academicJournals

Vol. 7(13), pp. 685-693, 8 April, 2013
DOI 10.5897/AJPP12.725
ISSN 1996-0816 © 2013 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Antispasmodic potential of leaves, barks and fruits of *Zanthoxylum armatum* DC

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Accepted 1 February, 2012

Zanthoxylum armatum **is a well known food product used as antispasmodic in traditional medicine system. In the present research work, the phytochemical composition of the leaves, barks and fruits was carried out showing that all parts are good source of alkaloids, saponin, tannins and flavonoids. The ethanolic and n-hexane extracts were tested for their antispasmodic potential using rabbit ileum tissues. The ethanolic and *n*-hexane extract of the leaves (ZLE and ZLH), barks (ZBE and ZBH) and fruits (ZFE and ZFH), demonstrated a dose dependant antispasmodic effect. In most of the tested extracts, the action of ethanolic extract was found more pronounced than the other. The most significant antispasmodic effect was observed with fruit extracts. In conclusion, the current study strongly supports the folkloric uses of the plant as antispasmodic.**

Key words: *Zanthoxylum armatum*, rabbit ileum, phytochemical studies, folkloric, antispasmodic.

INTRODUCTION

Developing countries have been facing morbidity and mortality of childhood due to diarrhea and other gastrointestinal problems. An estimated frequently 10 million deaths per year in children under age of 5 has been reported (Carlos and Saniel, 1990). In these countries, people are still relying on the herbal drugs for control of diarrhea despite of enormous development of all antidiarrheal medicines in the world (Ojewole, 2004; Agunu et al., 2005). The world health organization (WHO) highly appreciated the conventional medical practices for treatment and precautionary measure of diarrheal diseases (Atta and Mouneir, 2004).

Zanthoxylum armatum is a small xerophytic tree or shrub, with leaflet blades usually with prickles. The leaves are compound, imparipinnate with 3 to 7 foliolate and pellucid-punctate. The petiole and rachis are winged. The leaflets are sessile, elliptic to ovate-lanceolate with crenate or entire margins. The flowers are born axillary, minute and polygamous. Calyx is 6 to 8 acute lobed. Petals are absent. Male flowers are always with 6 to 8 stamens with rudimentary ovary, while female flowers are with 1 to 3 carpels. Ovary is 1 to 3 locular. Fruit is small drupes with red color, splitting into two when ripe. The seeds are rounded and shining black (Hassan-Ud-Din and Ghazanfar . *Z. armatum* prefers semi shady or no shade for growth. It grows wild in foothills starting from about 800 to 1500 m in Malakand, Swat, Dir, Hazara, Buner, Muree hills and Rawalpindi (Shinwari et al., 2006). In Pakistan, it is known as Dambrary, Tamur (Urdu) and Dambara (Pashtu). Its fruits and seeds are edible and used as potherb species. The plant is used for pneumonia and tick infestation (Sindhu et al., 2010). Young shoots are used as toothbrush and useful for curing gum diseases. The fruit is used for toothache,

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dyspepsia, as a carminative and stomachache. The seeds are used as condiment and flavoring agent. The wood is used to make walking sticks (Arshad and Ahmad, 2004; Abbasi et al., 2010). Powdered fruit, mixed with *Mentha* species and table salt is eaten with boiled egg for chest infection and other digestive problems (Islam et al., 2009). Recently, the leaves and fruits of this plant were tested for various pharmacological activities including antipyretic action (Barkatullah et al., 2011).

In this study, ethanolic and n-hexane extracts of the leaves, barks and fruits of *Z. armatum* were evaluated on isolated rabbit jejunum preparations, to rationalize the ethnopharmacological use of this important medicinal plant.

MATERIALS AND METHODS

Drugs and other chemicals

Acetylcholine (BDH Chemicals, Poole, England), potassium chloride (KCl; 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 L distilled water).

