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GC/MS analysis, anti-leishmanial and relaxant activity of essential oil of *Chenopodium ambrosioides* (L.) from Malakand region

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Abstract: Chenopodium ambrosioides is abundantly available in Malakand region. As constituents and concentrations of essential oils vary based on its geographical location, we carried our current study to extract and evaluate its possible relaxant activity in rabbits' jejunum and anti-leishmanial activity against promastigotes of Leishmania tropica. The essential oil was obtained from aerial fresh parts through steam distillation followed by GC/MS analysis. Antispasmodic activity was performed on spontaneous and KCl induced contractions. Curves for calcium concentration response (CCRCs) were prepared with and without different concentrations of essential oils and verapamil - a standard calcium channel blocker as per our reported procedures. GC/MS analysis indicated that the essential oil contains 4-carene (56.59%) and o-cymene (41.46%), the two most abundant compounds previously reported from this species. The LD50 value for acute toxicity is 279.66±2.2mg/kg. The essential oil have significant antileishmanial activity with LC50 of Log10 (1.83±0.0026) ×10-6mg/ml, potent relaxant activity on rabbits' jejunal preparations with respective EC50 = 1.46±0.15mg/ml for spontaneous activity. For KCl (80mM) induced contractions, EC50=0.26±0.02mg/ml. In CCRCs, the oil produced a right shift as exhibited by verapamil. More, its relaxant activity, which is mediated through calcium channel blocking mechanism, proves a rationale for its traditional use in gut spasm.

Keywords: *Chenopodium ambrosioides,* essential oil, anti-leishmanial, *Leishmania tropica,* antispasmodic, verapamil, calcium channel blocking.

INTRODUCTION

Medicinal plants are now getting more attention for development of new drug (Harvey, 2007, Rehecho *et al.,* 2011). According to a report of WHO, 80% global population uses traditional medicines for primary health care needs. As per WHO essential drug list, of the total 252 available market drugs, 11% drugs are derived from plants sources and about 25% of current drug products, used in USA, are originated from herbal sources (Veeresham, 2012). Natural products are thought to be comparatively safer than synthetic drugs (Karimi *et al.,* 2015). So there is need for investigations of new drug development from natural sources, preferably the plant sources.

The family of Chenopodiaceae is geographically distributed into East Mediterranean regions. Its species are frequently used as drugs for treatment of various diseases. Essential oil, terpenes and flavonoids are the most prominent constituents of Chenopodiaceae. The members of this family are present in North and South America, Europe, Asia, China and India. Different parts of different species of genus Chenopodium have been usually utilized in medication of a variety of disorders (Dembitsky *et al.,* 2008). *Chenopodium ambrosioides (C. ambrosioides)* usually known as "West Indian Goosefoot" or "Mexican tea" or "Epazote" or "American wormwood" belongs to family Chenopodiaceae. It is aromatic plant having notched red-shaded stem, elongated lanceolate, dentate leaves, green blooms, which has a solid camphoraceous fragrance. It frequently achieves height up to ~125cm. The inflorescences are paniculate elongates up to 40 cm, with various slim limbs inferring from the axilla of a bractea that have some tertiary extensions (Muhayimana *et al.,* 1998). In Pakistan, this plant is found in Malakand, Hazara, Kashmir and Baluchistan regions, where the flowering season is March and April, locally called "chelwai". The whole aerial parts are used for medicinal purposes (Shah, 2014). GC/MS analysis carried out in different countries like Iran showed that the essential oil mainly contains α- pinene 3.57%, α-terpinene 15.90%, camphor 12.42%, cis ascaridole 43.40%, trans ascaridole 6.38% (Omidbaigi *et al.,* 2005). In France, GC/MS analysis showed that it contain α-terpinene 72.7%, p-cymene 15.3%, ascaridole 7.2% (Muhayimana *et al.,* 1998). In Nigeria, GC/MS analysis showed that it contains α-terpinene 56%, p-cymene 15.5% and α-terpinyl acetate 15.7% (Onocha *et al.,* 1999). In

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India, it contain α- terpinene 63.6%, p-cymene 19.5% and ascaridole 6.2 (Gupta *et al.,* 2002). In china *α*-Pinene 1.3%, *δ*-4- Carene 1.9% and *ρ*-Cymene 12.7%, *Z*)-Ascaridole 29.7%, 3,4-Epoxy-*ρ*-menthan-2-one 4.1%, Carvacrol 4.9%, Isoascaridole 13.0 % and Caryophyllene oxide 2.2% (Chu *et al.,* 2011).

