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In vitro evaluation of anthelmintic efficacy of *Trichilia* and *Ajuga* species on *Ascaridia galli*

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Abstract

The study was designed to evaluate the effect of the methanol, chloroform, acetone and aqueous extracts of different parts of *Trichilia connaroides*, *Ajuga bracteosa*, *Ajuga macrosperma*, *Ajuga parviflora* of Indian Himalayan region and a reference drug albendazole on isometric contractions of the poultry worm *Ascaridia galli*. The frequency and amplitude of spontaneous muscular contractions of *A. galli* were recorded on the physiograph through force transducer. There was inhibition in amplitude and frequency of the contractile activity as compared to control in dose dependent manner by methanol extracts of seeds (IC_{50} 3.93±0.70), pericarps (IC_{50}7.09± 2.64), aqueous extract of roots (IC_{50} 6.40± 4.74) of *T. connaroides*, methanol extracts of *A. parviflora* roots (IC_{50} 16.79± 2.93) *A. macrosperma* roots (IC_{50}1.73± 0.02) and *A. bracteosa* aerial parts (IC_{50} 4.49± 0.72). These observations indicated the paralytic effect of the extracts on *A. galli*. There was no inhibition contractile activity by chloroform extract of seeds, acetone extract of leaves of *T. connaroides* and methanol extract of *A. bracteosa* roots on autorythmicity of *A. galli*.

Keywords: *T. connaroides*; *Ajuga*; *A. galli*; Albendazole; Autorythmicity.

Introduction

Helminthiasis or worm infestation is one of the major public health problems in the world. It is responsible for considerable economic losses to the livestock industry in developing countries. Other adverse effects of these parasites include loss of meat, wool and egg production [1]. Chemotherapy against helminths involves either selective inactivation of enzymes or blocking of specific receptors in the tissues of the parasites [2]**.** Consequently, there is an urgent need to develop newer, selective and eco-friendly agents to control helminth infections. Plant based anthelmintics can be both sustainable and environmentally acceptable.

Unlike synthetic anthelmintics, plant-based anthelmintics with different modes of action could be of value in preventing the development of resistence**.** Herbal drugs have been in use since ancient times for the treatment of a variety of acute and chronic parasitic diseases both in humans and in veterinary medicine [3]. Traditional system of medicine reports the efficacy of several plant products for eliminating helminths. Several essential oils and plant extracts have been found to possess anthelmintic activity.

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Indian system of medicine reports the efficacy of medicinal plants like *Chenopodium ambrosioides, Embelia ribes, Carica papaya, Mallotus phillippinensis, Butea frondosa* etc. for eliminating helminths [3]Himalayan region of India has been considered to be a rich repository of medicinal and aromatic plants since time immemorial. However, very little efforts have been made to study the biodiversity of plant species in this region. This paper presents the anthelmintic activity of *Trichiliaconnaroides ,Ajuga bracteosa, Ajuga macrosperma* and *Ajuga parviflora* a of this region on *Ascaridia galli* which is a common intestinal parasite of the fowl, causing considerable economic losses to poultry farmers. It has many characterstics similar to *Ascaris lumbricoides* which is pathogenic to human beings [4]

Trichilia connaroides:

Trichilia is a genus of trees, rarely shrubs [5]**.** It belongs to the family *Meliaceae* which contains 40 genera and 600 species [6.] Genus *Trichilia* is important because of medicinally important tetranortriterpenoids or limonoids. Limonoids show a wide range of biological activities including anti-feedant and growth regulatory properties in insects and antifungal, bactericidal and antiviral activities in laboratory animals and humans [7]. From the genus *Trichilia,* only *T. connaroides* Wight. and Arn. occurs in Indian subcontinent. It is distributed in Sub-Himalayan tract from Kumaun eastward, Sikkim up to 4000 ft, Khasia Hills, Manipur, E. Ghats in the forests of Godawari and Vizagapatnam up to 4,500 ft. W.Ghats from Poona Southwards through the Nilgiris and Anamalais to Tranvancore, up to 6,000 ft. Apart from India it is also distributed in Burma, Tonkin, Cambodia, Malay Peninsula and Sumatra [8]. The plant also possesses medicinal value. The bark and leaves possess bitter and tonic properties and a decoction of the leaves is taken in cholera 5**.** The roots of *T. connaroides* are used as a Chinese drug to treat arthritis, pharingitis, tonsillitis and other ailments