Animals

Local breed rabbits of either sex with weights ranging from 1.0 to 1.4 kg were used. The animals were kept for 14 days before starting the experiments at the "Animal House of the 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 were fed on standard diet and tap water. The animals were kept in fasting condition 24 h prior to the start of experiments with free excess to water.

Qualitative chemical identification tests

Various phytochemical tests were performed for detection of various constituents preliminary, using well established procedures (Trease and Evans, 1989; Muhammad and Saeed, 2011). In case of quantitative phytochemical study, alkaloids, saponin, tannins and flavonoid were determined following the published protocols (Harborne, 1998; Huang et al., 2010; Muhammad and Saeed, 2011; Barkatullah et al., 2012).

Rabbit's jejunum preparations for antispasmodic activity

Slaughtered animals were dissected to open abdomen and jejunum portion(s), 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. About 1.5 cm length tissue was mounted in 10 ml tissue bath containing Tyrode's solution and was stabilized for 25 to 30 min. All the processes were carried out at 37+1°C with constant aeration and kept under 1 g 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 (Gilani et al., 2005b; Ali and Shah, 2010). 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 the tissue was kept in a position of sustained contraction. 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 at 5 Hz×10 gain using input 1, rate 40 s^{-1} (Ali and Shah, 2010). Results were expressed as percentage of KCl induced contraction.

Statistics and interpretation

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.

RESULTS

Qualitative phytochemical screening

Qualitative phytochemical screening was carried out using different extracts of the leaf, stem bark and fruit of *Z*. *armatum*. The detail of this study is shown in Tables 1, 2 and 3, respectively.

Quantitative phytochemical screening

Bioactive constituents like alkaloids, sterol, saponins, tannins, phenols and flavonoids were quantitatively evaluated in leaf, bark and fruit of *Z*. *armatum*. The results revealed the presence of bioactive constituents in leaves comprising of alkaloids (15.60±0.10 mg/g), sterols (71.60±0.10 mg/g), saponins (21.57±0.12 mg/g), tannins (34.43±0.21 mg/g), phenols (11.66±0.33 mg/g), and flavonoids (13.68±0.66 mg/g). The bark contained alkaloids (19.60±0.10 mg/g), sterols (33.83±0.29 mg/g), saponins (14.78±0.10 mg/g), tannins (28.62±0.13 mg/g), phenols (16.48±1.33 mg/g), and flavonoids (18.33±1.22 mg/g), while the fruits contained alkaloids (25.07±0.21 mg/g), sterols (164.92±0.14 mg/g), saponins (28.60±0.10mg/), tannins (35.5±0.5 mg/g), phenols (21.68±0.44 mg/g) and flavonoids (22.8±1.33 mg/g) (Table 5). Comparative statistics of these bioactive constituents in the leaves, bark and fruit of *Z*. *armatum* are as shown in Figure 3.

Antispasmodic activity

Antispasmodic potential of all samples from *Z*. *armatum* is shown in Figures 1 and 2. The effect of ethanolic extract of *Z. armatum* leaves (ZLE) was found to start from 0.3 mg/ml and reached to maximum at a dose of 10 mg/ml.

Table 1. Preliminary phytochemical screening of *Zanthoxylum armatum* leaves.

Constituent	Test name	Ethanolic extract	Hexane extract
Carbohydrates	Fehling's test	+	-
	Molisch's test	+	-
	Benedict's test	+	-
Protein	Ninhydrin test	-	-
	Millon's test	-	-
Alkaloids	Wagner's test	+	+
	Mayer's test	+	+
	Hager's test	+	+
Phytosterol and Triterpenoids	Salkowski's test	+	+
	Liebermann-Burchard test	+	+
Phenol	Ferric chloride test	+	-
Flavonoids	Lead acetate test	+	+
	Alkali test	+	+
	Shinoda's test	+	+
Tannins	Gelatin test	+	+
	Ferric chloride test	+	+
	Alkali test	+	+
Saponins	Frothing test	-	-
Anthocyanins	HCl test	+	-
Glycosides	Killer-Kiliani test	-	-
Fixed oil and fats	Spot test	+	+
	Alkali test	+	+
Volatile oil	Spot test	+	+

Table 2. Preliminary phytochemical screening of *Z. armatum* bark.

