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Full Length Research Paper

Phytopharmacological assessment of medicinal properties of *Psoralea corylifolia*

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Psoralea corylifolia **(Leguminosae) is traditionally used for the treatment of skin conditions, fever and as anthelmintic. The aim of present study was to standardize the crude extract of *P. corylifolia* and determine its pharmacological activities. The crude extract of *P. corylifolia* was standardized by high performance liquid chromatography (HPLC), Fourier transform infra red (FT-IR), Fourier transform near infra red (FT-NIR) and ultra violet (UV) spectrophotometric methods. Pharmacological study of the crude extract of *P. corylifolia* and its fractions were carried out on smooth muscle of rabbit intestine. The results revealed quick decrease in the normal intestinal movement followed by a gradual dose dependant increase in the rhythmic activity of intestine. The antispasmodic response of the crude extract was found most significant at 20 mg/ml (78.13%). The chloroform fraction of the crude extract exhibited maximum antispasmodic response at 10 mg/ml (45.16%). *n*-butanol and aqueous fractions produced 39.29 and 13.79% antispasmodic effect, respectively, whereas, ethyl acetate fraction produced spasmogenic effect (21.9%). The crude extract of *P. corylifolia* exhibited positive antifungal activity against *Candida albicans* and positive antibacterial activity against *Staphylococcus aureus*. Significant analgesic effect was also observed with 300 and 500 mg/kg dose at $P < 0.05$. The analgesic effect of the plant extract (500 mg/kg) was found to be higher than that of diclofenac sodium (50 mg/kg; positive control). Brine shrimp lethality assay was performed and the plant extract did not exhibit any toxicity.**

Key words: *Psoralea corylifolia*, high performance liquid chromatography (HPLC), standardization, smooth muscles, analgesic, antibacterial, antifungal.

INTRODUCTION

Psoralea corylifolia is distributed all over Pakistan (Khan, 1975), especially in Baluchistan's coastal area, Peshawar and Jhelum (Baquer, 1989). It is also found in Bengal, Bombay and all over the plains of India (Nadkarni, 1976). Some species of it are also found in America and China (Vaidya, 1992).

In Unani system, it has been used in the treatment of fever, skin conditions; particularly leucoderma and internal ulcers. It has also been used as an anthelmintic and sedative (Nadkarni, 1976). The seeds of *P. corylifolia* are laxative, stimulant and aphrodisiac. They are also used for the management of leprosy, leucoderma and inflammatory diseases of the skin and bilious disorders. The seeds are also known to produce anticancer activity and posses immune modulatory properties (Latha and Pannikar, 1999). Externally, the seeds are used for skin diseases. An ointment made of the powdered seeds and *Cassia tora* with limejuice is very effective for the

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treatment of ringworm infection. *Psoralea* oil is reported to turn gray hair to black (Khan, 1975).

P. corylifolia has been found to exhibit a strong antibacterial effect against the skin streptococci. On voluntary muscles, the essential oil extracted from *P. corylifolia* has been reported to produce stimulant effect at high dilutions (Panda, 2000). The drug has been considered to be so efficacious in leprosy, which was why it was given the name '*Kushtanashini*' (leprosy destroyer). *P. corylifolia* has been used in leucoderma and psoriasis; it is administered orally or applied locally on the skin. It is also used in the treatment of intestinal amoebiasis. It heals wounds and ulcers (Vaidya, 1992). The seeds yield essential oil, psoralen, resin, terpenoid oil, isopsoralen and psoralidin (Baquer, 1989; Khan, 1975). The seeds also contain a crystalline solid, furocoumarin. From the pericarp, psoralidin and isopsoralen have been identified. Psoralens are considered to be the active principles that induce pigmentation (Wang et al., 2009).

The aim of the present study was to standardize the crude extract of *P. corylifolia* by preliminary phytochemical screening. An important part of the study was to determine the antispasmodic activity of the plant extract using an *in vitro* system. The extract of *P. corylifolia* has already been reported to be an affective antimicrobial and antifungal agent (Somasundaram et al., 2010); therefore, it was further evaluated for the antifungal and antibacterial activity against a few selected fungi and bacteria. Anti-inflammatory effect of the plant extract on skin has been reported earlier (Gidwani et al., 2000) and this led towards exploring the analgesic activity of the drug in mice, in the present study.

MATERIALS AND METHODS

Identification and extraction

Psoralea corylifolia was purchased from the local market. The plant was identified by Professor Dr. Mansoor Ahmad, of the Department of Pharmacognosy, University of Karachi, Karachi, Pakistan. The voucher specimens were deposited in the Department of Pharmacognosy. The dried seeds of the plant were chopped into small pieces. The chopped material was macerated with ethanol and was kept for percolation for 15 days at room temperature (Ahmad and Salama., 1985 and Liu et al., 2004). The extract was then filtered and evaporated under reduced pressure in rotary evaporator to yield a dark green residue.

Phytochemical screening of crude extract of *P. corylifolia*

The phytochemical screening of the crude plant extract was performed using standard chemical tests as described (Harborne, 1973; Trease and Evans, 1989). Ultra violet (UV) spectroscopy was performed using Lambda-20 Perkin Elmer (USA). Fourier transform infra red (FT-IR) spectrophotometric analysis was performed using spectrum one (USA), Fourier transform near infra red (FT-NIR) spectrophotometric analysis was done using NTF One (USA). The methods described by Andreia et al. (2011, 2006) were used for FT-NIR and for FT-IR analysis, respectively.

High performance liquid chromatography (HPLC) was done by using a modified method of Christie (1997). For HPLC, Agilent 1100 series (Germany) was used, the mobile phase contained water and methanol (HPLC grade) in the ratio of 633:367, respectively and the sample was prepared in the concentration of 2 g/10 ml. ODS-C18 column (4.6 × 250 mm) was used and the solvent flow rate was kept at 0.7 ml/min. Detection was carried out with UV detector at 225 and 325 nm. Table 2 and Figur es 1 to 6 give a detailed report obtained from all the analytical methods used.