Ajuga* species*:

The plants of genus *Ajuga* belong to the family Lamiaceae. It is a large family comprising about 220 genera and almost 4000 species [10]. The increasing number of crops in this family (1959:38, 1986: 129 and 1999:174) reflects the intensification of taxonomical and ethnobotanical research in this field [11]. It has worldwide distribution growing under wide variety of soil and climate, but more abundant in Mediterranean regions and in the hills. Genus *Ajuga* is widely reported to have the clerodane and neo-clerodane diterpenoids with various biological activities [10-11]. The plants of this genus are used as folk medicinal plants as antihelmintic, hypoglycemic, antifungal, anti-tumor and antimicrobial agents [12]**.**

A. bracteosa is a perennial herb in the western Himalayas from Kashmir to Nepal. The leaves of this plant are used as stimulant, diuretic, aperient and as a substitute for cinchona. It acts as cardiostimulant in animals and shows anticancer activity in rats and mice [13].

A. macrosperma, found in Himalayan region up to Bhutan, Khasi Mountains, Cittagaon in Burma and China [14]**.** It is traditionally used to alleviate fever and remove phlegm by the Dai minority of Yunnan Province, China [15]**.**

A. parviflora grows in the temperate Kumaon region of the Indian Himalaya at 4000-6000 foot elevations [16]. It has found diverse medicinal uses in indigenous systems of medicine. It has been used as an astringent and for the treatment of swollen wounds, diarrhoea, reheumatism, fever, eye trouble and for the diseases of bladder [17-18]**.**

Materials and Methods

Chemicals used- Chloroform, Methanol, Acetone, NaCl, KCl, $CaCl_2$, $MgCl_2.6H_2O$, Na_2HPO_4, Glucose, $NaHCO_3$ were procured from E-Merk (India) Limited Mumbai. Reference drug albendazole (200mg/5 ml) was procured from Lupin Drug Company, India.

Collection and identification of plants

T. connaroides was collected from Kumaun region, India and identified at Forest Research Institute (FRI) Dehradun vide herbarium no. M-29 .Three species of *Ajuga* namely *A. parviflora*, *A. bracteosa* and A. *macrosperma* were collected from Kumaun region and identified at Forest Research Institute (FRI) Dehradun and herbariums are deposited at the Department of Chemistry at G.B.P.U.A & T Pantnagar for the future reference.

Extraction

T. connaroides

Seeds and pericarps of *T. connaroides* were subjected to the repeated soxhlet extraction by hexane followed by extraction with chloroform and methanol separately. Leaves were also subjected to repeated soxhlet extraction by acetone. Roots were extracted in boiling water for three times. Rotary vaccum evaporator concentrated the extracts. The percent yields(w/w) of chloroform extract of seeds, methanol extract of seeds and pericarps, acetone extract of leaves and aqueous extract of roots were 2.2, 1.4, 1.25, 1.0 and 1.25 respectively.

***Ajuga* species**

A. macrosperma and *A. parviflora* roots, *A. bracteosa* roots and aerial parts were subjected to repeated soxhlet extraction by methanol separately**.** Rotatory vaccum evaporator concentrated the extracts and their percent yields (w/w) were 11.25, 25, 39.47 and 33.2 respectively. *A. galli* worms were collected from the intestine of freshly slaughtered fowl *(Gallus gallus)* received for postmortem examination at Animal Disease Diagnostic Centre, Pantnagar. They were stored in Tyrode solution at 40 □ 1^0C.

