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In-vitro antioxidant activities, anti-nociceptive and neuropharmacological activities of *Polygonum hydropiper*

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Abstract
In the present study in-vitro antioxidant activities, anti-nociceptive assessment and neuropharmacological study of the leaf extract of *Polygonum hydropiper* was performed. In-vitro antioxidant activity of the extracts of *P. hydropiper* was performed using DPPH free radical scavenging, cupric reducing antioxidant capacity, total antioxidant capacity, total phenol and total flavonoid content determination assays; anit-nociceptive activity was done by using acetic acid induced writhing method and neuropharmacological activities were assessed by open field test and swimming test. The plant's leaf extracts exhibit potent antioxidant activities; significant anti-nociceptive activity and neuropharmacological activity.
Keywords: *Polygonum hydropiper*, in-vitro antioxidant activities, anti-nociceptive activity, neuropharmacological activities.

1. Introduction

For decades, the utilization of herbal plants has drawn avalanche of interest as they could accommodate therapeutic response and are promising candidate to be developed as pharmaceutical products. Free radicals have been accused of initiating many serious diseases (Malorni *et al*., 1998; Robert and Meunier, 1998; Pauli, 2005; Shah, 2005; Rios and Recio, 2005). These free radicals drive oxidative stress and transform the pathophysiological condition of the patient by acting on immune system. It has been known that phenolic and flavonoid compounds of the plant extracts are responsible for antioxidant (Da-Silva *et al*., 2006; Majhenic *et al*., 2007; Pereira *et al*., 2007). On the other hand, anti-inflammatory activities of plant extracts are vastly investigated now a day to develop more selective and efficacious pharmaceutical lead compound (Fábio *et al*., 2008). Neurophamacological tests of plant extracts are also investigated in animal model to develop more alternatives from plant source for the treatment of various neuropsychological and neurodegenerative diseases (Gomes *et al*., 2009). The genus *Polygonum* has particular medicinal importance with many plants being investigated and found having pharmacological property such as anti-inflammatory and diuretic agent (PED, 2009).

Polygonum hydropiper (Fam. Polygonaceae) locally known as Bishkatali, is an erect or ascending herb with stem decumbent at base, nodes below swollen, linear-lanceolate leaves and small pink flowers in very slender erect or decurved racemes, commonly found in wet places, particularly near the banks of canals and ditches all over the country. Traditionally juice of leaves is used in pain, headache, toothache (Rahman *et al*., 2002), liver enlargement, gastric ulcer, dysentery, loss of appetite, dismenorrhoea and rheumatoid arthritis (Yang *et al*., 2012). Juice of paste is applied to wounds, skin diseases and painful carbuncles. Root is used as stimulant. Preparation of the plant is used to cause premature abortion. Leaf paste is also capable of stopping external haemorrhage (Ghani, 2003).

In the present study we investigated the antioxidant property of *P. hydropiper* using DPPH free radical scavenging, cupric reducing antioxidant capacity, total antioxidant capacity, total phenol and total flavonoid content determination assays. Further, we studied the antinociceptive and neuropharmacological properties of this plant using leaf extracts. To our knowledge, no such work has been done to incorporate these three important assays together for this plant and thus we believe it will be a sufficient addition on the current knowledge about *P. hydropiper*.

2. Materials and methods

2.1 Plant materials collection and identification

Whole plant sample of *P. hydropiper* was collected from Tongi, Gazipur, Dhaka, Bangladesh in June 2012. Then the plant sample was submitted to The National Herbarium of Bangladesh, Mirpur, Dhaka for its identification.

One week later its voucher specimen was collected after its identification (Accession No.37756) which was identified and authenticated by taxonomist of the National Herbarium of Bangladesh.

2.2 Plant materials preparation

Whole plant of *P. hydropiper* was separated in different parts and sun dried for 7 days. Then leaves were taken & oven dried for 3 hours at 40^0C and then 800gm dried leaves were grinded in coarse powder using high capacity grinding machine. About 715gm of grinded powders were sieved to get fine powder. Finally 630gm of fine powder obtained which was then stored in air tight container with necessary marking for identification and kept in cool, dark and dry place for further investigation.