Constituents	Test name	Ethanolic extract	Hexane extract
Carbohydrates	Fehling's test	+	+
	Molisch's test	+	+
	Benedict's test	+	-
Protien	Ninhydrin test	-	-
	Millon's test	-	-
Alkaloids	Wagner's test	+	-
	Mayer's test	+	-
	Hager's test	+	-
Phytosterol and Triterpenoids	Salkowski's test	+	+
	Liebermann-Burchard test	+	+
Phenol	Ferric chloride test	+	-
Flavonoids	Lead acetate test	+	-
	Alkali test	+	+
	Shinoda's test	+	+

Table 2. Contd.

Tannins	Gelatin test	+	+
	Ferric chloride test	+	+
	Alkali test	+	+
Saponins	Frothing test	-	-
Anthocyanins	HCl test	+	-
Glycosides	Killer-Kiliani test	-	-
Fixed oil and fats	Spot test	+	+
	Alkali test	+	+
Volatile oil	Spot test	+	+

Table 3. Preliminary phytochemical screening of fruit of *Z. armatum*.

Constituent	Test name	Ethanolic extract	Hexane extract
Carbohydrates	Fehling's	+	+
	Molisch's	+	+
	Benedict's	+	-
Protien	Ninhydrin	+	-
	Millon's	+	-
Alkaloids	Wagner's	+	-
	Mayer's	+	-
	Hager's	+	-
Phytosterol and Triterpenoids	Salkowski's	+	+
	Liebermann-Burchard	+	+
Phenol	Ferric chloride	+	-
Flavonoids	Lead acetate	+	-
	Alkali	+	-
	Shinoda's	+	
Tannins	Gelatin	+	+
	Ferric chloride	+	-
	Alkali	+	-
Saponins	Frothing	-	-
Anthocyanins	HCl	-	-
Glycosides	Killer-Kiliani	-	-
Fixed oil and fats	Spot	+	+
	Alkali	+	+
Volatile oil	Spot	+	+

n-hexane extract of *Z. armatum* leaves (ZLH) showed relaxant effect in the spontaneous tissue treatment. The ethanolic extract of *Z. armatum* bark (ZBE) showed significant effects observed from 3 to 10 mg/ml concentration. In the case of n-hexane extract of *Z. armatum* bark (ZBH), the antispasmodic effect was

Table 4. EC_{50} (half maximal effective concentration values) for Ethanolic and n-hexane extract of leaf, bark and fruit of *Z. armatum*.

Part used	Extract	EC_{50} value spontaneous rabbit jejunum	EC_{50} value for KCl induced contraction
Leaves	ZLE	0.23	6.81
	ZLH	6.13	4.45
Bark	ZBE	4.00	6.00
	ZBH	5.5	4.5
Fruit	ZFE (without atropine)	1.69	7.49
	ZFE (with atropine)	8.86	3.18
	ZFH	0.74	5.00

Table 5. Quantitative chemical analysis of *Zanthoxylum armatum*.

Extract	Flavonoids	Phenol	Alkaloids	Tannins	Saponin	Sterol
ZLE	13.68±0.66	11.66±0.33	15.60±0.10	34.43±0.21	21.57±0.12	71.60±0.10
ZBE	18.33±1.22	16.48±1.33	19.60±0.10	28.62±0.13	14.78±0.10	33.83±0.29
ZFE	22.8±1.33	21.68±0.44	25.07±0.21	35.5±0.5	28.60±0.10	164.92±0.14

All values are mean ± SEM of three determination and are expressed in mg/g.

observed at concentration of 3 mg/ml, while the most significant effect was observed at 10 mg/ml. The ethanolic extract of *Z. armatum* fruit (ZFE) produced strange effect in both cases (spontaneous and in potassium induced contraction). This extract was just like control up to 1 mg/ml and at higher concentrations, a good right ward shift was produced. The EC_{50} values (half maximal effective concentration values) for ethanolic and *n*- hexane extract of leaf, bark and fruit of *Z. armatum* are shown in Table 4.