Isometric mounting of *A. galli* and mechanical recording of the spontaneous muscular activity

The worm *A. galli* was mounted isometrically on a tissue bath of 4 ml capacity in Tyrode solution (Composition in mM: NaCl 136, KCl 5, $CaCl_2$ 2, $MgCl_2.6H_2O$ 0.98, $NaH_2PO_4.2H_2O$ 0.36, Glucose 5.5, $NaHCO_3$ 11.9 and PH adjusted to 7.4) maintained at 40 ± 1ºC and allowed to equilibriate for 30 min without any tension. During the equilibration period the bath fluid was changed once every 10 min. Isometric contractions were made after applying a tension of 0.5 g using force transducer (T-301, Pt-1979) and spontaneous muscular activity was recorded using ink writing physiograph (Biodevice, India) at 0.25mm/sec chart speed. Control recordings were made for 15 min before the addition of a drug. Three parameters namely frequency (total no. of contractions in 10 min), amplitude (average of all peaks per 10 min or average tension) of spontaneous muscular contractions and baseline tension (average of all minimum levels of contractions used for measuring amplitude) of the isometrically mounted *A. galli* were measured.

Effect of different extracts and albendazole on autorythmicity of *A. galli*

Cumulative doses of the extracts at a dose rate of 3.125 -100 mg/ml dissolved in Tyrode solution and of albendazole at a dose rate of 0.32-2.56mg/ml were added in the tissue bath containing the isometrically mounted worm. Each dose was allowed to act for 15 min with continuous recording of rhythmic movements of *A. galli*. The effects of various concentrations of plant extracts on frequency and amplitude of spontaneous muscular contractions and on base line tension of the mounted worm were recorded and compared with the control.

Statistical analysis

The results are presented as mean ± standard error of the mean. To measure the significance one way T test was applied.

Results and Discussion:

The effects of different extracts and the reference drug albendazole on autorythmiity of *A. galli* are presented in Table 1-11 and Fig 1-3. The acetone extracts of leaves and chloroform extract of seeds of *T. connaroides* could not alter any of the three parameters of autorythmicity of A. *galli*, amplitude, frequency and base line tension when used at dose rates of 3.125-50mg/ml bath solution.

Methanol extracts of pericarps (MPE) and seeds (MSE) and aqueous extract of roots (ARE) of *T. connaroides* decreased the amplitude and frequency of autorythmicity of *A. galli* dose dependently with a highest effect at the concentration of 50mg/ml. MPE and MSE also reduced base line tension of *A. galli* contractility significantly and dose dependently at the dose rate of 3.125-50mg/ml (Table 1-5 and Fig 1). An inhibition in the amplitude and frequency of rhythmic contractions of *A. galli* and base line tension was shown by methanolic extract of *Ajuga bracteosa* aerial part (BAE), *Ajuga macrosperma* roots (MRE) and *Ajuga parviflora* roots (PRE). Methanol extract of *Ajuga bracteosa* roots (BRE) could not alter the contractility of *A. galli*. The maximum decrease in frequency and amplitude in autorythmicity of *A. galli* was observed at the cumulative doses of 12.5 mg/ml with BAE, 50 mg/ml with MRE and 100mg/ml with PRE.

Among all the above-mentioned extracts, the methanol extract of *A. macrosperma* roots (MRE) has been found to possess maximum anthelmintic efficacy with the minimum IC_{50} value (1.73 ± 0.02). Albendazole, a well-proven anthelmintic of benzimidazole group [19] showed comparatively better response for inhibiting amplitude, frequency and base line tension in dose dependent manner. The maximum decrease in frequency, amplitude and base line tension was observed at the dose of 2.56 mg/ml. Albendazole was found to possess lowest IC_{50} value (0.74± 0.05). The IC_{50} values of all the extracts are presented in Table 12.

