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Chemical Composition and Biological Screening of Essential Oils of *Zanthoxylum armatum* DC Leaves

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Abstract

Essential oil (ZVO) from *Zanthoxylum armatum* was extracted through hydro-distillation and tested for various biological activities. A total of 34 chemical constitutes were identified through GC-MS, the major constituents of ZVO were beta- Linalool (53.05%), Bergamot mint oil (12.73%), alpha-Limonene diepoxide (11.39%), alpha- pinene (4.08%), beta- Myrcene (3.69%) and D-Limonene (3.10%). ZVO showed significant antispasmodic effect and relaxed the isolated rabbit jejunum in spontaneous as well as in potassium chloride induced contraction. The maximum effect was observed against *M. leutus* followed by *B. subtilis* with percent zone of inhibition 28.45 and 20.45, respectively. A concentration dependent effect was also observed against available species of fungi. The maximum effect was observed against *M. canis, C. albicans and C. glabrata* with percent activity 84.87, 83 and 79, respectively. The oils were also found to have Cytotoxic and Phytotoxic potential.

Keywords: Essential oil; *Zanthoxylum armatum;* Antispasmodic

Introduction

Zanthoxylum armatum is a small xerophyte, tree or shrub having leaflet blades usually with prickles. Leaves are compound, imparipinnate with 3-7 foliolate and pellucid-punctate. Petiole and rachis are winged. Leaflets are sessile, elliptic to ovate-lanceolate with crenate or entire margins. Flowers born axiliary, minute and polygamous. Calyx 6-8 was acute lobed, petals absent. Male flowers had 6-8 stamens with rudimentary ovary. Female flowers were with 1-3 carpels. Ovary was 1-3 locular. Fruit was small drupes with red color, splitting into two when ripe. Seed are rounded and shining black. *Zanthoxylum armatum* prefers semi shady or no shade for growth. It grows wild in foothills starting from about 800m up to 1500m in Malakand, Swat, Dir, Hazara, Buner, Muree hills and Rawalpindi [1]. It is locally known as Dambara. Its fruits and seeds are edible and used as potherb species. The plant is used for Pneumonia and tick infestation [2]. Young shoots are used as toothbrush and useful for curing gum diseases. Fruit is used for toothache, dyspepsia, as a carminative and stomachache. Seeds are used for condiment and flavouring agent. Wood is used to make walking sticks [3,4]. Powdered fruit is mixed with *Mentha* sp. and table salt, eaten with boiled egg for chest infection and digestive problems [5]. Recently, we have tested the leaves and fruits of this plant for various pharmacological activities including antipyretic action [6].

Materials and Methods

Drugs and other chemicals

Acetylcholine (BDH Chemicals, Poole, England), Potassium chloride (E. Merck Germany), Tyrode's solution (Prepared from its constituents with their respective concentrations (mM) NaCl 136.9, KCl 2.68, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.90, $CaCl_2$ 1.8, and glucose 5.55 dissolved in 1 litre distilled water).

Essential oil extraction

A Modified Clevenger type apparatus were used for the extraction of essential oil from the leaves of *Zanthoxylum armatum* through hydro- steam distillation. The leaves were thoroughly washed, cut into small pieces, placed in distillation flask and subjected to hydro-steam distillation for about 4 hours. The steam and vaporized oil were condensed into liquid by a vertical condenser and collected in measuring tube. Being immiscible and lighter than water, the volatile oil separated out as an upper layer. The oil was then separated from water and collected in small bottles, dried with anhydrous sodium sulphate, sealed, labelled and stored in light resistant vials at 4–6°C for further use [7].

Animals

Local breed rabbits of either sex with weights in the range of 1.0-1.4 kg were used. The animals were kept for 14 days before starting the experiments at the "Animal House of Department of pharmacy, University of Malakand" under standard conditions mentioned in the "Animals Bye-Laws 2008 of the University of Malakand (Scientific Procedures Issue- 1)." and fed with standard diet and tap water. The animals were kept in fasting condition for 24 hours prior to the experiments; they were only provided with free access to water.