DISCUSSION

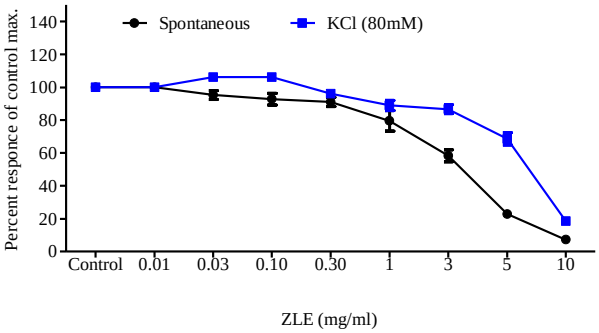
Several active constituents of pharmacological importance may be available in a single plant (Ming et al., 2005). It is suggested that preliminary phytochemical screening is important and useful for isolation of pharmacologically active principles from a single plant (Sugumaran and Vetrichelvan, 2008). The qualitative and quantitative analysis of *Z*. *armatum* showed that this plant might be a rich source of bioactive constituents for future use in pharmaceutical preparations.

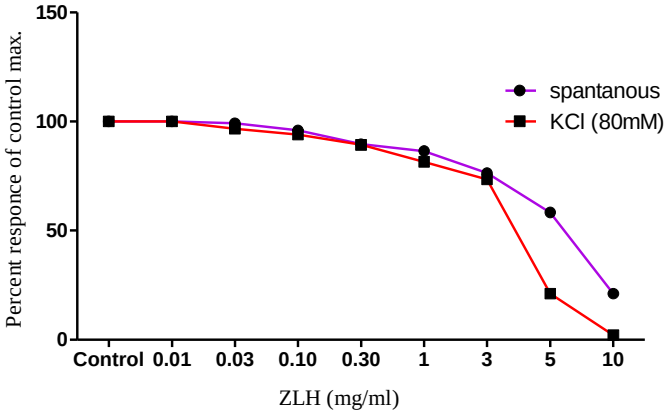
Ethanolic and *n*-hexane extracts of leaves (ZLE and ZLH), barks (ZBE and ZBH) and fruits (ZFE and ZFH) of *Z*. *armatum* were evaluated on the isolated rabbit jejunum for possible antispasmodic effect, providing scientific proof for its ethnopharmacological use as an antispasmodic drug. All the samples were tested against spontaneous and KCl induced contracted smooth muscle of the isolated rabbit jejunum.

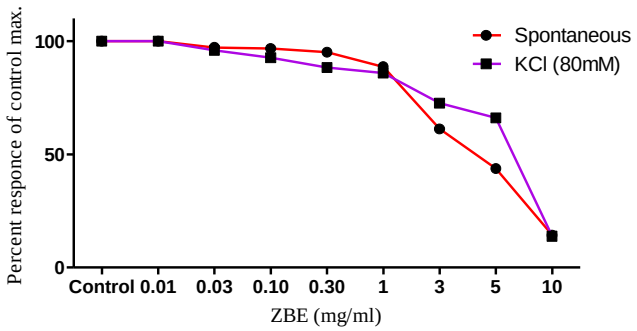
All samples produced antidiarrheal effect in a dose dependent manner. To determine the possible mode of action, the tissue was pretreated with high concentration of KCl (80 mM in final bath solution). KCl cause depolarization and keep the tissue in a position of sustained contraction (Farre et al., 1991). The test samples were then applied in cumulative manner to obtain a dose dependant curve and relaxation results were expressed as percentage of KCl induced contraction (Van Rossum, 1963).