C. ambrosioides is commonly used as anthelmintics, vermifuge, emmenagogue and abortifacient purposes (Al-kaf *et al.,* 2016). It is applied in treatment of digestive disorders, respiratory disorders, uro-genital disorders, diabetes and hypercholesterolemia. It is also used as soothing agent, antipyretic and antirrheumatic as well (Kuete, 2014). The extract of *C. ambrosiodes* of various parts are used for treatment of irregular menses, dysmenorrhea and as hemostatic (Ososki *et al.,* 2002). This oil is also known as *Chenopodium* oil, American wormseed oil or Baltimore oil. On scientific grounds this oil has been found to possess antifungal, antibacterial, anti-mycobacterial, antiviral, insecticidal and nematocidal potentials. It is also used in some skin disorder involving dermatophyte (Kuete, 2014). It possesses significant cytotoxic activity against human tumor HT29 cell line (Al-kaf *et al.,* 2016). This oil is also used in intestinal parasites, amoebic dysentery hook and round worms eradication; Ascaris infection (Kaul, 2000), possess allelopathic and acetylcholinesterase inhibitory activities (Fdil *et al.,* 2017). As constituents and concentrations of essential oils, as described above, vary country to country based on its geographical location, hence, we carried our current study to isolate and characterize the essential of *C. ambrosioides* of Malakand region as this specie is very abundantly available in Malakand region.

Objectives

This plant species has been used for gut spasms and keeping its traditional use as antispasmodic, this study was carried out to scientifically evaluate its possible relaxant activity. Since, the isolated essential oils showed *in vitro* antispasmodic activity in our laboratory, hence further investigated the oil for possible antidiarrheal activity as well.

MATERIALS AND METHODS

Ethical statement

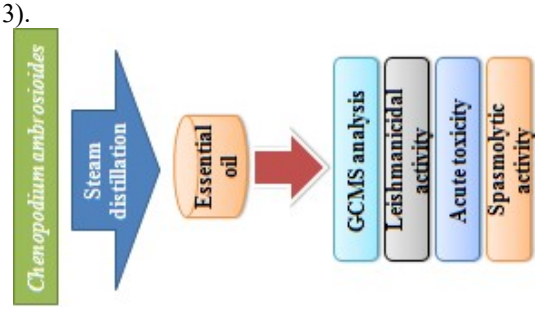
The proper use of animals in this study was approved by Advanced Study & Research Board via No: ASRB 000141/AA/IBMS/22/02/2014. Ethics Board of the Khyber Medical University also approved study protocols via No: Dir/KMUEB/AA/000080.

Study design

Experimental procedures

Plant collection, identification and extraction of essential oil

Fresh aerial parts of *C. ambrosioides* were collected in August-October, 2013 from Sayar valley Distt. Dir Lower, Malakand region, Khyber Pakhtunkhwa, Pakistan. Professor Jehandar Shah identified the plant. A voucher specimen (Ch. a-1-2013) has been placed in Department of Pharmacology IBMS, KMU, Peshawar, Pakistan. The fresh aerial parts of *C. ambrosioides* were washed properly with distilled water and then subjected to the steam distillation as per our reported procedure (Ali *et al.,* 2013).



Drugs and standards

Chemicals used were of analytical grade. Acetylcholine (BDH), England and rest of the chemicals were of E Merck grade, Germany were purchased. All stock and test solutions were prepared in double distilled water.

GC/MS Analysis

Essential oil was extracted from fresh aerial parts through steam distillation. GC/MS analysis was carried out at PCSIR laboratories, Peshawar, Pakistan. Area normalization procedure was used for analysis and GC/MS spectrum was obtained. The % contents of oil in the spectrum were determined using the mass library and, as stated earlier, area normalization technique was used as per our reported procedure (Ali *et al.,* 2013).

Pharmacological Screenings

Acute Toxicity

To investigate acute toxicity of essential oil, Swiss Albino mice (weighing 25-35g, purchased from NIH, Islamabad) of either sex were used for the experiments. Animals were acclimatized prior to performance of the experiments. A total of 45 animals were taken which were divided into 9 groups with 5 each. All the animals were given standard environmental conditions, food and water ad libitum. The essential oil in different doses was administered intraperitoneally. Acute toxicity was performed in three stages. In first stage groups (A-C) received exponential doses of essential oil of *C. ambrosioides* with test dose of 10, 100 and 1000mg/kg body weight. Its effects were observed. In the second stage, group D and group E received 300 and 600mg/kg essential oil of *C. ambrosioides.* As it showed mortality, so, the trials of the essential oil were further carried out in third stage (in three groups i.e. F-H) with test doses of 150, 200 and 250mg/kg (Ali *et al.,* 2014a). Group I was used as negative control. The number of death and symptoms of possible toxic effects of test samples were observed for 24 hours. LD_{50} was then calculated using graphical method.