15gm of powdered leaves were successively extracted in Soxhlet extractor at elevated temperature (40-60^{0}C) by using 300ml of methanol (solvent). Then by same process, ethanol, chloroform, petroleum ether and n-Hexane were extracted too. All extracts were filtered individually by Watman filter paper. Then all extracts were poured on petri dishes individually to evaporate liquid and proper dry. After drying, crude extracts were stored in those petri dishes and kept in refrigerator (0-4°C) for future investigation.

2.3 In-vitro antioxidant activities

i) DPPH Free radical scavenging activity

The free radical scavenging activity of the whole plant's extractives of *P. hydropiper* was evaluated by the published method (Braca *et al*., 2001). DPPH was used to evaluate the free radical scavenging activity (antioxidant potential).

ii) Cupric Reducing Antioxidant Capacity (CUPRAC)

The assay was conducted as described previously by Resat *et al.*, 2004. To 0.5 ml of plant extract or standard of different concentration solutions diluted from 5µg/ml to 200µg/ml, 1 ml of copper (II) chloride solution (0.01 M prepared from $CuCl_2.2H_2O$), 1 ml of ammonium acetate buffer at pH 7.0 and 1 ml of neocaproin solution (0.0075 M) were mixed. The final volume of the mixture was adjusted to 4.1 ml by adding 0.6 ml of distilled water and the total mixture was incubated for 1 hour at room temperature. Then the absorbance of the solution was measured at 450 nm using a spectrophotometer against blank which is made of same above mixture except extract or sample or standard. Ascorbic acid & BHT was used as a standard.

iii) Determination of Total Antioxidant Capacity

The total antioxidant capacity was evaluated by the phosphomolybdenum method by Prieto *et al.*, 1999.

All of extracts and standard were diluted by serial dilutions (12.5µg/ml to 200µg/ml). Then on each test tube containing 0.3ml of diluted solution of sample and standard, following reagent solutions were added as 1ml of 0.333% H_2SO_4 and 1ml 0.02295M Sodium Phosphate and then 1ml of 0.004M Ammonium Molybdate was finally added. Later it was incubated at 95^0C for 90 min to get proper reaction, then after incubation remove and kept at room temperature to cool. Later on, absorbance was taken at 695 nm against the blank which is made of same above mixture except extract or sample or standard. The antioxidant activity is expressed as the number of equivalents of ascorbic acid and was calculated by the following equation:

$$A=(c\times V)/m$$

Where,
A = total content of Antioxidant compounds, mg/gm plant extract, in Ascorbic acid Equivalent
c = the concentration of Ascorbic acid established from the calibration curve, mg/ml,
V = the volume of extract in ml,
m = the weight of crude plant extract, gm.

iv) Total phenolics analysis

Total phenolic content of whole plant's leaves extractives of *P. hydropiper* was measured employing the method described by Velioglu *et al*., 1998 and Demiray *et al*., 2009 involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard.

All of extracts and standard were diluted by serial dilutions as (6.25µg/ml to 200µg/ml) then, on each test tube containing 1ml of diluted solution of sample and standard, 5ml of (1:10 v/v) Folin -ciocalteu reagent and 4ml of 7.5% sodium carbonate solution were added. Then samples were incubated at 20^0C temperature for 60 min. Here, standard diluted solution–reagent mixture was incubated at 20^0C for 30 min. After incubation absorbance was taken at 765 nm, where blank was methanol.

The total content of phenolic compounds plant extracts in gallic acid equivalents (GAE) was calculated using the following equation:

$$C = (c \times V)/m$$

Where,
C= total content of phenolic compounds, mg/gm plant extract, in GAE
c= the concentration of gallic acid established from the calibration curve (mg/ml)
V = the volume of extract in ml.
m = the weight of crude plant extract in gm

v) Determination of Total Flavonoids Content

Aluminum chloride colorimetric method was used for Flavonoids determination by Wang *et al.*, 2000.

All of extracts and standard were diluted by serial dilutions as (6.25µg/ml to 200µg/ml) then, on each test tube containing 1ml of diluted solution of sample and standard, 3 ml of methanol, 0.2 ml of aluminum chloride (10% $AlCl_3$), 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water. It incubated at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with spectrophotometer against blank. Methanol served as blank. The total content of flavonoid compounds in plant methanol extracts in quercetin equivalents was calculated by the following equation:

$$C = (c \times V)/m$$

Where;
C = total content of flavonoid compounds, mg/gm plant extract, in quercetin equivalent,
c = the concentration of quercetin established from the calibration curve in mg/ml,
V = the volume of extract in ml and
m = the weight of crude plant extract in gm.