Gas chromatography- Mass spectrometery (GC-MS)

The essential oil from the leaves of *Zanthoxylum armatum* were analyzed by GC-MS Model QP 2010 plus (Shimadzu) operating in EI mode at 70 ev, equipped with a split-splitless injector (Split ratio, 1:50). Helium was used as carrier gas with flow rate of 1 ml/min. A capillary column (Length: 30 m, id: 0.25 mm, thickness: 0.25 µm, DB-5MS Agilent

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technologies, USA) treated with 95% dimethyl- and 5% biphenyl poly silphenylene. HPLC grade dichloromethane was used as reagent. The following conditions were maintained during the operation of GC-MS analysis. Inject temperature: 240 °C, Ion source temperature (EI): 240 °C, Interface temperature: 240 °C, Pressure: 80 KPa, GC program time: 46.67 minutes total, Solvent cut time: 2.5 minutes, MS start time: 3 minutes , MS end time: 46 minutes, Acquisition time: scan, M/Z: 40-500 [8].

Identification of the Components

The identification of the constituents was based on comparison of the retention times (RT) and mass spectra of the samples with those obtained from standards used. Relative percentage of compounds was calculated from the total chromatogram by using computer software [8].

Antispasmodic activity

Rabbit's jejunum preparations: Experiments on rabbit's jejunum preparations were carried out as following [9]. Slaughtered animals were dissected to open abdomen, and jejunum portion(s) were extracted and kept in freshly prepared Tyrode's solution, aerated with carbogen gas (5% Carbon dioxide and Oxygen mixture) to keep them alive and ready for use. Quiescent sub-maximal doses of acetylcholine (0.3 µM) to the tissues were used when needed for keeping the tissue viable and alive [10]. About 1.5 cm length tissue was mounted in 10 ml tissue bath containing Tyrode's solution and stabilized for 25-30 minutes. All the processes were carried out at 37+ 1°C with constant aeration and kept under 1 gram pressure. On attaining reproducible response, test samples at the doses of 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 5.0, and 10.0 mg/ml were applied to the bath solution [9,11]. The processes were repeated thrice (n=3) and fall in spontaneous activity was observed to be change of the sample tested. For the determination of possible mode of action, the tissue was pretreated with high concentration of KCl (80 mM in final bath solution). KCl cause depolarization and keep the tissue in a position of sustained contraction [12]. The extract was then applied in cumulative manner to obtain a dose dependent curve and relaxation. Intestinal responses data were recorded using Force Transducer (Model No: MLT 0210/A Pan Lab S.I.) attached with Power lab (Model No: 4/25 T) AD Instruments, Australia. Data was recorded at range of 20 mv, low pass 5 Hz X 10 gain using input 1, rate 40/S. Results were expressed as% of KCl induced contraction. Chart 5 (AD Instruments) was used to interpret the graph tracings. Student "t" test was used at 95% confidence interval (CI). 'P' values less or equal to 0.05 was considered as statistically significant [10].

Anti bacterial activities

Antibacterial activities of the plant were carried out by agar well diffusion method as used in our previous study [13]. Bacterial strains were first cultured on nutrient broth and incubated for 24 hours prior to experiments. Nutrient agar was melted, cooled to 40°C and poured into sterilized petridishes. Wells were then bored in media using 6mm diameter with the help of sterile metal cork borer and keeping a distance of 24 mm between two adjacent wells. 4-8 hour old bacterial culture was spread on the surface of nutrient agar with the help of sterilized cotton swab. These processes were repeated thrice turning the plate 60° between each streaking. About l00 µl of 3 mg/ ml of respective extract, dissolved in DMSO was then added to the wells. Other wells were supplemented with DMSO and 10 µg Imipenem served as positive and negative controls. The plates were then incubated for 24 hours at 37°C. The plates were then observed for zones of inhibition. All the experiments were conducted in triplicate.

Anti-fungal activities

Seven days old fungal cultures (PCSIR labs Lahore Pakistan), test samples, sabouraud dextrose agar,Dimethyle sulphoxide(DMSO), screw cap test tubes, micropipettes, autoclave, incubator, standard antibiotic (Miconazole). Twenty four mg of crude extract was dissolved in 1 ml sterile Dimethyl sulfoxide (DMSO) serving as a stock solution. 4 ml Sabouraud dextrose agar (SDA) growth media was transffered to each screw capped tube, under sterile conditions and autoclaved at 121°C for 15 minutes. These tubes were then allowed to cool to 50°C and 400 µg/ml test sample was added to non-solidify SDA tubes, which were then allowed to solidify at room temperature. Next each glass tube was inoculated with 4 mm diameter piece of inoculum removed from 7 days old fungal culture, whereas, agar streak was employed in case of non-mycelial growth. Other media supplemented with DMSO and miconazole antibiotic were used as a negative and positive control respectively. The tubes were incubated at 28 ± 1°C for 7 days. Cultures were observed twice weekly during incubation. Growth in the media was estimated by measuring linear growth (mm) in the in media loaded with sample, DMSO and miconozole respectively and then percentage inhibition of fungal growth was calculated as follows