Table 1: Various active constituents of essential oil of *C. ambrosioides* characterized by GC/MS analysis.

S. No	Name	R-Time	Area	Conc. (%)
1	α-phellandrene	9.845	5544	0.03
2	α-pinene	9.845	5544	0.03
3	β-myrcene	13.160	16122	0.07
4	gamma-terpinen	17.399	190572	0.86
5	D-limonene	15.595	122735	0.55
6	4-carene	14.815	12536605	56.59
7	o-cymene	15.280	9233633	41.46
8	p-cymen-8-ol	26.391	13422	0.06
9	P-mentha-1, 4(8) -diene.	19.157	7101	0.03
10	4-carene	14.139	10663	0.05

Table 2: Acute Toxicity of essential oil of *C. ambrosioides*

Dose and respective lethality			
1st stage	Group A (10 mg/kg)	Group B (100 mg/kg)	Group C (1000 mg/kg)
	All alive	All alive	All died
2nd stage	Group D 300 mg/kg	Group E 600 mg/kg	---
	All died	All died	---
3rd stage	Group F 150 mg/kg	Group G 200 mg/kg	Group H 250 mg/kg
	All alive	All alive	All alive

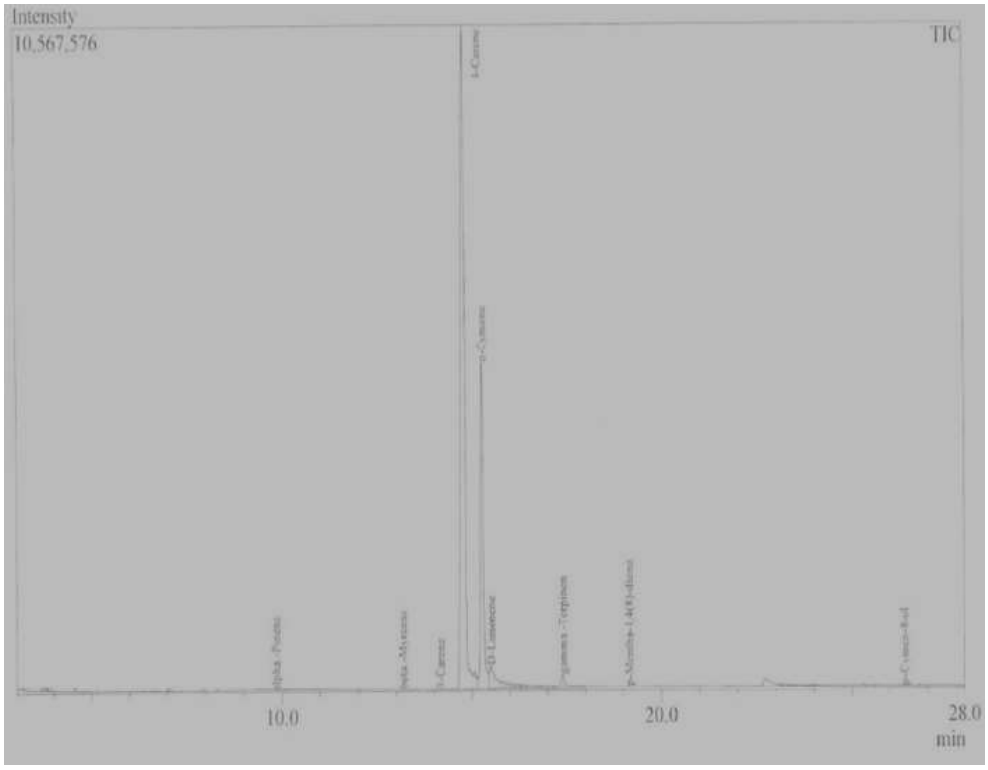


Fig. 1: Characterization of GC/MS analysis of essential oil of *Chenopodium ambrosioides*

In vitro anti leishmanial activity against clinical isolates of Leishmania tropica

Culture of the parasites

Pre-established *Leishmania tropica* strains KWH 23 were incubated at 24±1°C for 7 days in 199 medium having 10% Fetal Bovine Serum.

