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Research Article

EXPLORATION OF IN VITRO ANTIOXIDANT, THROMBOLYTIC ACTIVITY, NEUROPHARMACOLOGICAL AND ANTI-PYRETIC ACTIVITY OF LEAF EXTRACTS OF HOYA PARASITICA (WALL.)

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A B S T R A C T

The objective of the present study was to investigate the in vitro phytochemical screenings, thrombolytic, antioxidant, *in vivo* neuropharmacological and anti-pyretic activity of leaf extracts of *Hoya parasitica* Wall. Preliminary phytochemical screening was done for determining the nature of phytoconstituents or bioactive constitutes, which were assessed for their possible clot lysis, antioxidant, neuropharmacological and anti-pyretic activities as compared with the known drugs. Different classes of phytochemicals were present in the leaf extract. The methanol extract exhibited highest percentage of clot lysis (14.67%) as compared to 28.26% clot lysis produced by standard streptokinase. Among the three extracts, methanol and ethanol extracts showed relatively better cupric reducing antioxidant capacity with IC_{50} value (61.15 µg/ml) and satisfactory antioxidant potential in hydrogen peroxide scavenging assay with IC_{50} value (61.48 µg/ml) in comparison with L-ascorbic acid. Chloroform extract of *H. parasitica* was found to possess the highest total antioxidant capacity (1.86 mg/gm). Ethanol and chloroform extracts (100 and 200 mg/kg body weight) and methanol extracts (200mg/kg body weight) shortened the immobility period significantly (**p<0.01; ***p<0.001) in comparison with standard. In open field teat, ethanol extracts (100 & 200 mg/kg body weight) significantly (**P<0.01) decreased the rate of movement with time in a dose dependent manner. Significant (*p<0.05) antipyretic activity was observed by methanol and chloroform extracts in a dose dependent manner compared to standard. It can be concluded from the plant extracts of *H. parasitica* have significant thrombolytic, antioxidant, neuropharmacological and antipyretic activity which justifies its use as traditional medicine.

INTRODUCTION

Hoya parasitica Wall is a climbing epiphyte of the family Asclepiadaceae is one of 300 species in the genus Hoya locally known as Chera pata, Pargacha, Fassya gaas (Rahman *et al*., 2007) in Bangladesh. It is an evergreen tropical perennial shrub native to tropical wet forests and humid climate of southern Asia, Singapore, the Andaman Island (Khatun *et al*., 2014) Australia, Polynesia (Reza *et al*., 2007), Bangladesh (Sadhu *et al*., 2008). It is a parasite creeper with a fragrant flower. They are evergreen perennial vines or rarely shrubs. Its stems are stout or slender, glabrous. Its leaves are ovate elliptic or lanceolateactue or acuminate penduncles are solitary or in paris short or long slender or stout, pedicelsslender long glabourous, coronal processes longer than the corolla tube. The plant bears aesthetic flowers in May to June. Mukherjee *et al.,* (1986) reported the plant to contain triterpenic 3,4-seco acid 3,4-secolup-20(29)-en-3-oic acid, along with lupeol and lupenone from stem and Sadhu *et al.,* (2008) reported to contain an androstanoid, a sesquiterpene, and a phenolic compound, together with a known triterpene, dihydrocanaric acid. According to Khatun *et al.,* (2014) the steam extracts of *H. parasitica* was found to contain Reducing sugar, alkaloid, steroid, tannin, flavonoid, saponin.This plant has also been used in traditional medicine for the treatment of constipation (Hossan *et al*., 2009), bronchitis, diabetes, urinary tract disorders, frequent or infrequent urination, kidney disorders, bleeding, paralysis, rheumatic pain (Hanif *et al*., 2009; Biswas *et al*., 2010) fever, body pain (Khisha *et al*., 2012; Rahman *et al*., 2007), jaundice (Rahman *et al*., 2007), antirheumatic and in acute renal failure (Ahmed, 1997). *H. parasitica* has antibacterial, antinociceptive, cytotoxicity, activity and growth inhibitory effects of dihydrocanaric acid against both HeLa and SW480 cells (Ahmed *et al*., 2008;

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Khatun *et al*., 2014; Reza *et al*., 2007; Mukherjee *et al*., 1986). The leave extracts of *H. parasitica* demonstrated to have *in vitro* antioxidant activity, membrane stabilizing activity and *in vivo* gastrointestinal motility with no potential acute toxicity (Tania *et al*., 2016). As a part of our continuing studies on *H. parasitica*, the organic soluble materials of the leaf extracts of *H. parasitica* were evaluated for Phytochemical screening, thrombolytic, antioxidant, neuropharmacological and antipyretic activity.