Activity on smooth muscles of rabbit's intestine (jejunum)

Activity of the plant extract on smooth muscles was determined according to the method described (Mehjabeen et al., 2004). Rabbits of either sex, weighing approximately 1.0 to 1.5 kg (purchased form the animal house of Aga Khan University Hospital, Karachi and kept in the animal house of the Research Institute of Pharmaceutical Sciences at University of Karachi) were used in the experiments. The animals were sacrificed by a blow on the back of the neck. The abdomen was then opened immediately and caecum was pulled forward to display the length of the small intestine. The intestine was then cut from the animal and placed in an organ bath of 70 ml capacity, filled with Tyrode's solution. Organ bath circulating water was maintained at 37°C throughout the experiment. The perfusion solution was bubbled with a mixture of 95% oxygen and 5% carbon dioxide. The intestine segment was allowed to equilibrate before starting the experiments. The spontaneous movements of the intestine were recorded on Oscillograph using isotonic transducer.

The crude extract was diluted to 1, 5, 10, 15, 20 and 25 mg/ml doses which were evaluated *in vitro*, on the isolated rabbit intestine. Ethyl acetate, chloroform, *n*-butanol and aqueous fractions of the crude extract were also examined at the dose of 10 mg/ml.

Antibacterial activity

Antibacterial activity of *P. corylifolia* was determined by the method described (Naqvi et al., 1992; Ahmed et al., 2011; Ghalem and Mohamed, 2009.). *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia* were the selected organisms. The culture of organisms was maintained on stock culture agar and from the stock culture, a loop full of the culture was inoculated in nutrient broth. The broth seeds were incubated at 37 ± 1°C for 24 h. Modified soy agar Petri plates were prepared for testing the antibacterial activity of the extracts. Diluted culture of 0.1 ml was poured on each plate and the plates were dried for 30 min at 37°C. Wells of 6 mm diameter were cut with sterile cork borer in the inoculated agar. The wells were filled with the plant extract. 50% ethanol solution was used as control in the next well and amoxicillin was filled in the third well. The plates were incubated for 24 h at 37°C. At the end of incubation period, the inhibition zones were measured (Naqvi et al., 1992).

Antifungal activity

A study on antifungal activity was carried out against *Candida albicans* using the method described (Naqvi et al., 1992). The culture media used in this case was Sabouraud Dextrose Agar and the same procedure for inoculation of organism and antifungal assay was employed as used for antibacterial activity testing.

Analgesic activity

In present study, analgesia was assessed according to the method

Table 1. Phytochemical screening of crude extract of *P. corylifolia*.

Chemical	Results (present + or absent -)
Alkaloids	+
Tannins	+
Saponins	+
Sterols	-
Phenol	+
Proteins	-
Flavanoids	+

Table 2. Standardization of *P. corylifolia* crude extract by different techniques.

Crude drug extract	Method	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
P. corylifolia (Conc)	UV peaks-nm	223.62	282.56	290.64	329.03	368.44
P. corylifolia (Diluted)	UV peaks-nm	228.94	247.85	-	-	-
P. corylifolia	HPLC (225 nm), retention time (min)	1.919	2.579	3.002	3.132	3.382o
P. corylifolia	HPLC (325 nm), retention time (min)	1.911	-	-	-	-
P. corylifolia	FTIR peak-cm^{-1}	3336.95	2913.04	1701.08	-	-
P. corylifolia	FT-NIR peak-cm^{-1}	5861.11	5166.66	4305.55	-	-

Wavelength at which five peaks appeared when UV-visible spectrophotometry was done. It also shows the retention time of different constituents when HPLC was done at wavelength 225 and 325 nm. The frequency at which different peaks appeared during FTIR and FTNIR is also indicated.

of Di Stasi et al. (1989) and Indumathy and Kavimani (2011). Mice were divided into five groups with each group containing five animals. Each mouse was held in a suitable restrainer with the whole tail extending out. An area of the tail, 2 to 3 cm in length, was marked and immersed at 51°C. The withdrawl time of the tail from hot water (in seconds) was noted as the reaction time. Control animals recieved 10 ml/kg of 0.9% NaCl orally, while plant extracts in the dose of 300 and 500 mg/kg body weight were administered orally by intubation. The analgesic effect induced after administration of test and standard drugs was measured. The initial readings were taken immediately before administration of the test and standard drugs, and then at 30, 60, 90, 120, 150, 180 and 210 min.

Brine shrimp lethality bioassay

Lethality bioassay was performed according to the method described (Meyer et al., 1982; Arnason et al., 1989). Plant extract sample was prepared in three different dilutions 10, 100 and 1000 µg/ml. Brine shrimp (*Artemia salina*) nauplii were hatched in a specific tank. 10 shrimps were transferred to vials containing each concentration of the crude extract and then sea water was added to make the volume 5 ml. Later dry yeast suspension was added as food to each vial including control. The vials were kept for 24 h, then the active nauplii were counted and death percentage was calculated at each dose. The data was analyzed by Finney computer program to determine LD_{50} values. Etoposide was used as positive control in the assay.

Statistical analysis

The data obtained from smooth muscle activity and analgesic effect study were statistically analyzed by Student's t-test ($P < 0.05$), using 'GraphPad, QuickCalcs software (online)'.

RESULTS

The phytochemical screening of the crude extract of *P. corylifolia* showed the presence of alkaloids, tannins, saponin, phenols and flavonoids (Table 1). UV analysis was carried out on the concentrated (2 mg/10 ml, showed five peaks) and diluted solution (2 mg/100ml, showed two peaks) of crude extracts (Table 2 and Figures 1 and 2). HPLC, FTIR and FT-NIR finger prints are given in Table 2 and Figures 3 to 6.

The *in vitro* intestinal smooth muscle activity on *P. corylifolia* crude extract was carried out at 1, 5, 10, 15, 20 and 25 mg/ml doses (Table 3). The study was also carried out on its fractions along with the standard drugs (Table 4). The crude extract produced antispasmodic effect by decreasing the intestinal movement which was found to be highest at 20 mg/ml dose. The ethyl acetate, chloroform, *n*-butanol and aqueous fractions were tested at 10 mg/ml dose. All fractions exhibited antispasmodic effect except ethyl acetate fraction which produced an increase in the intestinal smooth muscle response.