% Mycelia inhibition = Gn - Gt / Gn x 100

Where, Gn= Mycelial growth in normal, Gt= Mycelial growth in test [14]

Cytotoxicity

The cytotoxic activity of essential oil from the leaves of *Zanthoxylum armatum* were tested using brine shrimp assay following recommended method used in our previous work [6,15]. About 20 mg of each extract was dissolved in 2 ml of respective solvent and from this solution transfer 5, 50 and 500 µl to vials (3 vials /concentration). This concentration was equivalent to 10, 100 and 1000 µg/ml, respectively. The solvent were allowed to evaporate overnight. 5 ml with seawater solution (38 g/L) were added to each vial. After 36 h of hatching and maturation of larvae as nauplii, 10 larvae were transferred to each vial using a Pasteur pipette. The vials were then placed at room temperature (25-27°C) under illumination. Other vials were supplemented with brine solution served as positive controls.

Phytotoxicity

The phytotoxic activity essential oil of the leaves of *Zanthoxylum armatum* were evaluated using *Lamna minor* as test species following recommended procedure used in our previous work [6,13]. 15 mg of respective extract was dissolved in 15 ml of respective solvent and from this solution transfer 5, 50 and 500 µl to the flask (3 flasks for each concentration). This concentration was equivalent to 10, 100 and 1000 µg/ml respectively. The solvent was allowed to evaporate overnight under sterilized condition in laminar flow. 20 ml of E. medium was added to each flask. Other flasks (3 for each) were supplemented with E. medium and standard drug (Atrazine) served as negative and positive control. To each flask ten plants with 2-3 fronds were transferred and kept all the flasks under about 12 h day light conditions. Plants were observed daily and on each seventh day the numbers of fronds were counted.

Statistical analysis

All the data expressed as mean ± SEM and the median effective concentrations (EC50) values are given as geometric mean with 95% confidence intervals (CI). One-way Analysis of Variance (one-way

ANOVA) followed by Tukey's post-test was used to determine the significant difference in various doses, P values < 0.05 (P < 0.05) were considered statistically significant. All the graphs, calculation and statistical analyses were performed using GraphPad Prism software version 4.00 for Windows, (GraphPad Software, San Diego California USA, http:// www.graphpad.com).

Results and Discussion

GC-MS Analysis of essential oil of *Zanthoxylum armatum* leaves

Essential oils from *Zanthoxylum armatum* leaf (ZVO) were hydro distillated and GC-MS analysis was carried out for identification of various components (Figure 1). The components with their respective terpenoid nature, percent concentration, retention time and charge to mass ratio are presented in Table 1. Out of the total 34 identified components, monoterpenes alcohol was the largest component (56.57%), followed by monoterpenes hydrocarbins (14.99%), Bergamot mint oil (12.73%), monoterpenes oxygenated (11.39%), monoterpenes acetate (1.9%), monoterpenes aldehyde (0.05%), and sesqueterpenes (2.67%). Among the monoterpene alcohol, beta- Linalool (53.05%) was the major constituents. Other significant components detected in ZVO were monoterpenes of hydrocarbon including alpha-Limonene diepoxide (11.39), alpha- pinene (4.08%), beta- Myrcene (3.69%) and D. limonene (3.1%). α-pinene has strong bactericidal and bacteriostatic potentials. As ZVO containing good amount of α-pinene, it might be used as a good antibacterial as well as bacteriocidal in various formulations. Minor components detected were the monoterpenes including p-meth-1-en-8-ol (2.47%), Geraniol acetate (1.32%), cis-beta-Ocimene (1.29%), Sabinene (1.24%) and p-meth-1-en-8-ol, acetate (1.05%) and sesqueterpenes i.e. Carryophyline (1.32%) and Germacrene- D (1.01%).