MATERIALS AND METHODS

Collection, Identification and Processing of Plant Samples

The leaves of *H. parasitica* were collected from Sylhet, Bangladesh and then plant sample was submitted to the National Herbarium of Bangladesh, Mirpur-1, Dhaka for its identification and the voucher specimen is DACB- 41159. Leaves were sun dried for seven days in order to remove the moisture contents and then ground into coarse powder using high capacity grinding machine (Jaipan designer mixer grinder, jaipan, India) which was then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

Extraction Procedure

The powdered plant parts (30 gm) were successively extracted in a soxhlet extractor at elevated temperature using 500 ml of distilled methanol (40-60) ºC which was followed by ethanol, and chloroform. After drying all extracts were labeled and kept in refrigerator at 4°C for future investigation.

Preliminary Phytochemical Screening

Different extracts of *H. parasitica* were subjected to preliminary phytochemical screenings for determining nature of phytoconstituents by using standard protocols (Tiwari *et al*., 2016).

Streptokinase (SK)

Commercially available lyophilized alteplase (Streptokinase) vial (Popular pharmaceutical Ltd.) of 15, 00,000 I.U, was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for in vitro thrombolytic activity evaluation.

Blood Sample

Blood (n=6) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 1ml of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

Thrombolytic Activity

The thrombolytic activity of all extracts was evaluated by the method developed by Prasad *et al*., (2006) and slightly modified by Sharif *et al*., (2014) using streptokinase (SK) as the standard.

Antioxidant Activity

Cupric reducing antioxidant capacity

The Cupric Reducing Antioxidant Capacity of all extracts was conducted as described previously by Shahriar *et al*., (2015).

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of all extracts was conducted as described previously by Bakhtiar *et al.*, (2015).

Determination of total antioxidant capacity

The total antioxidant capacity was evaluated by the phosphomolybdenum method (Jayaprakasha *et al*., 2004). 0.3ml of extract and sub-fraction in methanol, ascorbic acid used as standard (12.5-200µg/ml) and blank (methanol) were combined with 3ml of reagent mixture separately and incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance of each sample was measured at 695nm against the blank. 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.

Experimental Animal

For the experiment Swiss albino mice of either sex, 4-5 weeks of age, weighing between 20-30 gm were collected from ICDDR, B, Mohakhali, Dhaka. Animals were maintained under standard environmental conditions [temperature: (27.0±1.0) ºC, relative humidity: (55-65)% and 12 hour light/12 hour dark cycle] and free access to feed and water. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

Neuropharmacological Study

To check the neuropharmacological effects or side-effects of drug, two types of experiment is carried out which are forced swimming test and open field test.

Forced swimming test

According to Porsolt *et al.,* (1977) swimming test was performed with slight modification by Khandaker *et al*., (2016).

Open field test

According to previous work with slight modification open field test was performed to monitor behavioral responses in mice that were placed in a novel and bright arena (Gupta *et al*., 1971). 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 5 mice each. The first group was given 10ml/kg of 1% Tween 80 orally and served as control. Group 2 was served 2 mg of Clonazepam per kg of body weight and it served as standard. Groups 3, 4 received methanol extracst of the leaves of *H. parasitica* at 200 and 400 mg/kg of body weight, and groups 5, 6 received ethanol extracts of the leaves of *H. parasitica* at 200 and 400 mg/kg of body weight. Group 7, 8 received chloroform extracts of the leaves of *H. parasitica* at 200 and 400 mg/kg of body weight. 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 2min at 0, 30, 60, 90 and 120min subsequent to oral administration of the experimental crude extracts.