The crude extract also exhibited positive antibacterial and antifungal activity against *S. aureus* and *C. albicans,* respectively (Table 5). The results of analgesic activity are presented in Table 6. The crude extract of *P. corylifolia* was evaluated at 300 and 500 mg/kg doses in comparison with a standard analgesic drug (diclofenac

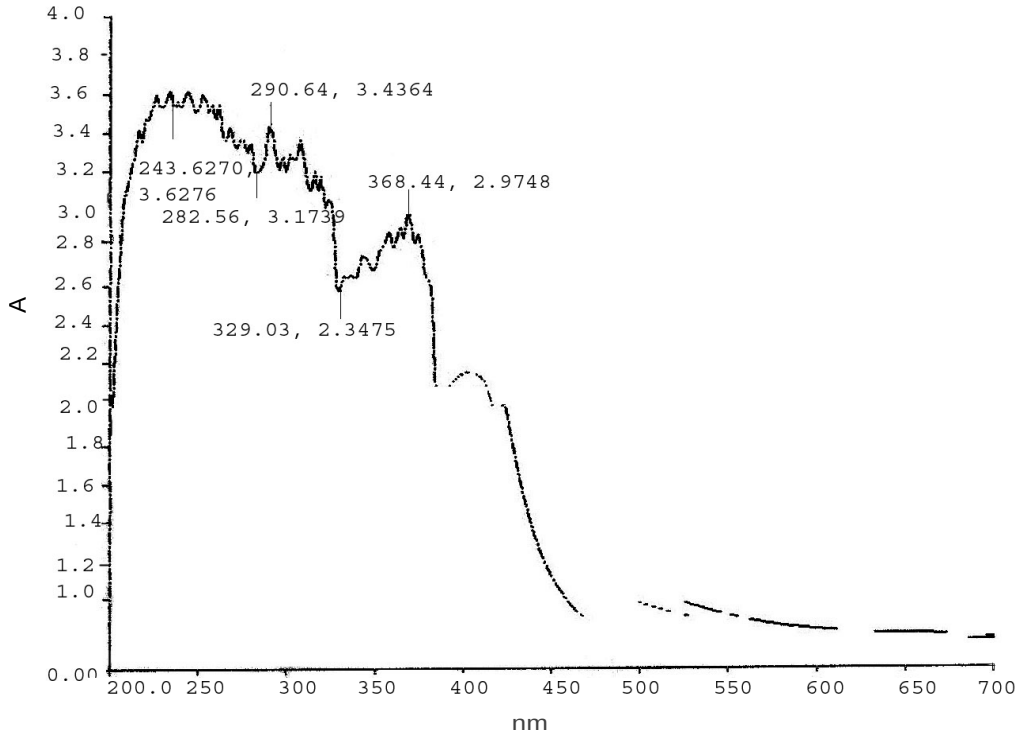

Figure 1. UV spectra of *P. corylifolia* (concentrated, 2 mg/10 ml).

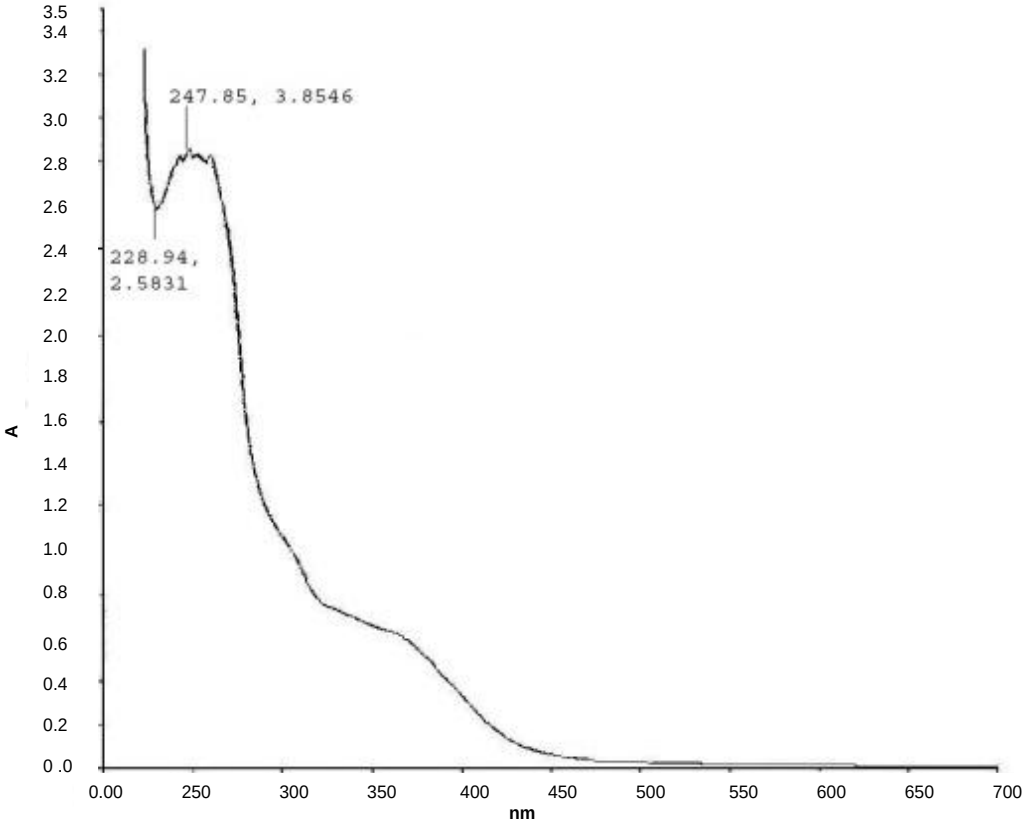

Figure 2. UV spectra of *P. corylifolia* (diluted, 2 mg/100 ml).