Antispasmodic effect

In the Present study, Essential oil of leaf (ZVO) of *Z. armatum* was evaluated on the isolated rabbit jejunum for possible antidiarrheal effect, which may be a cheaper and accessible source for treatment of diarrhea and will also provide a scientific proof for its ethno-pharmacological use as an antispasmodic drug. All the samples were tested against spontaneous and potassium chloride induced contracted smooth muscle of the isolated rabbit jejunum. The results of this all bioassays are presented in Figure 2 and 3. To determine the possible mode of action, the tissue was pre-treated with high concentration of KCl (80 mM in final bath solution). KCl cause depolarization and keep the tissue in a position of sustained contraction [16]. The test samples were then applied in cumulative manner to obtain a dose dependant curve and relaxation results were expressed as% of KCl induced contraction [16]. ZVO was also tested against spontaneous and Potassium chloride induced contracted smooth muscle of isolated rabbit jejunum. As compared to control, ZVO significantly relaxed the contracted smooth muscles in both the cases. The spasmolytic effect of the oils started from 0.03 mg/ml and showed 100% effect at 10 mg/ml dose. EC_{50} values for both spontaneous and KCl induced contraction for ZVO was found 0.22 and 0.73 mg/ml, respectively.

The contraction of smooth muscle of rabbit jejunum is due to increase concentration of the free calcium in cytoplasm, which stimulates the chemical mediators which are responsible for contraction. This increase in calcium level may be either due to influx via voltage dependent calcium channels or direct release of calcium from endoplasmic reticulum (calcium store). Thus a periodic depolarization is created

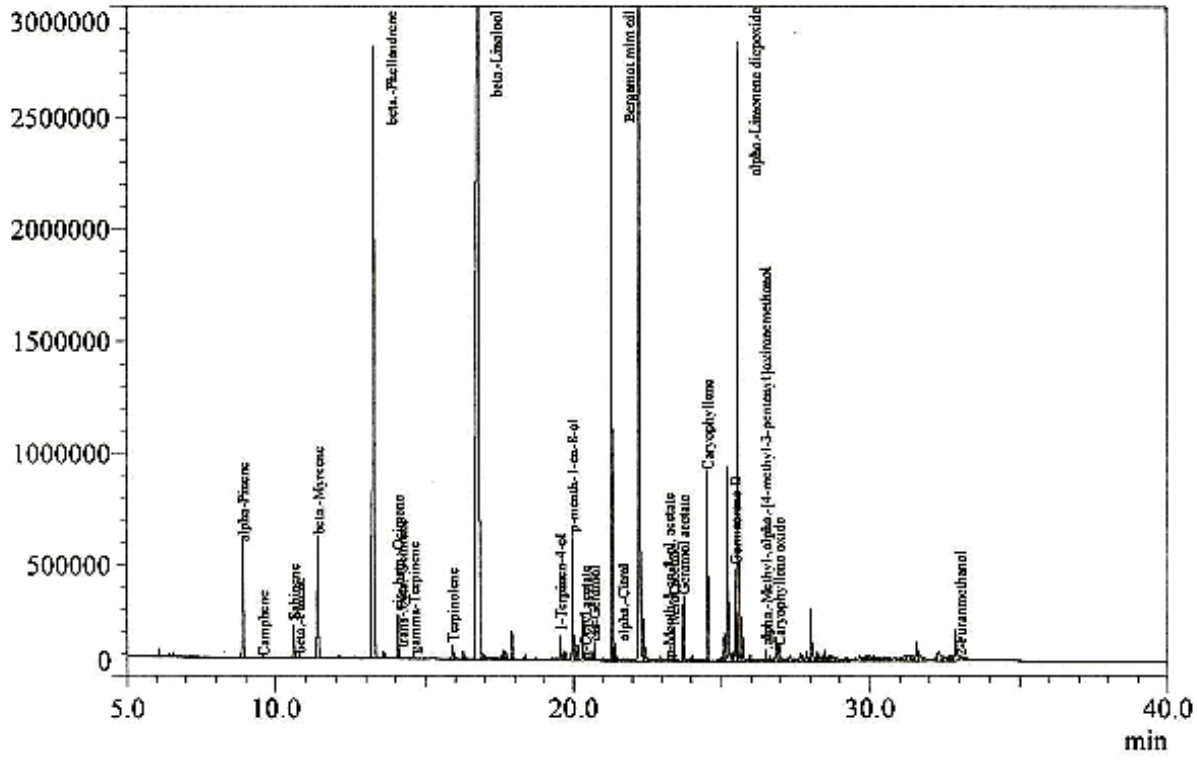

Figure 1: Typical GC-MS chromatogram of *Zanthoxylum armatum* leaf essential oil (ZVO) showing the separation of chemical components.