2.4 Animals and treatment

Swiss albino mice (*Mus musculus*) aged around 4 to 5 weeks and weighing 20-30 g of either sex were used for the research. The mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B) Mohakhali, Dhaka. The animals were maintained under standard hygienic conditions (temperature 27^0C ± 2^0C, relative humidity 55-65% and natural day night cycle) and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for one week prior to experiments.

i) Measurement of anti-nociceptive activity of *P. hydropiper*

Acetic acid induced writhing

The acetic acid writhing test in mice as described by Koster *et al.*, 1959 was employed with slight modification. Mice were divided into 6 groups of 6 mice each. The first group was given 10ml/kg of 1% Tween 80 i.p. and served as control. Group 2 was served as standard where Diclofenac Sodium has given to mice as dose of 150mg/kg of body weight. Groups 3, 4 received methanol extract of leaf of *P. hydropiper* 100mg/kg and 150 mg\kg of body weight. Groups 5, 6 received ethanol extract of leaf of *P. hydropiper* 100mg/kg and 150mg/kg of body weight. Thirty minutes later each mouse was injected intra-peritoneally with 0.7% acetic acid at a dose of 10 ml/kg body weight. Full writhing was not always completed by the animal, because sometimes the animals

start to give writhing, but they do not finish it. This incomplete writhing was taken as a half writhing. Accordingly, two half writhing were considered as one full writhing. The number of writhing responses was recorded for each animal during a subsequent 5 min period after 15 min intra-peritoneally administration of Acetic acid and the mean abdominal writhing for each group was obtained.

The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Mean no. of writhing (control)} - \text{Mean no. of writhing (drugs)}}{\text{Mean no. of writhing (control)}}$$

ii) Neuropharmacological Study
Open field test

According to Gupta *et al.*, 1971 with slight modification open field was performed to monitor behavioral responses in mice that were placed in a novel and bright arena. Rodents tend to stay away from brightly illuminated areas. The experiment also assesses a range of anxiety-induced, locomotor activity and exploratory behaviors. The animals were divided into 8 groups of 6 mice each. The first group was given 10ml/kg of 1% Tween 80 orally and served as control. Group 2 served as standard where Clonazepam has given to mice as dose of 2mg/kg of body weight. Groups 3, 4 methanol extract of *P. hydropiper* 100 and 150 mg/kg of body weight, and groups 5, 6 received ethanol extract of *P. hydropiper* 100 and 150 mg\kg of body weight. Group 7& 8 served as sample where chloroform extracts of *P. hydropiper* has given to mice as dose of 100mg/kg &150mg/kg of body weight.

The test was carried out according to the technique described by Gupta *et al.*, 1971 with slight modification. The open field apparatus is made of hardboard (60cm x 60cm; 40cm walls). Blue lines drawn on the floor divide the floor into thirty six squares 10cm x 10cm squares alternatively colored black and white and Central Square (10cm x 10cm) in the middle clearly marked. The number of squares visited by the animals was calculated for 2 min, at 0, 30, 60, 90, 120 and 150 min subsequent to oral administration of the experimental crude extracts.

Swimming Test
According to Porsolt *et al.*, 1977 swimming test was performed with slight modification. Animals were randomly divided into 8 groups with 6 mice on each group. Group 1 (control) received 1% Tween 80,10ml/kg orally. Group 2 received Imipramine of 10 mg/kg body weight which served as standard. Groups 3, 4 received sample where methanol extract of *P. hydropiper* 100 mg/kg and 150 mg/kg of body weight. Groups 5, 6 received sample where ethanol extract of *P. hydropiper* 100 mg/kg 150 mg/kg of body weight. Group 7, 8 served as sample where chloroform extracts of *P. hydropiper* has given to mice as dose of 100mg/kg &150mg/kg of body weight.

The forced swim test was carried out on mice individually forced to swim in an open acquire water tank apparatus (29cm x 19cm x 20cm), containing 9 cm of water at 25±1 °C. The total duration of immobility during the 6-min test was scored as described. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The duration of immobility was recorded. Decrease in the duration of immobility during the FST was taken as a measure of antidepressant activity.