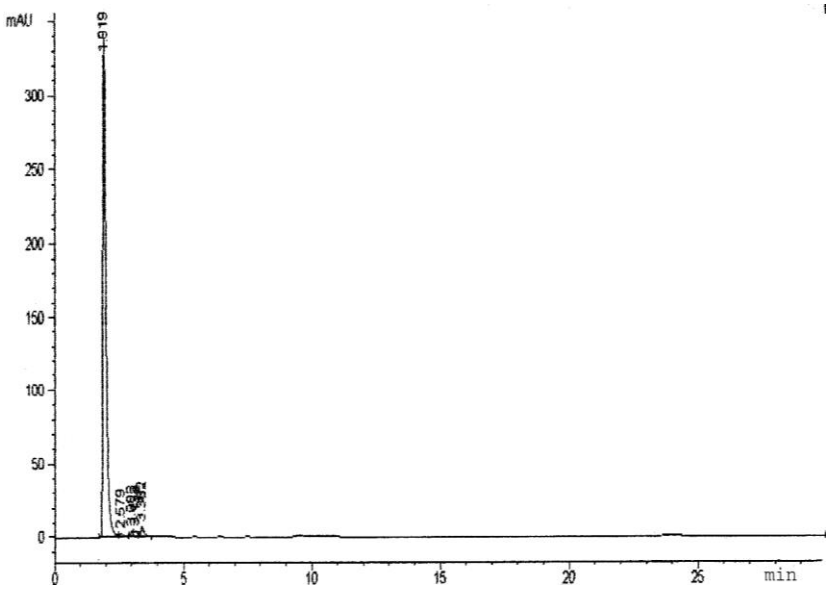

Figure 3. HPLC spectra of *P. corylifolia* at 225 nm.

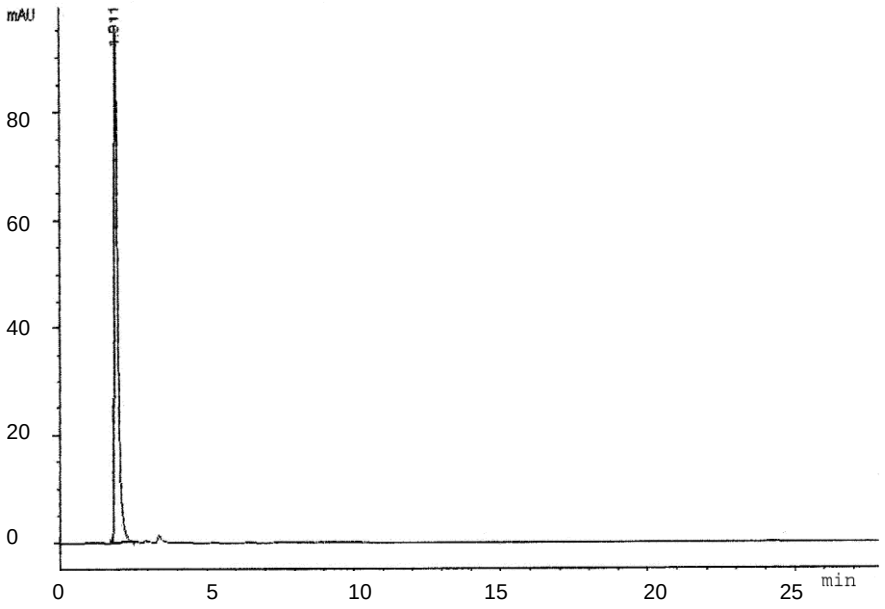

Figure 4. HPLC spectra of *Psoralea corylifolia* at 325 nm.

Na 50 mg/kg).

Brine shrimp lethality assay was performed to determine the acute toxicity of the crude extract of *P. corylifolia* in different concentrations. It showed no activity at all concentrations (Table 7).

DISCUSSION

P. corylifolia which is locally called as Babchi has been used as anthelmintic, laxative, stimulant, in leprosy and in skin diseases (Jeyakumar and Jayabalan, 2002). Preliminary phytochemical studies on crude extract of *P. corylifolia* can be used for the identification, confirmation and authentication of the drug in future.

P. corylifolia crude extract and fractions were subjected to smooth muscle activity testing on rabbit intestine. The results revealed the presence of antispasmodic effect in fractions and the crude extract of the plant, except the ethyl acetate fraction which produced slight spasmogenic