Samples Preparations and testing

In vitro anti leishmanial activity was performed according to the procedure described with slight modifications. Serially diluted the stock solutions. First row of 96 well plate having 198µL of 199 medium containing Promastigotes of *Leishmania tropica* and rest of the wells

had 180µL of 199 medium. Each well of first row in plate was added with 2µL of test sample, mixed and diluted serially to make final volume 180µL. Incubated microtitre plates for 24 hr at 24±1°C. Performed the experiments in triplicate. Transferred 20µL culture to improved Neobar counting chamber after 24 hr and live Promastigotes were counted under light microscope (Nabi *et al.,* 2012). LC_{50} of the essential oil determined using Graph Pad Prism and Microsoft XL- sheet.

Table 3: *In vitro* anti leishmanial activity of essential oil of *C. ambrosioides* against clinical isolates of *Leishmania tropica.*

S. No	Concentrations (v/v)	% lethality
1	1×10^{-2}	100
2	1×10^{-3}	100
3	1×10^{-4}	100
4	1×10^{-5}	100
5	1×10^{-6}	100
6	1×10^{-7}	23
7	1×10^{-8}	0
8	1×10^{-9}	0

Relaxant and calcium channel blocking activity

Preparation of physiological and test solutions

Three different physiological solutions known as Normal Tyrode's solution, Potassium Normal and Potassium Rich Tyrode's solutions were prepared on the same days of experiments. All these physiological solutions were prepared in double distilled water. Essential oil was suspended in distilled water using 0.01% carboxymethylcellulose (CMC) as suspending agent.

Antispasmodic effects of essential oil of C. ambrosioides using rabbits' jejuna preparations

The antispasmodic effect of the essential oil was carried out using rabbits' jejunums as per our reported work (Ali *et al.,* 2011b). All the experiments were carried out in triplicate. Briefly describing, rabbits' of either sex, obtained from National Institute of Health Islamabad, were fasted overnight before starting of experiments with free access to water. They were subjected to cervical dislocation. Their abdomens were dissected and jejunums were carefully removed without injury. Mesentery was carefully removed from the jejunums. They were placed in Petri dish, containing Tyrode's solution with regular carbogen gas supply. Jejunums were cut into small portions of about 1.5-2cm. Tissues were fixed in organ bath having 15ml of Tyrode's solution having continuous supply of carbogen gas maintained on 37°C. After stabilizing the tissues, the essential oil were tested at various concentrations i.e. 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0 and 10.0 mg/ml (Ali *et al.,* 2011a). Effects of 0.01% solution of CMC was also tested that served as negative control.

Effects of essential oil of C. ambrosioides on KCl-induced contractions

To investigate possible mechanism of action for relaxing effect of the essential oil it was tested in 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0 and 10.0mg/ml concentrations on sustained contractions produced by KCl (80 mm) in rabbit's jejunum (Ali *et al*., 2014a). It response was recorded.

Effects of essential oil of C. ambrosioides on calcium response curves

As the oil relaxed 80mM KCl-induced contraptions, hence, it provided an intimation for possible involvement of voltage gated calcium channel inhibition. Thus to investigate further mechanism of relaxant activity, CCRCs were constructed in the rabbits' jejunal preparations for possible right shifts using verapamil as standard calcium channel blocker (Ali *et al.,* 2014a, Nabi *et al.,* 2012, Ali *et al.,* 2011a). Briefly describing, calcium curves were constructed in tissues incubated with different concentrations of essential oil of *C. ambrosioides* in calcium free medium. Tissues were initially stabilized with Normal Tyrode's solution for 30-40 minutes. Then washed twice with Potassium normal and five times with Potassium Rich Tyrode's solutions to decalcify the tissues. Standard control curves were constructed using concentration (1-256) × 10^{-4} M concentrations of calcium chloride in the absence of essential oil. Tissues were treated with essential oil of *C. ambrosioides* of 0.1-10mg/ml, after an hour of incubation, calcium chloride curves were constructed. Similarly, CCRCs were also constructed in absence and presence of verapamil. EC_{50} value of essential oil of *C. ambrosioides* with its respective control was checked for possible right shift which were compared with EC_{50} of CCRCs of verapamil.

STATISTICAL ANALYSIS

Data were analyzed with Graph Pad prism 5 version 5.01 software, applying Dunnett's test were used to find statistical significance and were presented as mean ± SEM. Results of effects P value less than 0.05 were considered to be statistically significant.

RESULTS

Percent yield

A yellowish essential oil was obtained with % yield is 0.1%. Then it was placed in air tight close container and refrigerated for further screening.

