methyl ester	8060	3.97	12.640	87.00
11.	C16:0; Palmitic acid, methyl ester	126636	62.41	14.632	87.00
13.	C17:0; Margaric acid, methyl ester	7190	3.54	16.935	87.00
15.	C18:0; Stearic acid, methyl ester	37154	18.31	19.632	87.00
31.	C22:0; Behenic acid, methyl ester	6830	3.37	34.331	87.00
32.	C22:1; Eruccic acid, methyl ester	5849	2.88	34.903	97.00

TABLE-3
QUANTITATIVE RESULTS OF FATTY ACID METHYL ESTERS OF ETHYL ACETATE
(3 % ETHYL ACETATE AND HEXANE SOLVENT SYSTEM) SAMPLE BY GC-MS

Peak#	Name	Area	Conc. (%)	Retention time (min)	m/z
1	C6:0; Hexanoic acid, methyl ester	55302	1.30	3.069	87.00
2	C8:0; Caprylic acid, methyl ester	4971	0.12	4.967	87.00
3	C10:0; Capric acid, methyl ester	2501	0.06	6.803	87.00
4	C11:0; Undecanoic acid, methyl ester	578	0.01	7.658	87.00
5	C12:0; Lauric acid, methyl ester	10064	0.24	8.555	87.00
6	C13:0; Tridecanoic acid, methyl ester	3157	0.20	9.638	87.00
7	C14:0; Myristic acid, methyl ester	52592	1.24	10.985	87.00
9	C15:0; Pentadecanoic acid, methyl ester	68563	1.61	12.645	87.00
11.	C16:0; Palmitic acid, methyl ester	2837158	66.68	14.665	87.00
12.	C16:1c; Palmitoleic acid, methyl ester	8947	0.21	15.181	97.00
13.	C17:0; Margaric acid, methyl ester	61259	1.44	16.940	87.00
15.	C18:0; Stearic acid, methyl ester	348324	8.19	19.645	87.00
16.	C18:1c; Oleic acid, methyl ester	345228	8.11	20.198	97.00
18	C18:1n8T; Octadecenoic acid, methyl ester	23847	0.56	20.432	97.00
19.	C18:2c; Linoleic acid, methyl ester	45603	1.07	21.777	95.00
23.	C20:0; Arachidic acid, methyl ester	100036	2.35	27.208	87.00
27.	C21:0; Heneicosanoic acid, methyl ester	10115	0.24	30.885	87.00
31.	C22:0; Behenic acid, methyl ester	133278	3.13	34.338	87.00
34.	C23:0; Tricosanoic acid, methyl ester	37746	0.89	37.596	87.00
35.	C24:0; Tetracosanoic acid, methyl ester	108992	2.56	40.684	87.00

TABLE-4
QUANTITATIVE RESULTS OF FATTY ACID METHYL ESTERS OF ETHYL ACETATE (5 % ETHYL ACETATE AND HEXANE SOLVENT SYSTEM) SAMPLE BY GC-MS

Peak#	Name	Area	Conc. (%)	Retention time (min)	m/z
1.	C6:0; Hexanoic acid, methyl ester	2125	0.59	3.068	87.00
2.	C8:0; Caprylic acid, methyl ester	1127	0.31	4.966	87.00
3.	C10:0; Capric acid, methyl ester	1576	0.44	6.805	87.00
5.	C12:0; Lauric acid, methyl ester	5275	1.47	8.557	87.00
6.	C13:0; Tridecanoic acid, methyl ester	1535	0.20	9.310	87.00
7.	C14:0; Myristic acid, methyl ester	12797	3.57	10.983	87.00
9.	C15:0; Pentadecanoic acid, methyl ester	28435	7.92	12.657	87.00
11.	C16:0; Palmitic acid, methyl ester	221256	61.66	14.634	87.00
13.	C17:0; Margaric acid, methyl ester	6015	1.68	16.935	87.00
15.	C18:0; Stearic acid, methyl ester	42153	11.75	19.634	87.00
16.	C18:1c; Oleic acid, methyl ester	17664	4.92	20.175	97.00
19.	C18:2c; Linoleic acid, methyl ester	11373	3.17	21.773	95.00
31.	C22:0; Behenic acid, methyl ester	9064	2.53	34.334	87.00

TABLE-5
DIAMETER OF ZONE OF INHIBITION (mm) OF SAMPLES AGAINST BACTERIAL STRAINS

Sample No.	*Escherichia coli*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Bacilus subtilus*	*Staphylococcus epidermidis*
1	10	12	12	16	10
2	10	08	14	00	12
3	10	12	12	10	12
4	16	10	14	10	00
DMSO	00	00	00	00	00
Streptomycin	26	28	26	30	30

Sample No. 1 = *n*-Hexane (10 %), 2 = Ethyl acetate (1 %), 3 = Ethyl acetate (3 %), 4 = Ethyl acetate (5 %)

TABLE-6
MINIMUM INHIBITORY CONCENTRATIONS (MIC: µg/mL) OF TEST SAMPLES

Sample No.	Minimum inhibitory concentrations (µg/mL)				
	Escherichia coli	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Bacilus subtilus*	*Staphylococcus epidermidis*
1	0.6±0.05	0.4±0.02	0.40±0.03	0.40±0.05	0.6±0.02
2	0.6±0.06	0.9±0.04	0.2±0.02	-	0.4±0.01
3	0.6±0.02	0.4±0.01	0.4±0.03	0.6±0.05	0.4±0.04
4	0.2±0.04	0.6±0.02	0.1±0.02	0.6±0.02	-
Streptomycin	0.02±0.001	0.06±0.001	0.03±0.04	0.014±0.001	0.09±0.003

Sample No. 1 = *n*-Hexane (10 %), 2 = Ethyl acetate (1 %), 3 = ethyl acetate (3 %), 4 = Ethyl acetate (5 %)

TABLE-7
PERCENTAGE INHIBITION (mm) OF SAMPLES AGAINST FUNGAL STRAINS

Sample No	*Aspergillus niger*	*Aspergillus parasiticus*	*Trycophyton horzianum*	*Rizopus tolenapur*	*Aspergillus flavus*
1	36	35	15	31	30
2	25	36	00	30	10
3	26	22	10	00	00
4	00	20	30	18	10
Miconazole	100	100	100	100	100

Sample No. 1 = *n*-Hexane (10 %), 2 = Ethyl acetate (1 %), 3 = Ethyl acetate (3 %), 4 = Ethyl acetate (5 %)

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