Antipyretic Activity

Forty two Swiss Albino mice of both sexes (20-30) gm were randomly divided into 7 groups and fasted overnight before the experiment with free access to water. The normal body temperature of each mouse was measured rectally at predetermined intervals and recorded. Fever was induced according to the method described by Hambourger and Smith, (1935). A lubricated thermometer probe was inserted 3-4 cm deep into the rectum and fastened to the tail by adhesive tape. Temperature was measured on digital thermometer. After measuring the basal rectal temperature, animals were injected subcutaneously with 10 ml/kg body weight of 20% w/v brewer's yeast in NSS in the dorsum of the mice. Mice were then returned to their housing cages. Eighteen hours after brewer's yeast injection, the animals were again restrained for rectal temperature recording, as described previously. Only mice that showed an increase in temperature of at least 0.5˚ to 1˚C were used for this study. The extracts at the doses of 100 & 200 mg/kg body weight were administered orally to four groups of animals. The control group received 10ml/kg body weight dose of vehicle (0.9% NaCl solution) and the standard group received paracetamol (50 mg/kg body weight) orally. Rectal temperature was measured at 1 hr intervals for 4 hr after the extract/drug administration (Opo *et al*., 2016). The rectal temperature of normal mice (normothermic) was also measured at 1 hr. intervals for 7 hr. as stated by Chomchuen *et al*., (2010). The results are expressed as percentage of the pre-drug temperature recorded for the same animals using the formula of Makonnan *et al*., (2003).

Statistical Analysis

Data was expressed as Mean ± Standard deviation. IC50 values for antioxidant activities by the extracts were calculated from the dose - response curve by using Microsoft Excel 2010. The results were analyzed statistically by ANOVA followed by Dunnet's test. Results below *p<0.05, **p<0.01 and ***p<0.001 are considered statistically significant.

RESULTS

Phytochemical Screening

The leaf of *H. parasitica* showed either presence or absence of different phytochemicals. The results are listed below in the Table 1.

Table 1 Analysis of phytochemical constituents of various leave extracts of *H. parasitica*

Phytochemicals	Name of Extracts		
	Methanol	Ethanol	Chloroform
Alkaloids	+	+	+
Carbohydrates	+	+	+
Saponins	+	+	-
Glycosides	+	+	+
Flavonoids	+	+	+
Phenols	+	+	-
Tannins	+	+	+
Steroids	+	+	+

(+) Presence & (-) Absent

Thrombolytic Activity

The extractives of *Hoya parasitica* were assessed for thrombolytic activity and the results are presented in Table 2. Addition of 100 µl Streptokinase (30,000 I.U.) to the clots along with 90 minutes of incubation at 37°C, showed 28.26% clot lysis. After treatment of clots with 100 µl methanol, ethanol, chloroform extract of *H. parasitica*, clot lysis 14.67%, 11.65%, 13.12% was obtained respectively.

Table 2 % Clot lysis by differentl extracts of *H. parasitica* and standard

Samples	% of RBC lysis
Methanol	14.67±1.75
Ethanol	11.65±0.92
Chloroform	13.12±1.48
Control	8.72±1.75
Streptokinase	28.26±1.81

(Values are expressed as mean ± S.D)

Antioxidant Activity

Cupric reducing antioxidant capacity

All the methanol, ethanol and chloroform extracts showed dose dependant reducing capacity. Among the three extracts of *H. parasitica,* methanol (61.15 µg/ml) and ethanol (55.57 µg/ml) extracts showed relatively better cupric reducing antioxidant capacity compared to the standard L-ascorbic acid (Table 3).

Table 3 IC_{50} Values of different extracts of leaves of *H. parasitica* in cupric reducing antioxidant capacity

Samples	Inhibition
L-Ascorbic Acid	48.09
Methanol	61.15
Ethanol	55.57
Chloroform	46.30

Hydrogen peroxide (H_2O_2) scavenging activity

Scavenging of hydrogen peroxide of different leave extracts of *H. parasitica* is presented in Table 4. The standard, L-ascorbic acid showed 91.43% inhibition. Among the three extracts, ethanol and chloroform extracts showed highest inhibition 87.25% and 82.34% respectively, whereas the methanol extract showed significant result (77.88% inhibition).

Table 4 IC_{50} Values of different extracts of leaves of *H. parasitica* in H_2O_2 scavenging assay

Samples	IC_{50} Value (µg/ml)	% Inhibition
L-Ascorbic Acid	79.94	91.43%
Methanol	56.01	77.88%
Ethanol	61.48	87.25%
Chloroform	52.67	42.34%

Total antioxidant capacity

The crude methanol, ethanol and chloroform extracts of *H. parasitica*, were subjected to assay for total antioxidant capacity following standard protocol and the obtained results were represented in Table 5. Among all extracts, chloroform extract of *H. parasitica* leaves was found to possess the highest total antioxidant capacity (1.86 mg/gm) followed by ethanol (1.77 mg/gm) and methanol extracts (1.67 mg/gm).