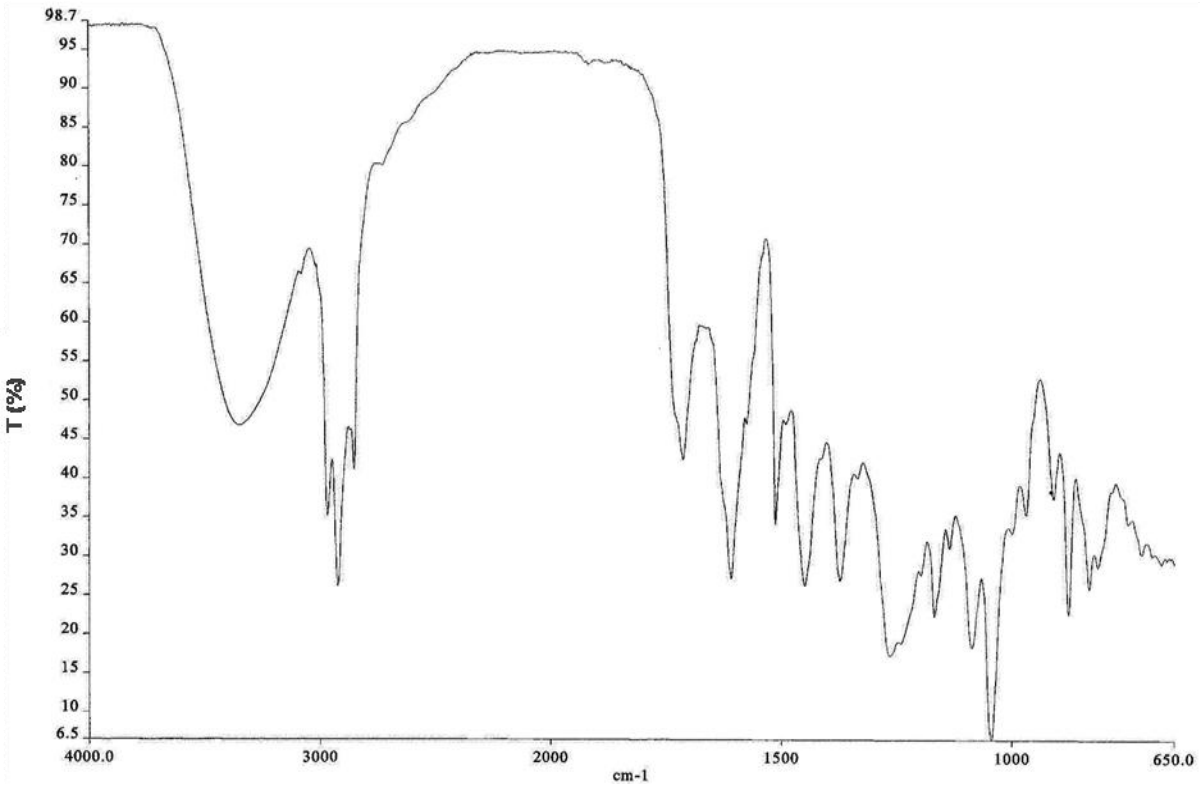

Figure 5. FTIR spectra of *P. corylifolia.*

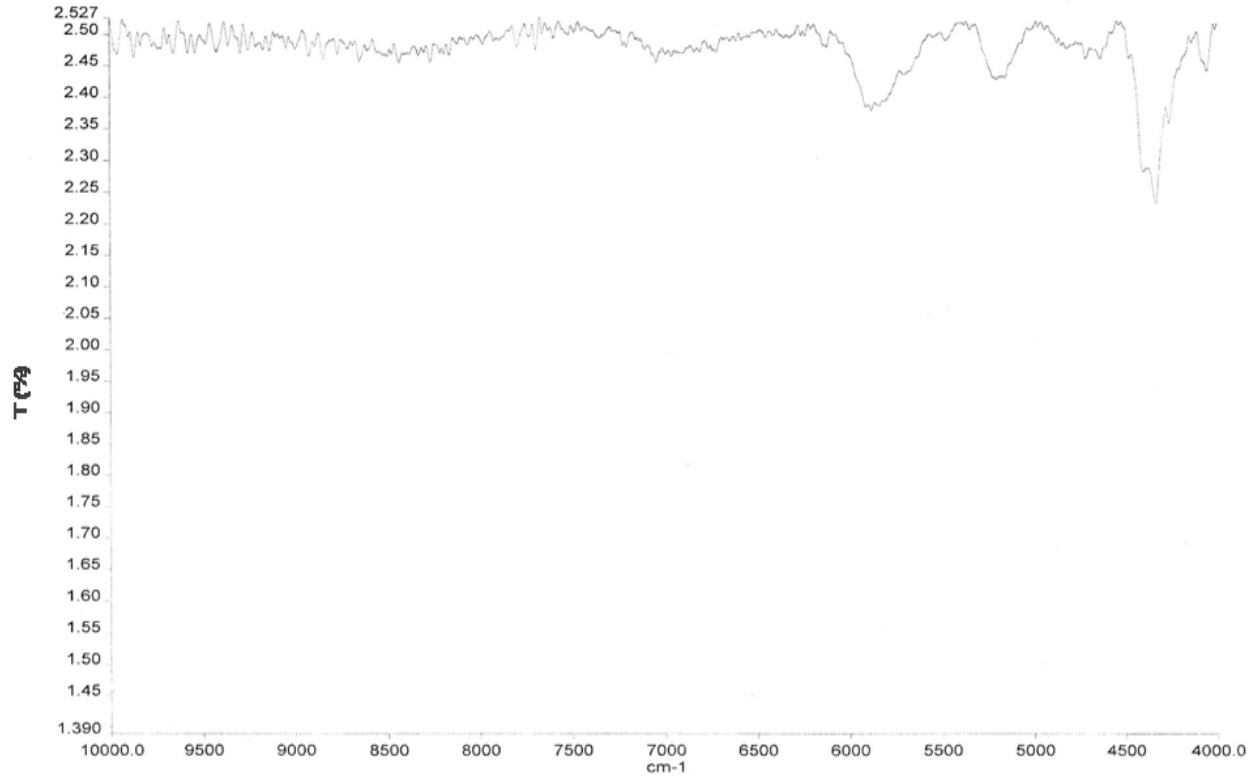

Figure 6. FTNIR spectra of *P. Corylifolia*.

Table 3. Dose related response of crude extract of *P. corylifolia* on isolated rabbit's intestine.

Dose (mg/ml)	Control (cm)	Response (cm)	Response (%)	t- value
1	1.000 ± 0.058	0.833 ± 0.033	16.67	4.583
5	1.033 ± 0.033	0.633 ± 0.033	38.71	14.142
10	1.133 ± 0.033	0.533* ± 0.033	52.94	21.213
15	1.033 ± 0.033	0.467* ± 0.033	54.84	18.031
20	1.067 ± 0.033	0.233** ± 0.033	78.13	22.981
25	1.233 ± 0.033	0.733* ± 0.367	40.54	2.671

The results are expressed in Mean ± SEM, *Significant at P < 0.05, **Highly significant at P < 0.05.

Table 4. Effects of different fractions of *Psoralea corylifolia* on isolated rabbit's intestine.

Fraction	Dose (mg/ml)	Control (cm)	Response (cm)	Response (%)	t- value
Ethyl acetate	10	1.067±0.067	1.300 ± 0.058	21.88%	6.262
Chloroform	10	1.033±0.033	0.567* ± 0.033	45.16%	15.839
n- butanol	10	0.933±0.033	0.567 ± 0.033	39.29%	11.667
Aqueous	10	0.967±0.033	0.833 ± 0.033	13.79%	5.091

The results are expressed in Mean ± SEM, at P ≤ 0.05, * Significant at P < 0.05; **Highly significant at P < 0.05.

Table 5. Antimicrobial activity of *P. corylifolia* against fungi and bacteria.

Antifungal and antibacterial activity				
C. albicans	***A. niger***	***S. aureus***	***E. coli***	***K. pneumoniae***
+	**-**	**+**	**-**	**-**

+Sign indicates that bactericidal or fungicidal activity was observed and -sign indicates that no effect was produced.

Table 6. Analgesic activity of crude extract of *P. corylifolia* (tail flick time in seconds).