GC/MS Analysis

The GC/MS analysis showed that essential oil contain *4*-carene 56.59% and the second most abundant compound *o*-cymene 41.46% that are reported for the first time from the species. Details of constituents are mentioned in table 1 (fig. 1).

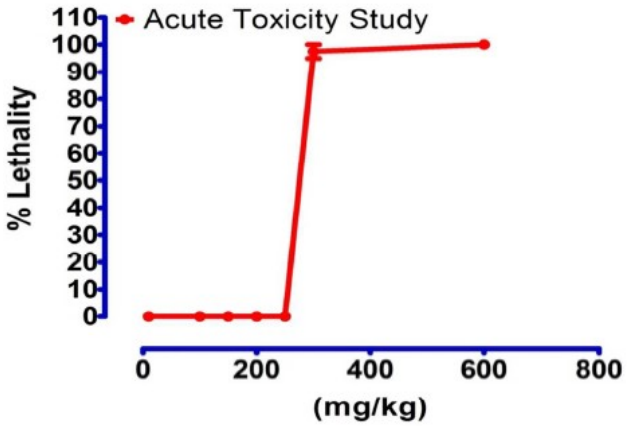


Fig. 2: Graphical presentation of acute toxicity of essential oil of *C. ambrosioides*.

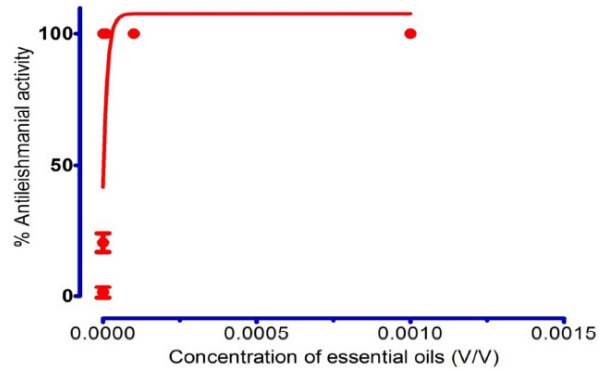


Fig. 3: Graphical presentation of anti-leishmanial activity of essential oil of *C. ambrosioides*.

Acute Toxicity

Results of acute toxicity are mentioned in table 2. Percent lethality is expressed in fig. 2. LD_{50} values for acute toxicity is 261.66±12.66mg/kg. The essential oil is safe in test subjects up to 250mg/kg. However, further increased dose killed all the experimental animal with100% mortality (fig. 2, table 2). The essential oil of the medicinal plants is very potent, so for its pharmacological actions are concerned, yet its safety is questionable. Essential oil in test dose of 300mg/kg killed all the test animals which prove its absolute toxicity. This work determined the safe dose range of essential oil *C. ambrosioides.* Hence, prospectively, we will design our experiments in safe dose range to screen the essential oil for possible antispasmodic and antileishmanial activities.

Experimental animals

Swiss Albino mice (weighing 25-35g, purchased from NIH, Islamabad) were used for acute toxicity testing. Local breed rabbits (weighing 1.2-2kg) of either sex were used in this study.

Housing and husbandry

The experimental animals were kept at Institute of Basic Medical Sciences, KMU, Peshawar, at animal house. Animals were housed in respective cages, overnight fasted prior to start of experiments. The animals were kept at temperature around 25 ±2°C with light and dark cycle of about 12 h each and a relative humidity of 50-60%.