3. **Results and discussion**

3.1 In-vitro Antioxidant Activities

(a) Free radical scavenging activity
All the extractives of *P. hydropiper* were subjected to free radical scavenging activity using DPPH by using ascorbic acid (ASA) and *tert*-butyl-1-hydroxytoluene (BHT) as reference standards. In this investigation, the ethanol extract (EE) showed significant free radical scavenging activity with IC_{50} value of 12.211 µg/ml.

(b) Cupric Reducing Antioxidant Capacity (CUPRAC)

Reduction of Cu^{2+} ion to Cu^{+} was found to rise with increasing the concentrations of different extracts. The standard ascorbic acid and BHT showed highest reducing capacity. Among the extracts the methanol extract of *P. hydropiper* showed maximum reducing capacity that is comparable to ascorbic acid and BHT (Figure 1).

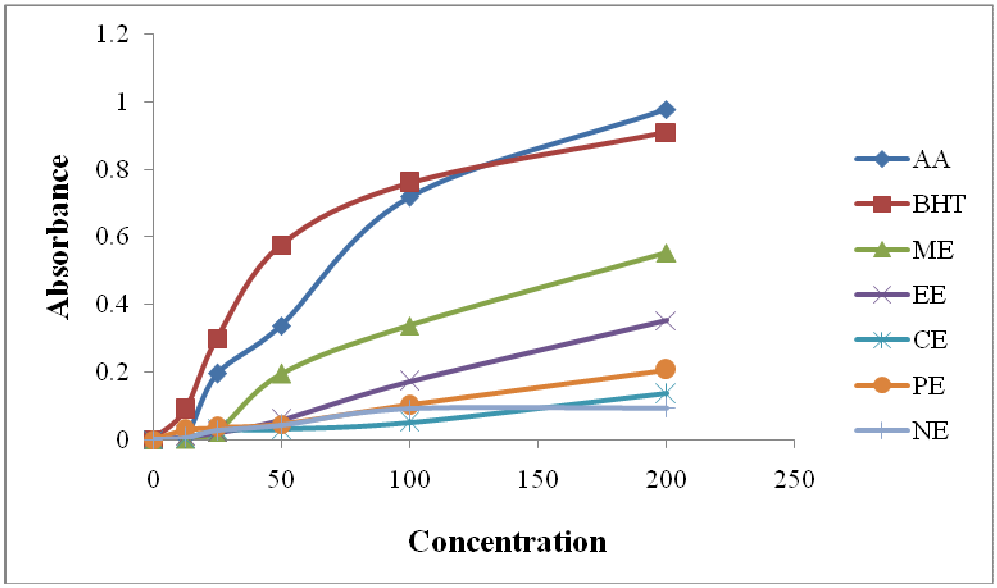

Fig 01: Comparative reducing power of extracts, Ascorbic acid and Butylated hydroxytoluene

(c) Total Antioxidant Capacity

Total antioxidant capacity of the different extracts of *P. hydropiper* was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid Ethanol extract of *P. hydropiper* was found to possess the highest total antioxidant capacity (Table 1).

Table 01: Total antioxidant capacity of the different extracts of *Polygonum hydropiper*

Name of Extracts	Total Antioxidant Capacity (mg/gm, Ascorbic Acid Equivalent)
Methanol	7.32±4.26
Ethanol	6.06±3.32
Chloroform	5.18±2.23
Petroleum ether	4.98±2.29
n-Hexane	4.7±2.11

Values are the mean of duplicate experiments and represented as mean ± SD.

Total antioxidant capacity of the extracts was found to decrease in the following order: Methanol extract > Ethanol extract > Chloroform extract > Pet-ether extract > n-Hexane extract.

(d) Total phenolic analysis

Total phenolic contents of the different extracts were determined by using the Folin-Ciocalteu reagent and were expressed as Gallic acid equivalents (GAE) per gram of plant extract. The total phenolic contents of the test fractions were calculated using the standard curve of Gallic acid. Ethanol extract was found to contain the highest amount of phenols (Table2).

Table 02: Total phenol contents of the different extracts of *Polygonum hydropiper*

Name of Extracts	Total Phenol Contents (mg/gm, Gallic Acid Equivalent)
Methanol	1.58±0.61
Ethanol	1.63±0.68
Chloroform	1.17±0.03
Petroleum ether	1.17±0.08
n-Hexane	1.24±0.05

Values are the mean of duplicate experiments and represented as mean ± SD

Phenol contents of the extracts were found to decrease in the following order: Ethanol extract > Methanol extract > n-Hexane extract > Petroleum ether extract > Chloroform extract.