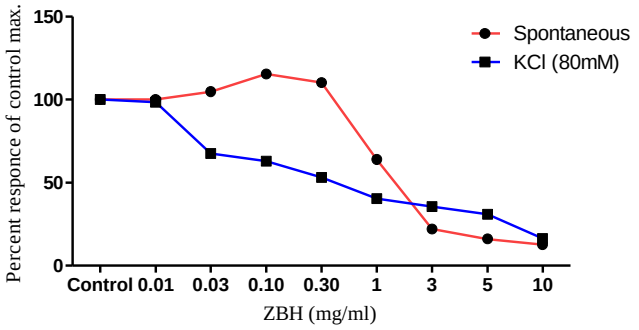
The effect of ZLE was dose dependent in both spontaneous and KCl induced contractions. The ZLH inhibited the depolarization of the smooth muscles of the jejunum, caused by high concentration of KCl solution (80 mM) (Ahmad, 2010). The ZBE produced spasmolytic effect in both spontaneous as well as in the KCl induced contraction. In case of ZBH, the relaxation of smooth muscle of potassium induced contraction was more than the spontaneous. To confirm that the spasmolytic effect of ZFE was due to possible calcium channel blockage, another series of experiments were performed, in which a pretreated atropine isolated tissues was treated with KCl (80 mM) that opens the voltage operated calcium channels, releasing extra-cellular calcium into the cytosol creating depolarization of the tissue (Gilani et al., 2005a). ZFE was found to relax the KCl depolarized tissue and was found to be calcium channel blocking agent at a dose range of 0.1 to 5.0 mg/ml (Ahmad, 2010) (Figure 1).

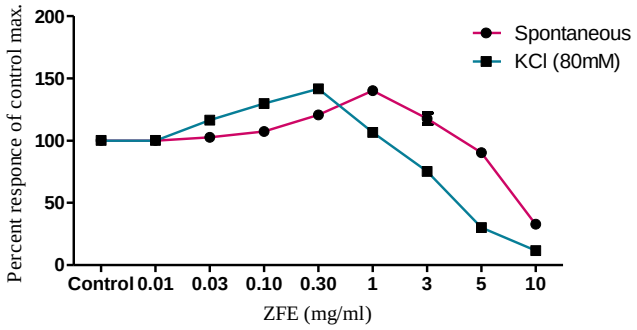
The contraction of smooth muscle of rabbit jejunum is due to increase concentration of the free calcium in cytoplasm, which stimulates the chemical mediators responsible for contraction. This increase in calcium level may be either due to influx via voltage dependent calcium channels or direct release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

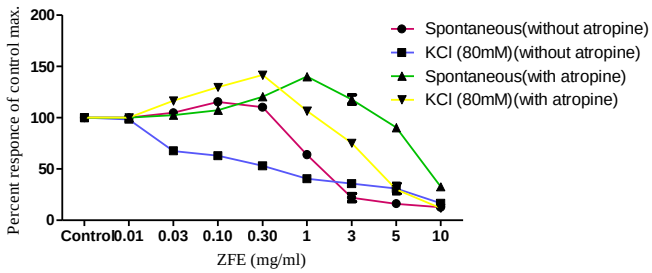












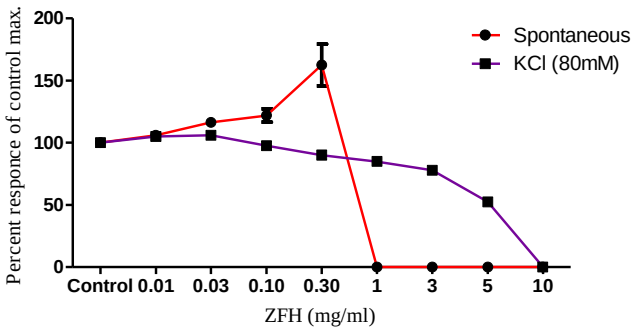


Figure 1. Dose response curve of the ZLE, ZLH, ZBE, ZBH, ZFE, and ZFH on isolated rabbit's jejunum preparations. All values are mean±SEM (n=5).