Table 5 Total Antioxidant Capacity of different extracts of leaves of *H. parasitica*

Samples	Total Antioxidant Capacity (mg/gm, Ascorbic Acid Equivalent)
Methanol	1.67±0.06
Ethanol	1.77±0.07
Chloroform	1.86±0.08

(Values are expressed as mean ± S.D)

Neuropharmacological Study

Forced swimming test

Forced swimming test was performed to evaluate the effect of anti-depressant effect of leaf extracts of *H. parasitica* on mice. After investigation of leave extracts of *H. parasitica,* following data were observed (Table 6). During the test ethanol and chloroform extracts at doses of 100 and 200 mg/kg body weight and methanol extract of 200mg/kg body weight shortened the immobility period significantly (**p<0.01; ***p<0.001) in comparison with standard and exhibited a dose dependent antidepressant activity.

Table 6 Effect of different extracts of leaves of *H. parasitica* in forced swimming test

Group	Duration of immobility (sec)
Control	116.33±3.29
Clonazepam	88.17±9.29
Methanol 100 mg/kg	71.17±4.38
Methanol 200 mg/kg	44.17±2.45**
Ethanol 100 mg/kg	40.66±2.92**
Ethanol 200 mg/kg	28.66±1.76**
Chloroform 100 mg/kg	31.00±1.15**
Chloroform 200 mg/kg	21.33±1.83***

Values are expressed as mean ± S.D. (n=6), *p<0.05; **P<0.01; ***P<0.001 significant when compared with the corresponding value of control

Open field test

The crude methanol, ethanol and chloroform extracts of *H. parasitica*, were subjected to assay for open field test following standard protocol and the obtained results were represented in Table 6 and Table 7. This experiment was performed to assay general locomotor activity levels. In the present study it was observed that both the doses of ethanol extracts of 100 & 200 mg/kg body weight significantly (**P<0.01) decreased the rate of movement with time in a dose dependent manner when compared with corresponding value of standard (Table 7). These extracts also decreased the frequency of standing, entrance into center and stool count at the same time (Table 8). So it can be said that ethanol extract of *H. parasitica* have the ability to relieve stress and had an anxiolytic effect on the rodents.

Anti-pyretic Activity

From the Table 9, the present study revealed that methanol extracts (100 and 200 mg/kg) and chloroform extracts of 200 mg/kg body weight showed significant level (*p<0.05) of lowering pyrexia from elevated level, compared to standard drug Paracetamol.

DISCUSSION

Natural antioxidants, particularly those present in fruits and vegetables, spices and medicinal herbs have attracted increasing interest among consumers and the scientific community as they protect against various diseases for centuries (Halliewll, 1996; Shi *et al*., 2010; Vinson *et al*., 1998; Ganthavorn and Hughes, 1997; Zheng and Wang, 2001; Stoilova *et al*., 2007). The occurrences of different secondary metabolites are mainly responsible for wide range of biological application of plants (Laboni *et al*., 2017). In the present study *Hoya parasitica* was found to contain alkaloids, carbohydrates, saponins, glycosides, flavonoids, phenols, tannins and steroids. This was similar to the study conducted previously by Reza *et al.,*(2007). The chloroform extracts found to contain no saponins and phenols.

Maximum percentage clot lysis was observed when clots treated with fibrinolytic enzyme streptokinase, which was used as reference. Clots when treated with 100 µl sterile distilled water (control) showed only negligible clot lysis (8.72 %). The mean difference in percentage of clot lysis between standard & control was found to be statistically significant. The comparison of standard with control clearly demonstrated that clot dissolution does not occur when water was added to the clot. In this study, the methanol extract of *H. parasitica* revealed highest thrombolytic activity 14.67% compared to the extracts of ethanol and chloroform.

The method of cupric reducing antioxidant capacity is based on the principle of increase in the absorbance of reaction mixtures, because increase in absorbance indicates the increase in antioxidant capacity, thus increase in reducing power of the sample (Jayaprakasha and Rao, 2000). Compounds with reducing power indicate that they are electron donor and can reduce the oxidized intermediates, thus they can act as primary and secondary antioxidants. Cupric reducing antioxidant capacity was highest in methanol extracts (61.15µg/ml) and lowest in chloroform extracts (46.30µg/ml).

Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. The generation of even low

Table 7 Effect of Different Extracts of *H. parasitica* in open field test (Movement)

	Movement				
Groups	-30 mins	+30 mins	+60 mins	+90 mins	+120 mins
Control (1% Tween 80)	35±4.01	39±3.22	34.16±8.84	34.16±4.75	21.66±3.98
Clonazepam	34.4±3.75	27±2.43	25.83±2.46	24.16±1.34	16.33±3.06
Methanol Extract 100 mg/kg	60±4.08	49.33±3.00	45.66±2.98	40.16±2.24	36.5±2.04
Methanol Extract 200 mg/kg	52.5±2.04	55.33±3.63	49±5.20	46.83±3.12	32±2.96
Ethanol Extract 100 mg/kg	47.33±3.35**	55±4.40	39.5±1.76**	22.83±1.74	17.33±3.04**
Ethanol Extract 200 mg/kg	43.16±2.94**	43.16±5.38	39.33±1.76**	24.16±4.30**	14.16±2.60**
Chloroform Extract 100 mg/kg	44.33±4.46	39.5±7.62	33.16±3.16	28±3.66	19.66±3.13
Chloroform Extract 100 mg/kg	46.83±2.83	44.16±6.10	34.16±6.12	28.83±3.71	20.33±4.30

Values are expressed as mean ± S.D. (n=6), *p<0.05; **P<0.01; ***P<0.001 significant when compared with the corresponding value of control

Table 8 Effect of Different Extracts of *H. parasitica* in open field test (Standing, entrance into center and stool count)

Standing					
Groups	**-30 mins**	**+30 mins**	**+60 mins**	**+90 mins**	**+120 mins**
Control (1% Tween 80)	0.66±0.51	3±1.75	3.16±2.04	2.16±2.13	2±1.41
Clonazepam	0.83±0.68	5±1.03	5.16±1,64	4.16±0.68	4.66±2.04
Methanol Extract 100 mg/kg	0.83±0.68	5.33±1.04	4.16±1.72	4.5±1.76	4±2.36
Methanol Extract 200 mg/kg	0.16±0.37	3.83±1.74	3.5±1.47	4.3±1.37	4.66±0.81
Ethanol Extract 100 mg/kg	0.66±0.74	3.16±2.40*	3.33±1.63	4.16±1.34	3.83±1.72*
Ethanol Extract 200 mg/kg	0.5±0.83*	3.5±1.87	3.83±2.13	3.33±1.36*	3.5±1.37
Chloroform Extract 100 mg/kg	0.66±0.81	3±0.89	3.16±1.32	2.5±1.04	2.16±1.60
Chloroform Extract 100 mg/kg	0.5±0.54	2.5±1.04	2.83±1.32	1.66±1.10	3.2±2.71
Entrance into center					
Groups	**-30 mins**	**+30 mins**	**+60 mins**	**+90 mins**	**+120 mins**
Control (1% Tween 80)	1±0	0.66±0.81	0.66±0.52	0.5±0.54	0.5±0.54
Clonazepam	1±0	0.83±0.37	0.83±0.57	0.16±0.37	0.5±0.54
Methanol Extract 100 mg/kg	0±0	0±0	0.16±0.40	0.33±0.51	0.83±0.75
Methanol Extract 200 mg/kg	1±0	0.66±0.84	1±0.63	0.5±0.54	0.83±0.47
Ethanol Extract 100 mg/kg	1±0	0.33±0.47	0.66±0.81	0.5±0.54	0.16±0.83
Ethanol Extract 200 mg/kg	1±0	0.5±0.83	0.33±0.74	0.5±0.54	0.0±0.0
Chloroform Extract 100 mg/kg	0±0	0.33±0.47	0±0	0.5±0.54	0.5±0.47
Chloroform Extract 100 mg/kg	0±0	0±0	0±0	0±0	0.83±0.75
Stool count					
Groups	**-30 mins**	**+30 mins**	**+60 mins**	**+90 mins**	**+120 mins**
Control (1% Tween 80)	0.83±0.68	0±0	0±0	0.51±0.83	0.83±0.98
Clonazepam	0±0	0±0	0±0	0.16±0.37	0.16±0.37
Methanol Extract 100 mg/kg	0.83±0.68	0.5±0.54	0.16±0.37	0±0	0.16±0.40
Methanol Extract 200 mg/kg	0.83±0.68	0.16±0.37	0.5±0.76	0.66±0.81	0.16±0.37
Ethanol Extract 100 mg/kg	0.5±0.54	0.33±0.47	0.33±0.51	0.33±0.81	0±0
Ethanol Extract 200 mg/kg	0.5±0.54	0.66±0.51	0.16±0.37	0±0	0±0
Chloroform Extract 100 mg/kg	1.33±1.37*	0.5±0.76*	0.16±0.37*	0±0*	0.16±0.37*
Chloroform Extract 100 mg/kg	0.66±0.47	0±0	0±0	0±0	0.33±0.74