S. N	Name	Compound type	Concentrtation	R/time	m/Z
1	alpha-phelendrene	Monoterpene hydrocarbon	0.02	8.617	
2	alpha- pinene	Monoterpene hydrocarbon	4.08	8.914	93
3	Camphene	Monoterpene hydrocarbon	0.03	9.59	93
4	Sabinene	Monoterpene hydrocarbon	1.24	10.61	93
5	Beta – Pinene	Monoterpene hydrocarbon	0.16	10.795	93
6	beta- Myrcene	Monoterpene hydrocarbon	3.69	11.411	93
7	D-Limonene	Monoterpene hydrocarbon	3.1	13.219	68
8	beta – phellandrene	Monoterpene hydrocarbon	0.41	13.282	40
10	Trans-beta-Ocimene	Monoterpene hydrocarbon	0.24	13.602	93
11	cis-beta-Ocimene	Monoterpene hydrocarbon	1.29	14.094	93
12	3-Carene	Monoterpene hydrocarbon	0.15	14.608	93
13	gamma turpentine	Monoterpene hydrocarbon	0.16	14.608	93
14	Terpinolene	Monoterpene hydrocarbon	0.32	15.921	93
15	alpha-Methyl-alpha-[4-methyl-3-pentenyl] oxirane methanol	Monoterpene alcohol	0.03	15.994	59
16	beta- Linalool	Monoterpene oxygenated	53.05	16.813	71
17	1-Terpinene-4-ol	Monoterpene alcohol	0.51	19.56	71
18	p-meth-1-en-8-ol	Monoterpene alcohol	2.47	19.997	59
19	n-octyle acetate	Monoterpene acetate	0.01	20.4	43
20	Cis Geraniol	Monoterpene alcohol	0.39	20.711	41
21	Brgamot mint oil	Mixture of acetate and alcohol monoterpenes	12.73	21.31	93
22	Alpha-citral	Monoterpene aldehyde	0.05	21.693	69
23	3-Nonanol,1,2;6,7-dipoxy-3,7-dimethyle acetate	Sesqueterpene	0.03	23.239	43
24	p-meth-1-en-8-ol, acetate	Monoterpene acetate	0.03	23.239	121
25	Nerol acetate	Monoterpene acetate	0.54	23.409	69
26	Geraniol acetate	Monoterpene acetate	1.32	23.744	69
27	Caryophyllene	Sesqueterpene	1.39	24.553	41
28	beta-Farnesene	Sesqueterpene	0.02	24.85	69
29	alpha-Limonene diepoxide	Monoterpene oxygenated	11.39	25.58	43
30	Germacrene D	Sesqueterpene	1.01	25.496	161
31	trans-Nerolidol	Sesqueterpene	0.14	26.516	69
32	Caryophyllene oxide	Sesqueterpene	0.08	26.962	41
33	p-Cimene	Monoterpene hydrocarbon	0.1	32.842	40
34	2-Furanmetahnol	Monoterpene alcohol	0.12	33.035	40

Table 1: GC-MS profile of essential oils of the leaves of *Zanthoxylum armatum* (ZVO).