(e) Total Flavonoid Content

Aluminium chloride colorimetric method was used to determine the total flavonoid contents of the different extracts of *P. hydropiper* Total flavonoid contents was calculated using the standard curve of quercetin (and was expressed as quercetin equivalents (QE) per gram of the plant extract. Petrolium ether extract of *P. hydropiper* was found to contain the highest amount of flavonoid (Table 3).

Table 03: Total flavonoid contents of the different extracts of *Polugonum hydropiper*

Name of Extracts	Total Flavonoid Contents (mg/gm, Quercetin Equivalent)
Methanol	2.66±0.8
Ethanol	3.147±1.5
Chloroform	3.313±1.8
Petroleum ether	3.587±1.7
n-Hexane	2.96±1.3

Values are the mean of duplicate experiments and represented as mean ± SD.
Flavonoid contents of the extracts were found to decrease in the following order: Petrolium ether extract > Chloroform extract > Ethenol extract > n-Hexane extract > Methnol extract.

3.2 Pharmacological Investigations

The results of acetic acid induced Writhing test for different extracts of *P. hydropiper* are presented in Table 4.

Table 04: Effect of different extracts of *P. hydropiper* in Acetic Acid Induced Writhing test

Group	Doses (mg/kg)	No of Writhing	% of Inhibition
Control	10 ml/kg of 1% Tween 80	3.07±0.77	-
Std (Diclofenac)	100	2.17±0.90	29.32*
Methanol (ME)	100	2.10±0.88	31.6*
	150	2.10±0.36	31.6*
Ethanol (EE)	100	2.32±0.52	24.43*
	150	2.07±0.28	32.57*

ME=Methanol extract of *P. hydropiper*, EE= Ethanol extract of *P. hydropiper*. Number of writhing values are mean ± S.E.M., (n=6). *p< 0.05, significantly different from control which is done by independent t-test.

Methanol and Ethanol extracts inhibited writhes in a dose dependent manner. But ethanol extract at 150 mg/kg showed highest inhibition (32.57%) (p< 0.05) which is even higher than the standard drug (29.32%) (p< 0.05). Literature revealed that the acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids (Voilley, 2004). The constriction response of abdomen produced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics. It has been associated with prostanoids in general, for example, increased levels of PGE2 and PGF2a in peritoneal fluids as well as lipoxygenase or cyclooxygenases products (Ahmed *et al*., 2006) and acid sensing ion channels (Dhara *et al*., 2006).

Open Field test

After investigation with leaf extract of *P. hydropiper* data of open field test (movement), Open Field test (Centre), Open Field test (Standing) and Open Field test (Stool) are presented in Table 5, Table 6, Table 7 and Table 8 respectively.

Table 05: Effect of different extracts of *P. hydropiper* in Open Field test (Movement)

Group	Doses (mg/kg)	-30 min	+30 min	+60 min	+90 min	+120 min	+150 min
Control	-	16.33±11.57	25.17±5.05	31.17±10.59	34.17±19.77	37.8±15.65	23.8±11.74
Std (Clonazepam)	2	4.33±3.09	24.5±5.06	27±5.97	22.83±6.04	24.17±3.29	16.33±7.50
Methanol Extract	100	5.33±2.43	25±6.32	20.33±6.39	14.5±9.52	15±10.58*	21.67±9.84
	150	4±1.53	24.17±11.22	11.33±6.68*	9.67±4.57*	7.5±4.92*	12±11.14
Ethanol Extract	100	5.5±3.10	24.83±4.45	12.67±2.28*	14±6.30*	17.5±7.65*	17.33±8.63
	150	3.5±0.5	24.33±6.62	14.67±7.80*	17.83±13.68	21.67±8.44	13±6.67
Chloroform Extract	100	4.83±2.54	2.83±9.3	14.17±8.95*	16±11.15	10.33±5.44*	14.67±8.22
	150	3.67±1.25	22±9.29	15.83±9.26*	17.5±4.89	11.83±9.87*	18.67±8.10

Values are mean ±S.E.M. (n=6). *P<0.05, significantly different from control; done by independent sample t-test