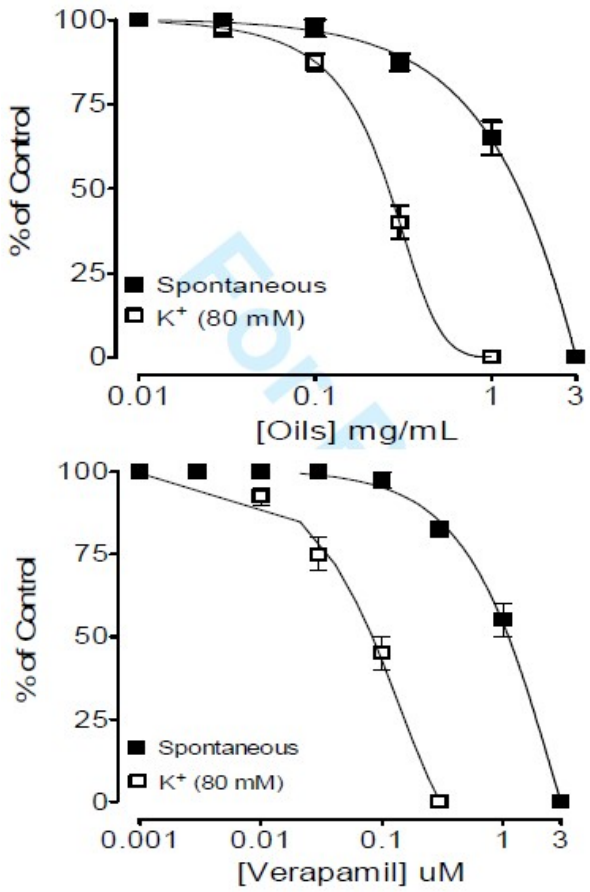


Fig. 4: To show effects of essential oil of *C. ambrosioides* on (A) spontaneous and (B) KCl-induced contractions in rabbits' jejunal preparations (*n*=4).

Sample size

A total of 45 animals were used in the acute toxicity study.

Allocating animals to experimental groups

The 45 animals were divided into 9 groups with 5 each. Acute toxicity was performed in three stages as:

First stage groups (A, B and C) received exponential doses of essential oil with test dose of 10, 100 and 1000 mg/kg body weight respectively. In second stage, group D and group E received 300 and 600mg/kg essential oil respectively. In third stage (F, G and H) received 150, 200 and 250mg/kg respectively (Ali *et al.,* 2014a). Group I was used as negative control.

In vitro anti Leishmanial activity of C. ambrosioides against clinical isolates of Leishmania tropica

Results of anti-leishmanial activity are expressed in fig. 3. When anti leishmanial activities were plotted versus concentrations of essential oil (v/v). It is evident from the fig. 3, essential oil of *C. ambrosioides* have very potent

anti-leishmanial activity with LC_{50} of Log10 (1.83±0.0026) ×10-6 mg/ml (*n*=4) table 3. The low LC_{50} value indicates that the essential oil is very potent against *Leishmania tropica*.

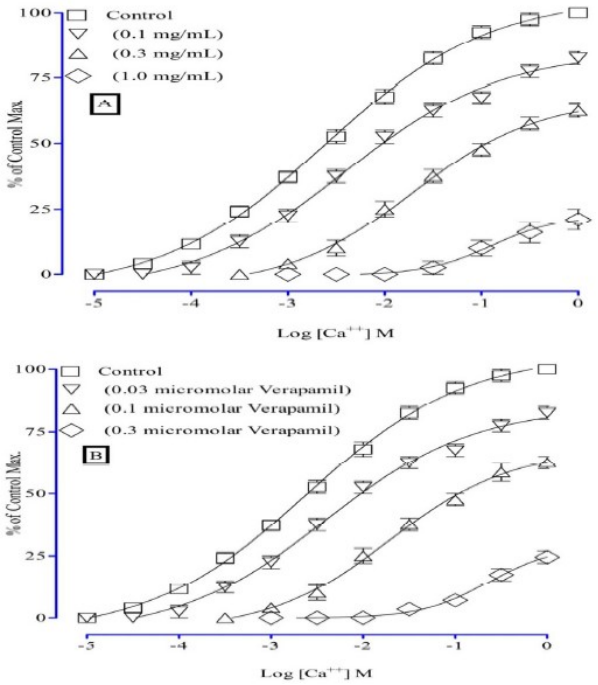


Fig. 5: (A) Calcium chloride curves in presence and absence of *C. ambrosioides*. (B) Calcium chloride curves in the presence and absence of verapamil. (Values represent the mean} SEM, *n* = 6).

Antispasmodic and calcium channel blocking activity of essential oil of C. ambrosioides using rabbit's jejunums

Effects of the essential oil have been shown in fig. 4 A and B. It is evident from the figs. that essential oil of *C. ambrosioides* relaxed spontaneous and KCl-induced contractions in rabbits' jejunum in 3 and 1 mg/ml, with respective EC_{50} of 1.46±0.14 mg/ml (*n*=4) and 0.26±0.02 mg/ml (*n*=4). The results of verapamil on spontaneous rabbits' jejunal preparations and on KCl-induced contractions are shown in fig. 4B with EC_{50} on spontaneous activity 1.08±0.08 mg/ml (*n*=4), and for the KCl-induced contractions EC_{50} = 0.08±0.01 mg/ml (*n*=4).