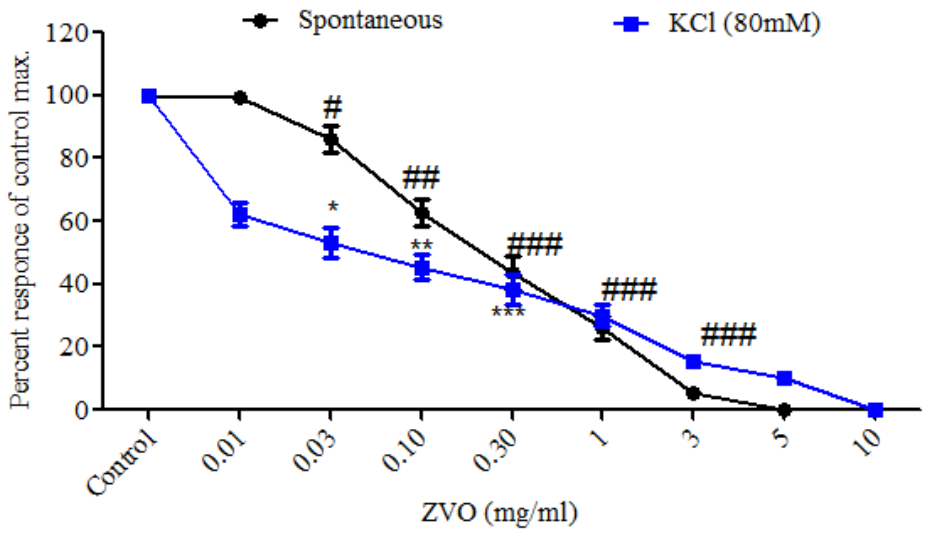

Figure 1: Inhibitory effect of ZVO on spontaneous and high K^+ induced contractions in isolated rabbit jejunum preparations ###P < 0.001; shows a comparison of concentration-dependent effects (specified effect compared with the effect of preceding dose) on spontaneous contractions, whereas **P < 0.01; ***P < 0.001; show comparisons of relaxant effects on K^+ (80 mM)-induced contractions.

Figure 3: Typical tracing showing inhibitory effect of crude extract of ZVO on the spontaneous contractions in isolated rabbit jejunum preparations

		M. leutus	*E.coli*	*S. aureus*	*P.multocida*	*P.aeruginosa*	*B.subtilis*	*S. viridans*
DMSO		-	-	-	-	-	-	-
Cifrofloxacin		28 ± 0.23	30 ± 0.10	24 ± 0.65	32 ± 0.24	-	-	22 ± 0.45
ZVO	125µg/ml	23.33 ± 0.58	14.83 ± 0.41	13.33 ± 0.58	13.67 ± 0.58	11.67 ± 0.41	15.83 ± 0.78	17.67 ± 0.58
	250µg/ml	25.23 ± 1.21	16.26 ± 0.85	13.78 ± 0.74	15.56 ± 1.08	12.45 ± 0.34	17.45 ±1.81	17.24 ± 2.23
	500µg/ml	28.45 ± 1.87	17.89 ± 1.67	14.02 ± 1.31	16.34 ± 1.91	14.67 ± 1.01	20.45 ± 1.41	18.54 ± 1.45

Table 2: Antibacterial effect of the essential oil of the leaves of *Zanthoxylum armatum.*

	M. leutus	*E.coli*	*S. aureus*	*P.multocida*	*P.aeruginosa*	*B.subtilis*	*S. viridans*
	Minimum inhibitory concentration MIC (mg/ml)						
ZVO	0.65	1.25	1.25	0.65	1.25	1.5	0.65

Table 3: MIC value of the leaf essential oil of Zanthoxylum armatum.

due to high speed action potential. When there is increase potassium concentration, the contraction of the smooth muscle will increase due to rapid action potential. When the calcium channel is blocked through calcium channel blocker agents, the contracted smooth muscle will relax [17]. In the present study, the extracts relaxed the contracted muscle, suggesting that the possible mode of action of this plant is either blocking the release of stored calcium from the sarcoplamic reticulum or blocking the calcium channel.

Antibacterial effect

Present study was also carried out for investigation of *Z. armatum* for such antimicrobial agents. Leaf essential oil of *Z. armatum* were tested against various gram positive and gram negative bacteria i.e. *Micrococcus leutus, Escherichia coli, Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Bacillus subtilis,* and *Streptococcus viridines* as shown in Table 2. The antibacterial actions of the extracts were compared with ciprofloxacin as standard drug. It was observed that ZVO has inhibitory effect against various gram positive and gram negative bacterial strains but the antibacterial potential was greatly varied among the extracts.

Antibacterial potential of ZVO was evaluated in the present study and it was found effective against all tested bacterial strains. *B. subtilis,* and *S. viridans* were found more susceptible as compared to other strain tested. The maximum effect was observed against *M. leutus* followed by *Streptococcus viridans* (17.67 ± 0.58 mm) and *B. subtilis* (15.83 ± 0.41mm). *S. viridans* and *S. aureus* which were found resistant against the other samples tested, affected significantly by ZVO. Essential oils from other plats like Cinnamon, Clove, Geranium, Lemon, Lime, Orange and Rosemary oils were found inhibitory to gram-negative bacteria (*E. coli*, *P. aeruginosa*, *P. vulgaris*) and gram-positive bacteria (*B. subtilis* and *S.aureus)*. *Citrus* peel oils have strong antimicrobial activity against various bacterial strains.