Table 06: Effect of different extracts of *P. hydropiper* in Open Field test (Centre)

Group	Doses (mg/kg)	-30 min	+30 min	+60 min	+90 min	+120 min	+150 min
Control		1±0	0.17±0.37	0.5±1.6	0.33±0.99	0.5±1.6	0.5±1.05
Std(Clonazepam)	2	1±0	0.17±0.37	0.33±0.47	0.83±0.37	0.17±0.37	0.5±0.5
Methanol Extract	100	1±0	0±0	0.17±0.37	0.17±0.37	0.33±0.47	0.83±0.69
	150	1±0	0.17±0.37	0.33±0.47	0±0	0.33±0.47	0.17±0.37
Ethanol Extract	100	1±0	0±0	0.33±0.47	0.17±0.37	0.67±0.75	0.33±0.47
	150	1±0	0.17±0.37	0.33±0.75	0.33±0.75	0.5±0.76	0.17±0.37
Chloroform Extract	100	1±0	0±0	0.17±0.37	0.33±0.47	0.17±0.37	0.5±0.5
	150	1±0	0±0	0.33±0.75	0±0	0.5±0.76	0±0

Values are mean ± S.E.M., (n=6)

Table 07: Effect of different extracts of *P. hydropiper* in Open Field test (Standing)

Group	Doses (mg/kg)	-30 min	+30 min	+60 min	+90 min	+120 min	+150 min
Control		0.17±0.37	2.83±2.19	3.5±1.98	3.83±2.19	2.83±2.27	2.17±1.57
Std(Clonazepam)	2	0.17±0.37	6.83±1.46*	5±1	5.17±1.34	4.16±0.69	4.67±2.05*
Methanol Extract	100	0.5±0.7	6±1.77*	4.5±0.94	1±2.99	4±1.8	6±2.15*
	150	0.25±0.37	4.75±1.46	3±1.35	3±1.15	2±0.69	3±2.13
Ethanol Extract	100	0.66±0.74	4.5±0.96	2.83±0.69	3.16±1.06	4.16±1.77	4±1.83
	150	0.16±.37	5±0.81	3.33±1.24	3.17±0.69	4.66±0.94	2.83±0.90
Chloroform Extract	100	0.16±.37	4.16±1.34	2.66±1.24	4±1.63	2.66±1.11	4±1.73
	150	0.5±0.76	4±1.63	3.83±1.95	3.33±1.37	2.83±2.11	4.16±1.67

Values are mean ± S.E.M., (n=6). *P<0.05, significantly different from control; done by independent sample t-test

Table 08: Effect of different extracts of *P. hydropiper* in Open Field test (Stool)

Group	Doses (mg/kg)	-30 min	+30 min	+60 min	+90 min	+120 min	+150 min
Control		0.83±0.68	0±0	0±0	0±0	0±0	1.67±1.21
Std(Clonazepam)	2	0.83±0.68	0±0	0±0	0±0	0.17±0.37	0.17±0.37
Methanol Extract	100	1.33±1.11	0.17±0.37	0.33±0.47	0±0	0.5±0.76	0.17±0.37*
	150	0.5±0.76	0.33±0.47	0±0	0±0	0.33±0.47	0.33±0.47*
Ethanol Extract	100	0.66±0.47	0.33±0.47	0±0	0±0	0.33±0.47	0.33±0.47*
	150	1±1	0±0	0±0	0±0	0.17±0.37	0.17±0.37*
Chloroform Extract	100	1.12±0.98	0.17±0.37	0.33±0.47	0±0	0.17±0.37	0.17±0.37*
	150	1±1.14	0.17±0.37	0.17±0.37	0.33±0.47	0.67±1.49	0.17±0.37*

Values are mean ± S.E.M., (n=6). *P<0.05, significantly different from control; done by independent sample t-test

Methanol extract increased locomotor activity in a dose dependent manner. Chloroform extract (100mg/kg) exerts decreased movement of rodents in a dose dependent manner with slight significance and also ethanol extract (100 and 150 mg/kg) exerts significant result whereas Clonazepam decreased movement. Clonazepam

and extracts failed to exert any effect on center entrance in the open field. Clonazepam and extracts failed to exert any effect on standing in the open field.