Chemotherapeutic agents available for treatment of helminth infection act mainly through three different mechanisms viz, by disruption of the neuromuscular physiology, by blocking the energy metabolism and by disturbing the highly efficient reproductive system of the parasites [20]. Several important anthelmintics cause paralysis of helminth parasites by disrupting one or the other aspect of their neuromuscular system. The spontaneous muscular activity was quantified in terms of frequency, amplitude of rhythmic contractions and baseline tension and these parameters were measured before and after drug treatment and values were compared.
Rapid and marked change in the spontaneous muscular activity of an isometrically mounted parasite by a drug indicates that the neuromuscular system of the parasites can be used to evaluate anthelmintic activity *in vitro* [1]**.** Thus it can be concluded that the extracts demonstrate a paralytic effect causing by progressive reduction in spontaneous muscular activity which may be associated with their inhibitory effect on the neuromuscular system of *A. galli*.

The inhibition caused by the *T. connaroides* extracts i.e. methanolic extract of seeds and pericarps and aqueous extract of roots on the autorythmicity may be attributed to the presence of polyphenols, as on phytochemical examination the plant revealed the presence of phenolic acids [21]. Phenolic acids are reported to have the activity against the worm infestation in human beings [22]. Moreover, the present study was also supported by the fact that polyphenols like tannins are well researched to produce anthelmintic effect [23]. There was no inhibition with chloroform extract of seeds and acetone extract of leaves.The activity found in the extracts of genus *Ajuga* i.e. methanol extract of *A. bracteosa* aerial parts, *A. macrosperma* and *A. parviflora* roots may be due to the presence of clerodane and neo-clerodane diterpenoids. Clerodane diterpenoids are reported as antifeedant, insecticidal, antiviral, antitumor and anti-microbial agents. They also exhibit the activity against jaundice, urinary diseases and rheumatism [11].
Similarly neo-clerodane diterpenoids are found to be antimicrobial, anti-mycobacterial, anti-plasmodial agents. They also have the cancer chemopreventive, hypoglycemic and hypotensive effects [10]**.** Since clerodane and neo-clerodane diterpenoids exhibit various biological activities. Therefore, they may be the active principles responsible for the inhibition of the amplitude and frequency of the autorythmicity of *A. galli.* Methanol extract of *A. bracteosa* roots was not effective.

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Table 1. Effects of acetone extract of leaves (AEL), chloroform extract of seeds (CES), methanol extracts of pericarps(MEP) and seeds (MES) and aqueous extract of roots (AER) of *connaroides* on amplitude (mm) of autorythmicity of *A. galli* (mean ± S.E, n=6)

Dose mg/mL	ALE	CSE	MPE	MSE	ARE
0(control)	6.06 ± 0.05	5.44 ± 0.15	12.15±1.37	9.8±1.90	11.82±4.10
3.125	7.80 ± 0.09 ^{z}	7.26 ± 0.09^{z}	8.68±0.89	5.4± 1.19^{x}	7.26±2.88
6.25	10.2 ± 0.09 ^{z}	9.36 ± 0.07^{z}	5.5±0.71^{z}	3.12 ± 0.64^{y}	4.30±0.73
12.5	12.53 ±0.13 ^{z}	10.1 ± 0.03^{z}	3.16 ±0.20^{z}	1.83 ± 0.30^{z}	2.766±0.34
25	13.33 ± 0.26 ^{z}	11.36 ± 0.11^{z}	2.3 ± 0.136^{z}	1.11 ± 0.042^{z}	0.626±0.124^{X}
50	13.6 ± 0.22 ^{z}	13.26 ± 0.07^{z}	0.59 ± 0.015^{z}	0.55 ± 0.03^{z}	

Student's t-test – P^{x} <0.05, P^{y} <0.01, P^{z} <0.001 vs. control (0mg/mL)

Table 2. Effects of acetone extract of leaves (AEL), chloroform extract of seeds (CES), methanol extracts of pericarps (MEP) and seeds (MES) and aqueous extract of roots (AER) of *T. connaroides* on amplitude (mg) of autorythmicity of *A. galli* (mean ± S.E, n=6)