Test groups	Time (min)								
	0	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**240**
Control	2.98 ± 0.44	1.8 ± 0.37	2.4 ± 0.24	1.8 ± 0.24	2.2 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	2.6 ± 0.24	2.6 ± 0.24
Diclofenac sodium 50 mg/kg	1.97 ± 0.13	2.6 ± 0.24	3.6* ± 0.24	4.12 ± 0.097	4.06** ± 0.096	3.3***** ± 0.199	2.3 ± 0.199	2.1 ± 0.1	2.1 ± 0.1
Treated	±1.6	±2.4	±3	±3.3*	±3.7*****	±4******	±3.3*****	±3	±2.7
300 mg/kg	0.24	0.24	0.00	0.20	0.20	0.16	0.20	0.00	0.20
Treated	±1.6	±3.4*	±3.8*****	±5******	±4.2******	±4.2******	±4.2******	±4.3******	±3.4*
500 mg/kg	0.40	0.24	0.25	0.32	0.20	0.34	0.34	0.30	0.24

Results are expressed as Mean ± SEM, *Significant at P < 0. 05; **Highly significant at P < 0.05.

Table 7. Toxicity testing of *P. corylifolia* by brine shrimp lethality assay.

Name of organism	Dose (µg/ml)	No. Of shrimps	No. of survivors	Reference drug
Brine shrimp *(Artemia salina)*	1000	30	30	Etoposide (LD_{50} = 7.4625)
	100	30	30	
	10	30	30	

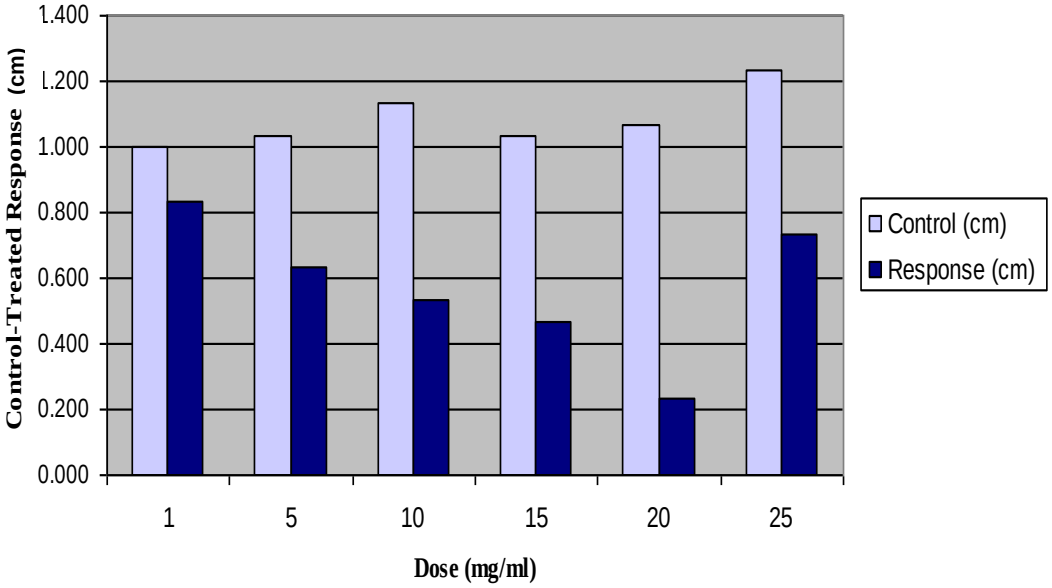

Figure 7. Comparison of control and treated response of crude extract of *P. corylifolia* on isolated smooth muscles of rabbit intestine.

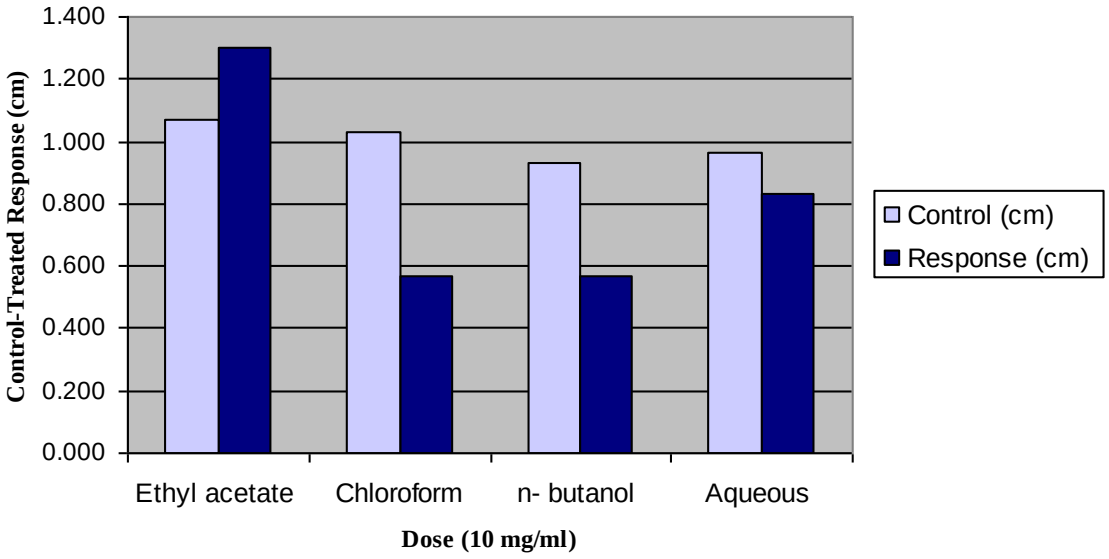

Figure 8. Comparison of control and treated response of fractions of *P. corylifolia* on isolated smooth muscles of rabbit.

effect. At the dose of 1 and 5 mg/ml, crude extract of *P. corylifolia* produced a quick decrease in the normal intestinal movement followed by a gradual increase in the form of curve (Table 3 and Figure 7). These results of smooth muscle activity testing at different doses indicated the presence of mild spasmolytic effect; this indicates the presence of more than one active constituent. For further evaluations, fractionation of the crude extract of *P. corylifolia* was done. The aqueous, *n*-butanol and chloroform fraction of *P. corylifolia* produced mild relaxation of the intestinal smooth muscles, whereas ethyl acetate fraction slightly increased the normal activity of the intestine (Table 4). Chloroform fraction showed the same effect as that of 1 and 10 mg dose of the crude extract (Table 4, Figure 8). The fractions were further administered with standard drugs atropine, adrenaline