The effect of higher dose of methanol (150mg/kg) and dose of ethanol (100 mg/kg) on defecation was similar to Clonazepam. Among all parameter only defecation showed Clonazepam like effect. So it is understood that the methanol (150mg/kg) and ethanol (100mg/kg) extract has not the ability to relieve stress and had an anxiolytic effect on the rodents like clonazepam did. But interesting effect of methanol and ethanol i.e. decreased defecation which was due to the physiological and environmental factor, or it might be chemical constitute present other than flavonoids and alkaloids in leaf extract of *P. hydropiper* (Voilley, 2004). Literature revealed that the originally proposed measuring aspects of rat behavior in a contained arena would indicate the emotional reactivity of the subjects. Many reports have validated open field tests as useful measures of emotional reactivity (Butterweck *et al*., 2000) for Turku aggressive mice. Others have not found differences in open-field activity despite differences in other anxiety measures (Blizard, 1981). Nevertheless, the open-field test remains a standard behavioral assay reported in the literature (Brown *et al*., 2009).The standard Open field test is commonly used to assess locomotor, exploratory and anxiety like behavior in laboratory animals (Crabbe *et al*., 1999).The open field test is designed to examine responses of mice or rats to a new and unfamiliar environment (novel environment).Rodents demonstrate anxiety, fear and curiosity when placed in a new environment (Walsh and Cummins, 1976). In response to the novel environment the rodents tend to explore the surrounding. The exploration capacity might be considered to be an index of anxiety although it is difficult to separate it from motor activity (Datusalia and Kalra, 2008). However, rodents are also afraid to go to the open and illuminated space which is also a sign of anxiety. So the novel environment induces anxiety and fear in rodents which is clearly demonstrated by their rearing, grooming, defecation, locomotor, and so on. These parameters are well utilized to assess anxiety and fear in rodents. Inhibition of such behaviors is indicative of centrally acting depressant or sedatives (Brown *et al*., 1992).

Swimming test
Test results for swimming test are given in Table 9.

Table 09: Effect of different extracts of *Polygonum hydropiper* in Swimming test

Group	Doses (mg/kg)	Duration of Immobility (s)
Control	-	34.14 ± 3.15
Std (Imipramine)	10	38.25 ± 0.74
Methanol Extract	100	37.70 ± 0.45
	150	39.83 ± 1.02*
Ethanol Extract	100	43.33 ± 0.70
	150	39.56 ± 0.52
Chloroform Extract	100	35.14 ± 0.30*
	150	37.06 ± 0.61

Values are mean ±S.E.M. (n=6). *P<0.05, significantly different from control; done by independent sample t-test

The higher dose of methanol extract (150mg/kg) and lower dose of chloroform (100mg/kg) (P<0.05) exert Immobile phase like Imipramine (10 mg/kg). It indicates the Anti-depressant like effect of these extracts.

Literature revealed that the FST was designed by Porsolt *et al.*, 1977 as a primary screening test or antidepressants. It is still one of the best models for this procedure. This is a low-cost, fast and reliable model to test potential antidepressant treatments with a strong predictive validity. However, the low face and construct validities should not forbid the use of this model for neurophysiological studies. It has a great sensitivity with all the antidepressant classes and all the mechanisms of action of treatments could be determined, but clinical correlations should be considered very carefully. When rodents are forced to swim in a confined place, they tend to become immobile after vigorous activity (struggling). This stressful inescapable situation can be evaluated by assessing different behavioral strategies and immobility during the test could be an efficient adaptive response to

the stress (Wang and Jiao, 2000). The development of immobility when the rodents are placed in an inescapable container of water reflects the cessation of persistent escape directed behavior. The CNS depressant effect of the extracts may be attributed to chemical constitute other than flavonoids and alkaloids because flavonoids are responsible for the decrease in immobile phase in the swim test (Butterweck *et al*., 2000) and so does alkaloid (Silva *et al*., 2005).

Conclusion

In this study, all five leaf extracts of *P. hydropiper* had potent antioxidant effects. Almost five extracts showed antioxidant activity but methanol, ethanol and pet-ether extracts showed highest antioxidant activity. Pharmacological studies with leaf extracts of *P. hydropiper* for analgesic activity and neuropharmacological investigation also showed significant results. Our current study is suggestive to future works on *P. hydropiper* with a consideration of compound isolation for particular activity and develop lead compound for therapeutic use.

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