Dose(mg/mL)	ALE	CSE	MPE	MSE	ARE
0(control)	151.6 ± 1.39	136.0 ± 3.93	607.5 ± 68. 94	245.6 ± 47.6	591±187.48
3.125	195 ± 2.41^{z}	181.6± 2.29^{z}	434.1 ± 44.67^{x}	135 ± 29.7^{x}	363.3 ± 131.8
6..25	255.0 ± 2.41^{z}	234.1 ± 1.90^{z}	275 ± 35.84^{z}	78.08 ± 16.0 ^{z}	215 ± 33.4
12.5	313.3 ± 3.20^{z}	252.5 ± 0.91^{z}	158.3 ± 10.13 ^{z}	45.79 ± 7.58 ^{z}	138.3 ± 15.75^{x}
25	333.3 ± 6.7^{z}	284.1 ± 2.93^{z}	115 ± 6.83 ^{z}	27.75 ± 1.05 ^{z}	31.3 ± 5.66^{x}
50	340.4 ± 5.64^{z}	331.6 ± 1.90^{z}	29.6 ± 0.785 ^{z}	13.8 ± 0.98 ^{z}	

Student's t-test – P^{x}<0.05, P^{y}<0.01, P^{z}<0.001 vs control (0mg/mL)

Table 3. Effects of acetone extract of leaves (AEL), chloroform extract of seeds (CES), methanol extracts of pericarps(MEP) and seeds (MES) and aqueous extract of roots (AER) of *T. connaroides* on % control amplitude of autorythmicity of *A. galli* (mean ± S.E, n=6)

Dose (mg/mL)	ALE	CSE	MPE	MSE	ARE
0(control)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100± 0
3.125	128.6 ± 2.68^{x}	134.30 ±5.39^{z}	72.47 ± 3.6^{z}	79.01 ± 28.61	55.93±3.03^{z}
6.25	168.2 ± 3.07^{z}	172.6 ± 3.73^{z}	45.5 ± 3.5^{z}	40.45 ± 9.76^{z}	48.27±6.25^{z}
12.5	206.6 ± 1.8^{z}	186.24 ± 4.58^{z}	27.69 ± 3.2^{z}	23.62 ± 5.35^{z}	35.63±8.38^{z}
25	219.0 ± 2.92^{z}	209.5 ± 4.93^{z}	20.08 ± 2.2^{z}	13.8 ± 2.94^{z}	9.86±3.14^{z}
50	224.0 ± 2.4^{z}	244.93 ±8.08^{z}	5.20 ± 0.59^{z}	6.92 ± 1.48^{z}	

Student's t-test – $P^{x}<0.05$, $P^{y}<0.01$, $P^{z}<0.001$ vs control (0mg/mL)

Table 4. Effects of acetone extract of leaves (AEL), chloroform extract of seeds (CES), methanol extracts of pericarps (MEP) and seeds (MES) and aqueous extract of roots (AER) of *T. connaroides* on frequeny (/min) of autorythmicity of *A. galli* (mean ± S.E, n=6)

Dose (mg/mL)	ALE	CSE	MPE	MSE	ARE
0(control)	4.75 ± 0.09	2.24 ± 0.11	4.01± 0.61	2.81± 0.20	5.69 ± 0.62
3.125	4.9 ± 0.28	2.53 ± 0.34	2.71 ± 0.65	1.56 ± .04^{z}	4.53 ± 0.46
6.25	6.35 ± 0.24^{z}	2.24 ± 0.60	4.13 ± 1.12	0.93 ± 0.13^{z}	3.08 ± 0.49^{z}
12.5	6.23 ± 0.29^{z}	2.93 ± 0.41	3.62 ± 1.01	0.60 ± 0.11^{z}	1.63 ± 0.07^{z}
25	6.53 ± 0.24^{z}	3.05 ± 0.58	2.33 ± 0.61^{x}	0.66 ± 0.09^{z}	0.46 ± 0.11^{z}
50	6.73 ± 0.24^{z}	2.95 ± 0.74	1.93 ± 0.57^{y}	0.36 ± 0.09^{z}	

Student's t-test – $P^{x}<0.05$, $P^{y}<0.01$, $P^{z}<0.001$ vs control (0mg/mL)