Minimum inhibitory concentration (MIC) values were also determined for the test samples. ZVO appeared to be most potent antibacterial agent, as it was effective in inhibiting growth of all bacterial strains with very low MIC values ranging from 0.65- 1.25 µg/ml with the lowest value for *M. leutus* and *S. viridians* (Table 3). MIC values acquired in the present study were very encouraging and further research will enhance opportunity for exploiting ZVO as a strong antibacterial agent, especially the volatile oil for treating halitosis (bad breath) caused by excessive growth of bacteria (Yaegak and Coil, 1999), urinary tract infection caused by *E. coli* and *P. aeruginosai* and skin infection caused by *M. luteus* and *B.subtilis*. These results are also found very promising as these extract especially the essential oil were found efficient in quite lower concentration.

Antifungal effect

In the present study leaf essential oils were evaluated for antimycotic potential against various fungal strains like *Trichophyton longifusis, Candida albicans, Fusarium solani, Microsporum canis, Aspergillus flavus* and *Candida glabrata*as. The results are shown in Table 4. A general trend of dose dependency was observed i.e. effect became more pronounced with increasing concentration of the various tested samples.

Antifungal activity of ZVO as a percent inhibition of mycelia growth showed a concentration dependent effect against all the fungi. Best anti-fungal effect was observed against *C. albicans* (66.67 ± 0.57) followed by *A. flauus* (55.33 ± 0.57) and *F. solani* (46.33 ± 0.33). Other fungal strains were also affected at 125 µg/ml concentration. Overall results showed that all fungal strains were inhibited by the ZVO.

Cytotoxicity effect

Brine Shrimp Toxicity bioassay is preliminary study for the detection and development of anti-cancer drugs. Brine shrimp lethality

	Concentration	Percent Inhibition of mycelia growth					
		T.longifusis	*C.albicans*	*F.solani*	*M. Canis*	*A. flavus*	*C.glabrata*
ZVO	125µg/ml	30.43	66.67	46.33	34.76	55.33	21.33
	250µg/ml	54.33	76.33	67	76.33	56.93	29.21
	500µg/ml	70.59	83.87	70.37	84.62	67.74	79.41

Data is presented as Mean ± SEM

Table 4: Antifungal effect of the essential oil of leaves of Zanthoxylum armatum.

Cytotoxic activity				
	No of BS	Living BS	Dead BS	LD_{50}
10	30	15 ± 1.00	15 ± 1.91	5.90
100	30	3.9 ± 1.00	26.1 ± 1.78	
1000	30	1.17 ± 1.08	28.83 ± 1.05	
Phytotoxic activity				
	No of fronds	Living fronds	Dead fronds	LD_{50}
10	52	26 ± 0.12	26 ± 1.34	2.30
100	52	20 ± 1.99	32 ± 1.78	
1000	52	22 ± 1.56	30 ± 1.11	

BS= Brine shrimp, data is presented as Mean ± SEM

Table 5: Cytotoxic and Phytotoxic effect of essential oils of the leaves of Zanthoxylum armatum.

tests were carried out to investigate preliminary cytotoxic potential of the crude ethanolic and *n*-hexane extract of leaf, bark, fruit and leaf essential oil of *Z. armatumi*. ZVO showed outstanding mortality rate (100%) at a dose of 1000 µg/ml with 15.90 LC_{50} values (Table.5). It is needed to carry further detailed investigations for identification and quantification of pharmacologically bioactive specific constituents from *Z. armatum*.

Phytotoxicity effect

All the parts showed significant dose dependant phyto inhibition (Table 5). The ZVO showed moderate inhibition at higher dose having FI_{50} of 2.30 µg/ml. These results suggested that all parts have some active principles with phytotoxic potential and *Z.armatum* can be a good herbicides or weedicides. Further study is needed to exploit its phytotoxic mechanism and also to identify and quantify the bioactive constituents. It might be helpful to investigate its efficacy in detail as a weeds, pests and disease control agent.

Conclusion

The findings of this research work suggest that the ZVO has antispasmodic, antimicrobial, cytotoxic and phytotoxic properties which may be due to the presence of various chemical constituents. The results strongly support the ethno medicinal use of this valuable plant in the treatment of diarrhea and various microbial infections. However more detail study is required to establish the active constituents responsible for these activities.

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