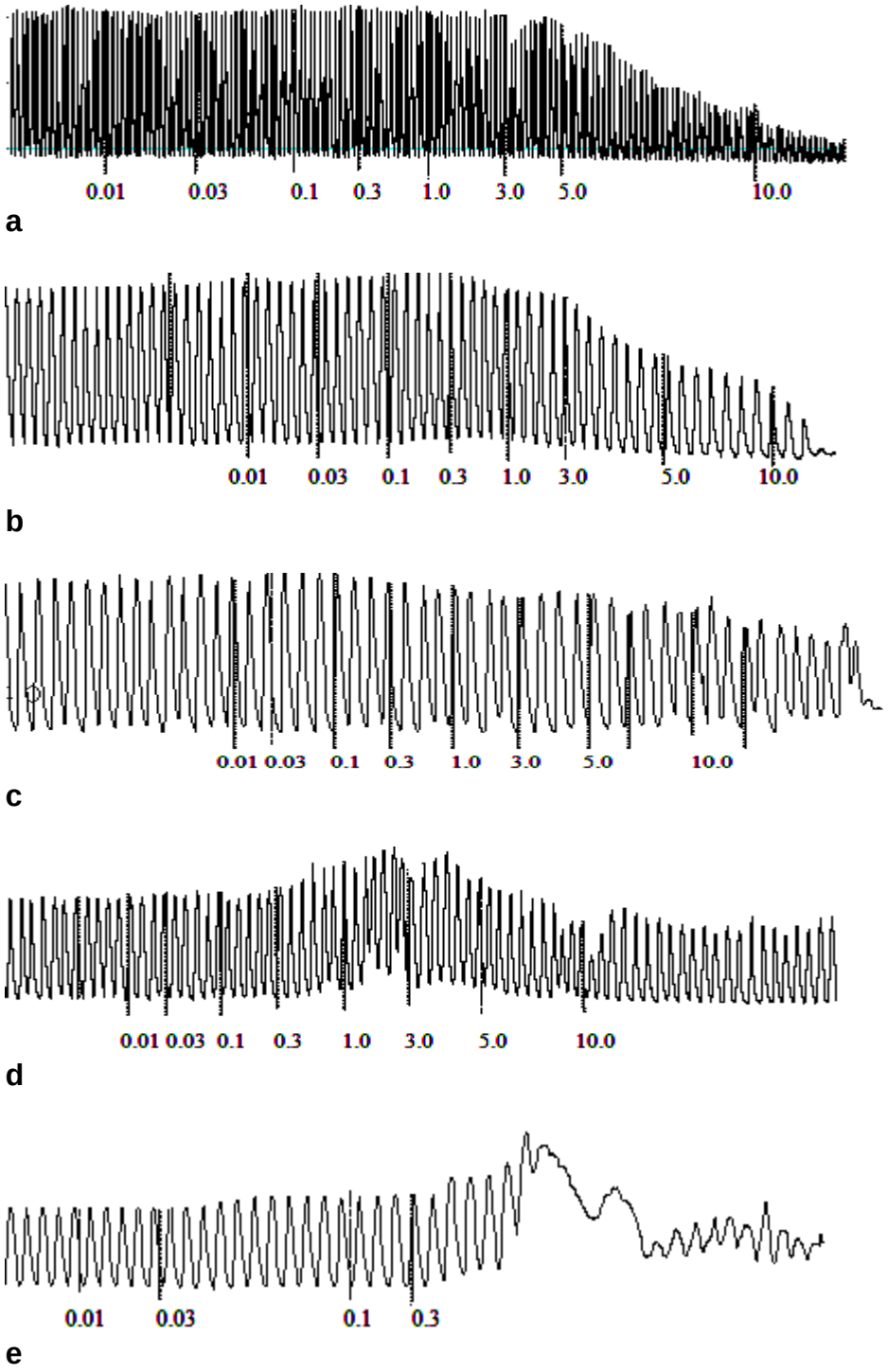


Figure 2. Antispasmodic effect of *Zanthoxylum armatum*. (a) Effect of ZLE (mg/ml) on isolated rabbit jejunum; (b) Effect of ZBE (mg/ml) on isolated rabbit jejunum; (c) Effect of ZBH (mg/ml) on isolated rabbit jejunum; (d) Effect of ZFE (mg/ml) on isolated rabbit jejunum; (e) Effect of ZFH (mg/ml) on isolated rabbit jejunum.

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 (Ali and Shah, 2010). In the present study, the extracts relaxed the contracted muscle, suggesting that the possible mode of action of this plant

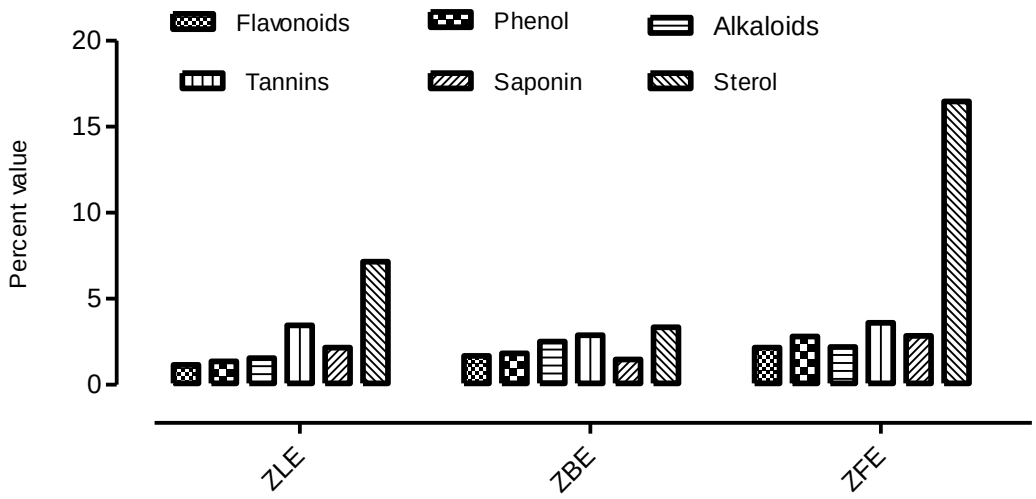


Figure 3. Bars represent percents values of flavonoids, phenols, alkaloids, tannins, saponins and sterols in ethanolic extracts *Zanthoxylum armatum* leaf (ZLE), bark (ZBE) and fruit (ZFE).

is either blocking the release of stored calcium from the sarcoplasmic reticulum or blocking the calcium channel. More or less similar studies have also been carried out by other workers. Ahmad (2010) carried out antispasmodic activities on isolated rabbit jejunum testing the crude methanolic extract of *Tylophora hirsutum*. The methanolic-aqueous extract of the aerial part of *Z. armatum* has been tested for muscles relaxation effect in gut, air passage way and in cardiovascular system (Gilani, 2005a). This study showed that this plant is an important source of active constituents both qualitatively and quantitatively. Further various experiments on this plant demonstrated that this plant has strong antispasmodic potential. Detail studies are required to explore this plant phytochemically and pharmacologically, which may prove this plant as cheaper and accessible source of valuable drugs.

ACKNOWLEDGEMENT

We are highly thankful to Higher Education Commission, Pakistan for providing the financial support for this research work.

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