Table 5. Effects of acetone extract of leaves (AEL), chloroform extract of seeds (CES), methanol extracts of pericarps(MEP) and seeds (MES) and aqueous extract of roots (AER) of *T. connaroides* on base line tension (mg) of autorythmicity of *A. galli* (mean ± S.E, n=6)

Dose (mg/mL)	ALE	CSE	MPE	MSE	ARE
0(control)	489.4 ±21.9	451.4 ± 0.06	557.0 ± 11.07	563.5 ± 14.59	459.3 ±12.92
3.125	509.0 ±9.82	482.7 ± 0.99^{z}	285.6 ± 12.3^{z}	454.0 ±16.84^{z}	655.8 ± 232.7
6.25	514.0 ±20.3	334.3 ± 2.03^{z}	119.9 ± 29.83^{z}	406.1 ±12.2^{z}	512.3 ±171.3
12.5	556.0±34.30	434.3 ±4.11^{z}	69.12 ± 14.07^{z}	437.0± 29.7^{z}	263.1 ± 3.64^{y}
25	591.0±45.7^{x}	557.4 ± 8.27^{z}	21.2 ± 15.8^{z}	415.0 ± 8.00^{z}	449.0 ± 18.7
50	590.0 ± 38.1^{x}	625.3 ± 16.6^{z}	9.47 ± 4.8^{z}	420.0 ± 7.83^{z}	

Student's t-test – $P^{x}<0.05$, $P^{y}<0.01$, $P^{z}<0.001$ vs control (0mg/mL)

Table 6. Effects of methanol extracts of *A. bracteosa* aerial part (BAE) and root (BRE), *A. macrosperma* root (MRE) and *A. parviflora* root (PRE) on amplitude (mm) of autorythmicity of *A. galli* (mean±S.E, n=6)

Dose(mg/ml)	BAE	BRE	MRE	PRE
0(control)	7.45 ± 0.81	3.36 ± 0.12	25.43± 0.89	15.24 ± 4.78
3.125	4.72 ± 0.70^{x}	3.68 ± 0.07^{x}	13.33 ± 0.55^{z}	13.12 ± 3.43
6.25	2.47 ± 0.22^{z}	2.877 ± 0.14^{x}	10.1 ± 0.28^{z}	11.88 ± 3.90
12.5	0.441 ± 0.022^{z}	2.81 ± 0.179^{x}	7.13 ± 0.35^{z}	9.3 ± 3.48
25		3.01 ± 0.151	4.6 ± 0.29^{z}	5.11 ± 2.258^{x}
50		3.09 ± 0.27	1.44 ± 0.100^{z}	3.13 ± 1.07^{x}
100		3.42 ± 0.07		0.74 ± 0.028^{y}

Student's t-test – $P^{x}<0.05$, $P^{y}<0.01$, $P^{z}<0.001$ vs control (0mg/mL)

Table 7. Effects of methanol extracts of *A. bracteosa* aerial part (BAE) and root (BRE), *A. macrosperma* root (MRE) and *A. parviflora* root (PRE) on amplitude (mg) of autorythmicity of *A. galli* (mean±S.E, n=6)

Dose(mg/ml)	BAE	BRE	MRE	PRE
0(control)	372.8 ± 40.9	84.13±3.10	1271.6±44.8	762.03 ± 239.4
3.125	236.1 ± 35.2^{x}	92.2±1.75^{x}	666.6±27.8^{z}	656.08 ± 171.9
6.25	123.9 ± 11.13^{z}	71.93±3.68^{x}	505±14.49^{z}	594.16 ± 195.2
12.5	22.0 ± 1.14^{z}	70.33±4.47^{x}	356.6±17.59^{z}	465.0 ± 174.3
25		75.30±3.78	230±14.66^{z}	255.8 ± 112.9^{x}
50		77.41±6.75	72.08±5.03^{z}	156.6 ± 53.61^{x}
100		85.6±1.87		37.04 ± 1.410^{y}

Student's t-test – $P^{x}<0.05$, $P^{y}<0.01$, $P^{z}<0.001$ vs control (0mg/mL)