Values are expressed as mean ± S.D. (n=6), *p<0.05; **P<0.01; ***P<0.001 significant when compared with the corresponding value of control

Table 9 Effect of Different Extracts of *H. parasitica* anti-pyretic activity

Group	Brewer's yeast induced temperature	Temperature after doses (^{0}F)		
		1 hour	2 hour	3 hour
Control	98.45±0.89	94.43±0.65	95.12±0.45	95.67±1.55
Paracetamol 50 mg/kg	97.82±0.61	94.25±1.31	95.38±1.58	94.53±1.15
Methanol 100 mg/kg	98.91±0.64*	94.10±0.81	94.60±0.51*	93.83±0.77*
Methanol 200 mg/kg	98.12±0.90*	94.08±0.89*	94.83±0.76	94.73±0.52*
Ethanol 100 mg/kg	97.28±0.30	93.60±0.92	94.37±0.75	95.83±0.24
Ethanol 200 mg/kg	97.25±0.57	93.22±0.88	93.14±0.74	92.08±0.24
Chloroform 100 mg/kg	97.75±0.52	94.40±1.45	95.85±1.03	95.10±0.93
Chloroform 200 mg/kg	97.50±0.69	95.18±0.95*	96.44±0.82	94.30±1.56*

Values are expressed as mean ± S.D. (n=6), *p<0.05; **P<0.01; ***P<0.001 significant when compared with the corresponding value of control

levels of H_2O_2 in biological systems may be important. Naturally occurring iron complexes inside the cell believed to react with H_2O_2 *in vivo* to generate highly reactive hydroxyl radicals and this may be the origin of many of its toxic effects. Methanol, ethanol and chloroform extracts showed noticeable radical scavenging activity with IC_{50} values 56.01, 61.48 and 52.67 µg/ ml respectively whereas the standard showed IC_{50} value of 79.94µg/ml (Table 4).

Total antioxidant capacity of the different extracts of *H. parasitica* was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents per gram of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid ($y = 1.025x - 0.961$; $R^2 = 0.878$). The total antioxidant capacity was highest in chloroform extracts 1.86 mg/gm and lowest in methanol extracts 1.67 mg/gm. Presence of total phenol content and flavonoid in the plant extracts may be a reason for this activity (Hossain *et al*., 2016). Ethannol and chloroform extracts showed decreased immobility with dose dependently like the standard clonazepam which indicates its antidepressant effect. The CNS depressant effect of the extracts may be responsible for chemical constitutes, as flavonoids are responsible for the decrease in immobile phase in the swim test and so does alkaloids as well which were also observed in the present study. The extract significantly decreased the locomotor activity as shown by the results of the open field. This activity is a measure of the level of excitability of the CNS and this decrease may be losely related to sedation resulting from depression of the central nervous system. Ethanol and chloroform extracts decreased movement of rodents in a dose dependent manner as well as decreased standing and entrance in the center of the open field significantly. The effect of both doses of methanol and chloroform on defecation was like clonazepam. Extracts of ethanol and chloroform were capable of lowering pyrexia from elevated level, compared to standard drug paracetamol, so it can be said that more of active principles responsible for the antipyretic activity might be available in these three extracts.

CONCLUSION

All the conducted experiments in the present study are based on crude extract and are considered to be preliminary and more sophisticate research is necessary to reach a concrete

conclusion about the findings of the present study. It can be concluded from the above findings, that the plant *H. parasitica* have significant thrombolytic, antioxidant, antidepressant and antipyretic activity. The plant has strong antioxidant activity. So, further scientific studies are necessary to elucidate detailed mechanism of action and isolate the responsible active principles.

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