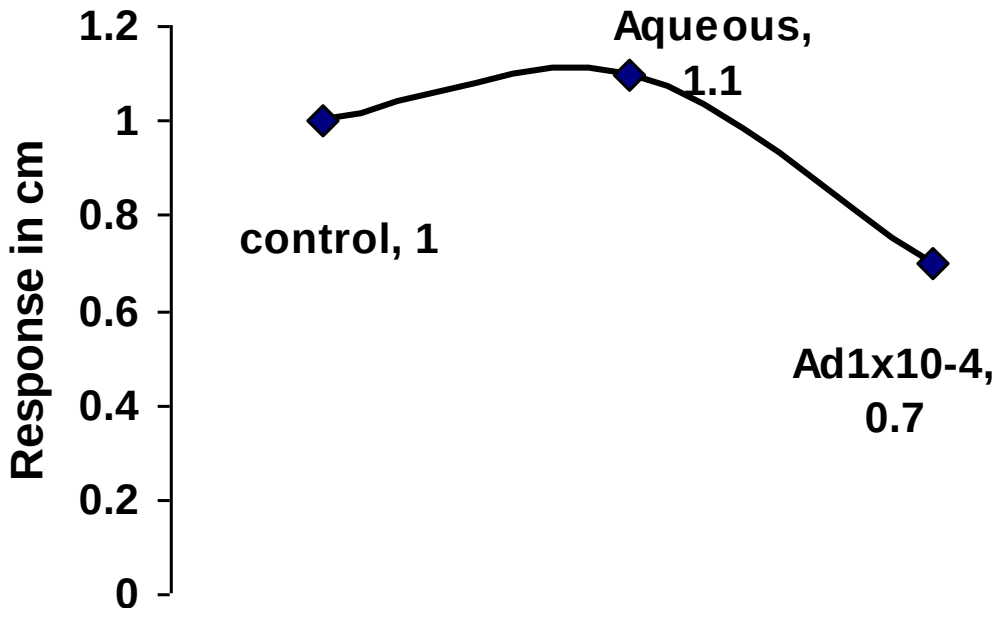

Figure 9. The effect of aqueous fraction of *P. corylifolia* post-treated with adrenaline (Ad)1 × 10^{-4} M.

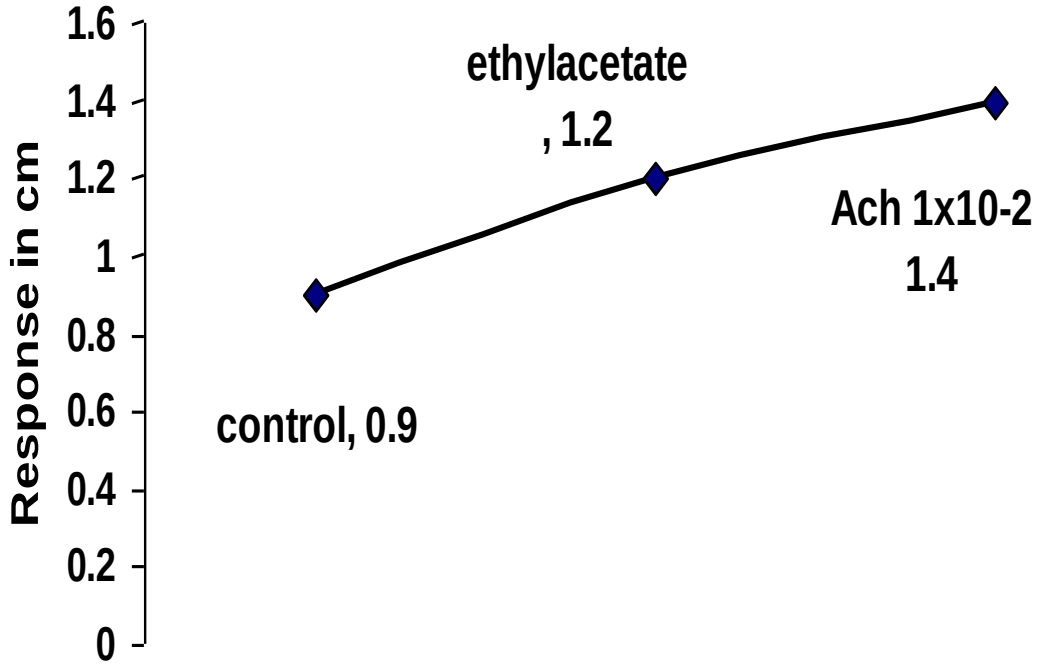

Figure 10. The effect of ethyl acetate fraction of *P. corylifolia* post-treated with acetylcholine (Ach) 1 × 10^{-4} M.

and acetylcholine at different concentrations. Figure 9 shows the effect of aqueous fraction post-treated with adrenaline at 1 × 10^{-4} M concentration; which overcomes the effect of aqueous fraction. Figure 10 shows the effect of ethyl acetate fraction of *P. corylifolia* post-treated with acetylcholine 1 × 10^{-4} M. Figure 11 shows the effect of chloroform fraction pre-treated with the acetylcholine in 1 × 10^{-4} M concentration, which showed an increase in the effect of acetylcholine. Figure 12 shows the effect of chloroform fraction of *P. corylifolia* pre-treated with

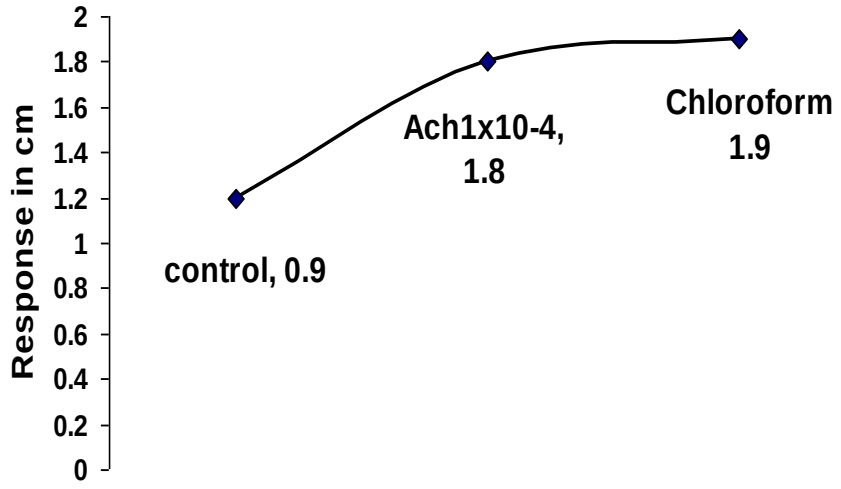

Figure 11. The effect of chloroform fraction of *P. corylifolia* pre-treated with acetylcholine (Ach) 1×10^{-4} M.