Table 8. Effects of methanol extracts of *A. bracteosa* aerial part (BAE) and root (BRE), *A. macrosperma* root (MRE) and *A. parviflora* root (PRE) on % control amplitude of autorythmicity of *A. galli* (mean±S.E, n=6)

Dose (mg/ml)	BAE	BRE	MRE	PRE
0(control)	100 ± 0	100 ± 0	100 ± 0	100 ± 0
3.125	62.31 ± 4.2^{z}	110.2 ± 4.11^{x}	52.36 ± 0.38^{z}	94.55±10.51
6.25	36.29 ± 6.32^{z}	86.10±5.31^{x}	39.80 ± 0.87^{z}	78.91±13.27
12.5	6.26 ± 0.67^{z}	83.15± 2.406^{y}	28.20 ± 1.73^{z}	56.5±8.65^{z}
25		89.27 ± 1.41^{y}	18.15 ± 1.19^{z}	27.04±4.37^{z}
50		91.25 ± 5.06	5.73 ± 0.52^{z}	19.96±2.14^{z}
100		102.96 ± 6.32		7.22±1.556^{z}

Student's t-test – $P^{x}<0.05$, $P^{y}<0.01$, $P^{z}<0.001$ vs control (0mg/mL)

Table 9. Effects of methanol extracts of *A. bracteosa* aerial part (BAE) and root (BRE), *A. macrosperma* root (MRE) and *A. parviflora* root (PRE) on frequency (/min) of autorythmicity of *A. galli* (mean±S.E, n=6)

Dose (mg/mL)	BAE	BRE	MRE	PRE
0(control)	4.28 ± 0.60	5.65 ± 0.37	3.98 ± 0.51	2.73 ± 0.106
3.125	4.31 ± 0.64	5.99 ± 0.28	3.42 ± 0.23^{x}	2.51 ± 0.91
6.25	2.22 ± 0.57^{x}	6.35 ± 0.14	3.04 ± 0.38	2.22 ± 0.61
12.5	1.46 ± 0.190^{z}	5.97 ± 0.46	3.38 ± 0.27^{x}	2.04 ± 0.45
25		6.03 ± 0.164	3.23 ± 0.53	1.69 ± 0.31^{y}
50		6.03 ± 0.12^{x}	2.84 ± 0.56	1.64 ± 0.28^{z}
100				1.52 ± 0.635^{x}

Student's t-test – $P^{x}<0.05$, $P^{y}<0.01$, $P^{z}<0.001$ vs control (0mg/mL)

Table 10. Effects of methanol extracts of *A. bracteosa* aerial part (BAE) and root (BRE), *A. macrosperma* root (MRE) and *A. parviflora* root (PRE) on base line tension (mg) of autorythmicity of *A. galli* (mean±S.E, n=6)

Dose (mg/mL)	BAE	BRE	MRE	PRE
0(control)	590.2 ± 41.92	528.5 ± 21.06	528.7 ± 25.4	478.7 ± 27.13
3.125	645.25 ± 131.4	500.3 ± 6.42	360.1 ± 42.45	603.5 ± 135.4
6.25	360.0 ± 100.21	510.4 ± 6.32	526.8 ± 8.59	647.6 ± 115.5
12.5	269.3 ± 49.37^{z}	538.6 ± 13.09	643.2 ± 35.31	585.5 ± 83.97
25		577.6 ± 9.27^{x}	476.15 ± 39.39	658.3 ± 145.34
50		629.4 ± 15.54^{y}	488.55 ± 50.97	466.7 ± 155.8
100		640 ± 19.7^{y}		517.2 ± 181.0

Student's t-test – $P^{x}<0.05$, $P^{y}<0.01$, $P^{z}<0.001$ vs control (0mg/mL)

Table-11. IC_{50} values of crude extracts of *Trichilia connaroides* and some species of genus *Ajuga.*