We constructed CCRCs also in presence of essential oil and compared with verapamil (Ali *et al.,* 2014a, Ali *et al.,* 2014b). Curves of calcium chloride with and without test samples are shown in fig. 5 A. According to fig. 5 A, EC_{50} (Log[Ca++]M) for control (in the absence of test drug) calcium chloride curves is -2.59±0.07 mg/ml *vs*. EC_{50} -2.035±0.06 mg/ml in presence of 0.1 mg/ml of essential oil. EC_{50} (Log[Ca++]M) for 0.3 mg/ml of essential oil is -0.9±0.01mg/ml. The right resembled the right shift of verapamil with an EC_{50} (Log[Ca++]M) for control -2.59±0.02 versus the EC_{50} (Log[Ca++]M) -1.0±0.01 in presence of 0.1µM of verapamil.

DISCUSSION

In this study, we evaluated the GC/MS, anti-leishmanial and relaxant potentials of the essential oil. A yellowish essential oil was obtained with % yield is 0.1%, mainly containing α- pinene 3.57%, α- terpinene 15.90%, camphor 12.42%, cis ascaridole 43.40%, trans ascaridole 6.38% (Omidbaigi *et al.,* 2005), p-cymene 15.3%, α-terpinyl acetate 15.7% (Omidbaigi *et al.,* 2005), *δ*-4-Carene 1.9 %, Carvacrol 4.9 %, Isoascaridole 13.0 % and Caryophyllene oxide 2.2 % (Gupta *et al.,* 2009).

The essential oil is safe in test subjects up to 250 mg/kg. However, further increased dose killed all the experimental animal with100% mortality. Essential oil in test dose of 300 mg/kg killed all the test animals which prove its absolute toxicity.

The essential oil have very potent anti-leishmanial activity with LC_{50} of Log10 (1.83±0.0026) ×10-6 mg/ml (*n*=4). The low LC_{50} value indicates that essential oil is very potent against *Leishmania tropica*. Further work shall be carried out to document it's *in vivo* anti-leishmanial activity either for cutaneous leishmaniosis or visceral leishmaniosis as we predicted a wide safe dose range of ≤ 250 mg/kg.

The tested oil has showed *in vitro* antispasmodic activity in our laboratory, hence further investigated the oil for possible antidiarrheal activity as well.

It relaxed spontaneous and KCl-induced *in vitro* contractions. As the test samples relaxed the KCl-induced contractions, usually predicts for the involvement of voltage gated calcium channels. However, it is not necessary that relaxing effect on KCl-induced contractions usually follow the voltage gated calcium channels. Thus we constructed CCRCs in the presence of essential oil and compared with the effects of verapamil (Gupta and Misra, 2006, Ali *et al.,* 2014a).

CONCLUSION

GC/MS analysis showed that essential oil of *C. ambrosioides* mainly contains 4- carene (56.59%) and o-cymene (41.46%), the two most abundant constituents that are first time reported from the species. More, essential oil of *C. ambrosioides* possesses significant anti-leishmanial. Relaxant activity of essential oil follows inhibition of voltage gated calcium channels.

Ethics approval and consent to participate

Advanced Study & Research Board (ASRB000141/AA/IBMS/22/02/2014) and Ethical Board (No. Dir/KMU-EB/AA/000080) of Khyber Medical University approved the study protocols. The experimental protocol was in accordance with the Animals Bylaws of Khyber Medical University, Peshawar.

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REFERENCES

Al-kaf AG, Crouch RA, Denkert A, Porzel A, Al-Hawshabi OS, Ali NAA, Setzer WN and Wessjohann L (2016). Chemical composition and biological activity of essential oil of Chenopodium ambrosioides from Yemen. *Am. J. Essent. Oil. Nat. Prod*., **4**(1): 20-22.

Ali N, Ahmed G, Shah SWA, Shah I, Ghias M and Khan I (2011a). Acute toxicity, brine shrimp cytotoxicity and relaxant activity of fruits of *Callistemon citrinus* curtis. *BMC Complement. Altern. Med.,* **11**(99): 1-8.

Ali N, Alam H, Khan A, Ahmed G, Shah WA, Nabi M and Junaid M (2014a). Antispasmodic and anti-diarrhoeal activity of the fruit of *Rosa moschata* (J). *BMC Complement. Altern. Med.,* **14**(485): 1-6.

Ali N, Shah I, Shah SWA, Ahmed G, Shoaib M, Junaid M, Ali W and Ahmed Z (2013). Antioxidant and relaxant activity of fractions of crude methanol extract and essential oil of *Artemisia macrocephala* jacquem. *BMC Complement. Altern. Med.,* **13**(96): 1-8.

Ali N, Shah S and Shah I (2011b). Preliminary phytochemical screening and antispasmodic activity of *Artemisia macrocephala* Jacquem. JYP, **3**(2): 125-128.