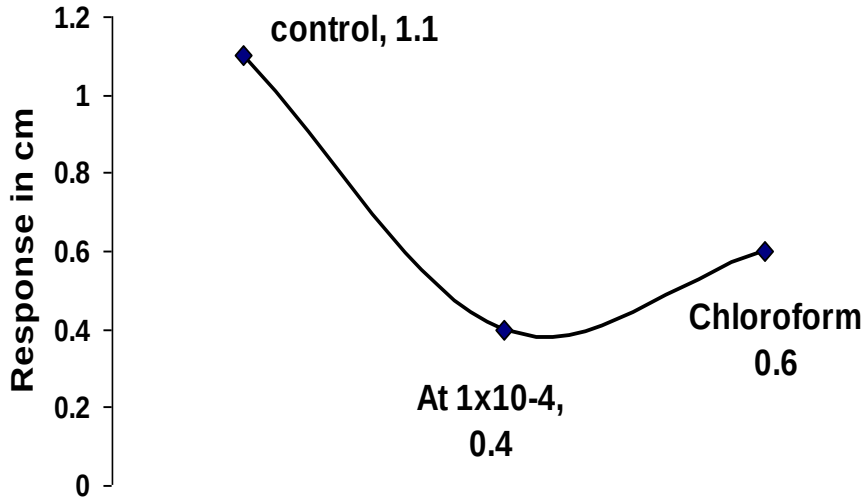

Figure 12. The effect of chloroform fraction of *P. corylifolia* pre-treated with atropine (At) 1×10^{-4} M.

atropine in 1×10^{-4} M concentration, here the effect of the test drug was not significant. The overall study showed that the crude extract of *P. corylifolia* was more effective than its different fractions, as in case of the fractions, no significant contraction or relaxation was observed. In order to evaluate its possible mechanism of action, the fractions were also treated with acetylcholine, atropine and adrenaline, and the results showed the possible involvement of muscarinic receptors. But this effect was not highly significant as observed in acetylcholine and atropine.

In vitro functional experiments have suggested that muscarinic receptors are predominant in mediating the contractile response in the gastrointestinal tract. Muscarinic (M_3) receptors sub type preferring antagonist, can inhibit contractions produced by muscarinic agonist (Eglen et al., 1990; Das et al., 1997). However, both radioligand binding and receptor antibody study from a variety of smooth muscle studies have shown that mostly, the receptors involved are the M_2 sub type (Eglen et al., 1996). In the intestine, excitatory responses induced by activation of motor neurons can be inhibited by atropine indicating the importance of cholinergic neurons in the normal regulation of physiological functions in this tissue (Kunze and Furness, 1999). As the crude extract showed initial inhibition followed by gradual contraction, it indicates the involvement of muscuranic receptors.

The antibacterial and antifungal activity of this plant

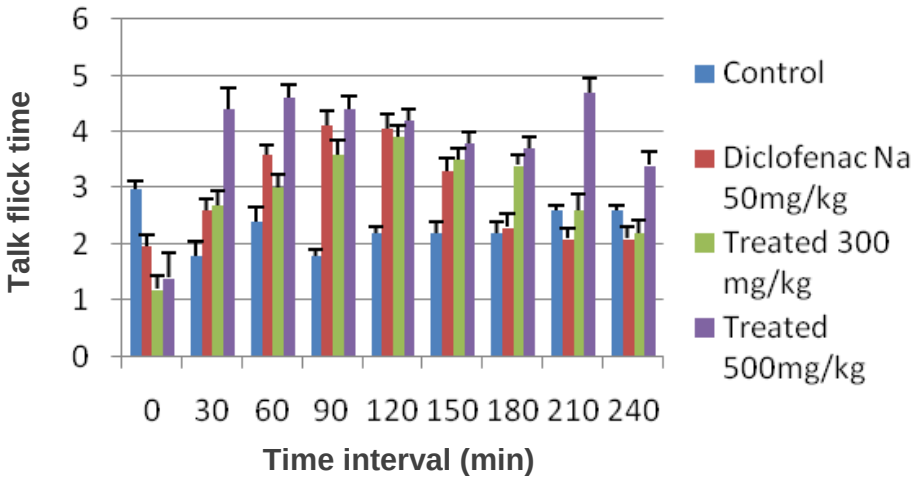

Figure 13. Analgesic activity. The tail flick time of mice kept as control, treated with diclofenac sodium and crude extract of *P. Corylifolia*.

was performed for its specific action against three selected organisms. No significant antibacterial activity was recorded against *E. coli* and *K. pneumoniae*, but it showed some antibacterial activity against *S. aureus.* The extract also displayed positive antifungal activity against *C. albicans* (Table 5). Analgesic activity was assessed in mice by tail flick method. The results were found significant on higher dose of the crude extract (Table 6 and Figure 13). The crude extracts of *P. corylifolia* showed analgesic potential which was observed at 300 and 500 mg/kg. The crude extract exhibited maximum response with 500 mg/kg dose, at 90 min, which was highly significant when compared with control and standard drug diclofenac sodium ($P < 0.05$). The results of Brine shrimp bioassay revealed a non toxic effect of the crude extract at 10, 100 and 1000 µg/ml (Table 7).

Conclusion

Conclusively, the crude extract of *P. corylifolia* and its fractions revealed quick decrease in the normal intestinal movement, followed by a gradual increase from the normal movement. These results at different doses indicated the presence of antispasmodic effect. The plant extract also showed a significant analgesic potential as well as antifungal and antibacterial activity against *C. albicans* and *S. aureus*, respectively.

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