S.No.	Plant Species	Extract	Plant part used	IC_{50} (mg/ml)
1.	*T. connaroides*	MeOH	seeds	3.93 ± 0.70
2.	*T. connaroides*	MeOH	pericarps	7.09 ± 2.67
3.	*T. connaroides*	Aqueous	roots	6.40 ± 4.74
4.	*A. parviflora*	MeOH	roots	16.79 ± 2.93
5.	*A. bracteosa*	MeOH	aerial parts	4.49 ± 0.72
6.	*A. macrosperma*	MeOH	roots	1.73 ± 0.02
7.	Albendazole	-	-	0.74± 0.05

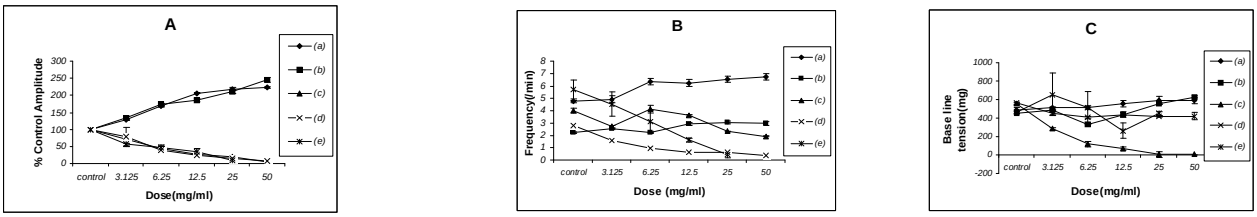

Fig. 1Effect of (a) Acetone extract of leaves (b) Chloroform extract of seeds (c) Methanol extract of pericarps (d) Methanol extract of seeds (e) Aqueous extract of roots of *T. connaroides* on (A) % Control amplitude (B) Frequency (C) Base line tension on autorythmicity of *A.galli*. (n=6)

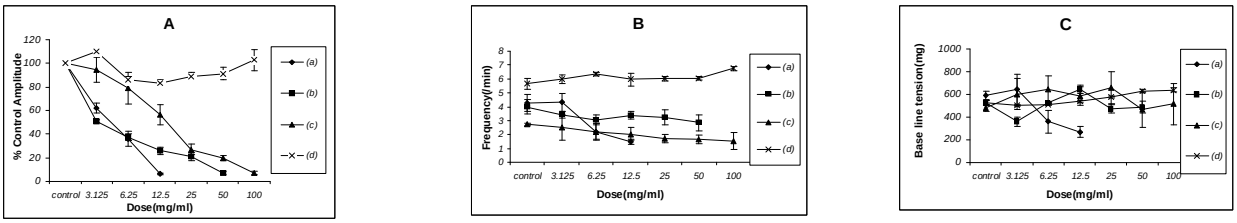

Fig. 2.Effect of methanol extracts of (a) *A. bracteosa* aerial parts (b) *A. macrosperma* roots (c) *A. parviflora* roots (d) *A. bracteosa* roots on (A) % control amplitude (B) Frequency (C) Base line tension on autorythmicity of A. galli. (n=6)

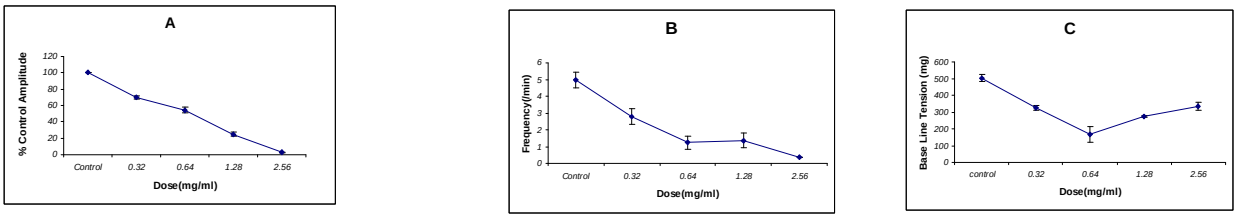

Fig. 3.Effect of Albendazole on (A) % Control amplitude (B) Frequency (C) Base line tension on autorythmicity of *A. galli*. (n=6)

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