Ali N, Shah SWA, Ahmed G, Shah I, Shoaib M, Junaid M and Ah W (2014b). Acute toxicity and antispasmodic activities of *Achillea wilhelmsii* C. Koch. *Pak. J. Pharm. Sci.,* **27**(2): 309-315.

Chu SS, Feng Hu J and Liu ZL (2011). Composition of essential oil of Chinese *Chenopodium ambrosioides* and insecticidal activity against maize weevil, *Sitophilus zeamais. Pest Manag. Sci.,* **67**(6): 714-718.

Dembitsky V, Shkrob I and Hanus LO (2008). Ascaridole and related peroxides from the genus Chenopodium. *Biomed. Pap. Med. Fac Uni Palacky Olomouc Czech Repub,* **152**(2): 209-215.

Fdil R, Derhali S, El Maliki S, Filali-Ansari N, Zefzoufi M, El Abbouyi A, El Khyari S, Sraidi K and Mouzdahir A (2017). Comparative analysis, antibacterial and antiradical activities of essential oils in leaves and fruits of *Chenopodium ambrosioides* of Morocco. *RJPBCS,* **8**(4): 1038-1044.

Gupta A, Smith S, Greenway F and Bray G (2009). Pioglitazone treatment in type 2 diabetes mellitus when combined with portion control diet modifies the metabolic syndrome. *Diabetes Obes. Metab,,* **11**(4): 330-337.

Gupta AK and Misra N (2006). Hepatoprotective activity of aqueous ethanolic extract of Chamomile capitula in paracetamol intoxicated albino rats. *Am. J. Pharmacol. Toxicol.,* **1**(1): 17-20.

Gupta D, Charles R, Mehta V, Garg S and Kumar S (2002). Chemical examination of the essential oil of *Chenopodium ambrosioides* L. from the southern hills of India. *J. Essent. Oil Res*.**14**(2): 93-94.

Harvey AL (2007). Natural products as a screening resource. *Curr. Opin. Chem. Biol,,* **11**(5): 480-484.

Karimi A, Majlesi M and Rafieian-Kopaei M (2015). Herbal versus synthetic drugs; beliefs and facts. *J. Nephropharmacol.,* **4**(1): 27-30.

Kaul VK (2000). Analysis of the essential oil of the leaves of the medicinal plant *Chenopodium ambrosioides* var. anthelminticum (L.) A. Gray from India. *Sci. Pharm.,* **68**(1): 123-128.

Kuete V (2014). Physical, hematological, and histopathological signs of toxicity induced by African medicinal plants. Toxicological Survey of African Medicinal Plants. Elsevier.

Muhayimana A, Chalchat J-C and Garry R-P (1998). Chemical composition of essential oils of *Chenopodium ambrosioides* L. from Rwanda. *J. Essent. Oil Res.,* **10**(6): 690-692.

Nabi S, Ahmed N, Khan MJ, Bazai Z, Yasinzai M and Al-Kahraman Y (2012). *In vitro* antileishmanial, antitumor activities and phytochemical studies of methanolic extract and its fractions of *Juniperus excelsa* Berries. *World Appl. Sci. J.,* **19**(1): 1495-1500.

Omidbaigi R, Sefidkon F and Nasrabadi FB (2005). Essential oil content and compositions of *Chenopodium ambrosioides* L. *J. Essent. Oil-Bear. Plants,* **8**(2):**154-158**.

Onocha PA, Ekundayo O, Eramo T and Laakso I (1999). Essential oil constituents of *Chenopodium ambrosioides* L. leaves from Nigeria. *J. Essent. Oil Res.,* **11**(2): 220-222.

Ososki AL, Lohr P, Reiff M, Balick MJ, Kronenberg F, Fugh-Berman A and O'Connor B (2002). Ethno-botanical literature survey of medicinal plants in the Dominican Republic used for women's health conditions. *J. Ethnopharmacol.,* **79**(3): 285-298.

Rehecho S, Hidalgo O, de Cirano MG-I, Navarro I, Astiasarán I, Ansorena D, Cavero RY and Calvo MI (2011). Chemical composition, mineral content and antioxidant activity of *Verbena officinalis* L. *LWT-Food Sci. Technol,* **44**(4): 875-882.

Shah H (2014). Antibacterial and antifungal activities of the crude extracts from the stem of *Chenopodium ambrosioides* Linn., an indiginous medicinal plant. *Afr. J. Pharm. Pharmacol.,* **8**(8): 231-234.

Veeresham C (2012). Natural products derived from plants as a source of drugs. Wolters Kluwer – Medknow Publications.