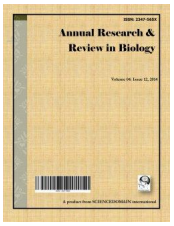

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Pharmacological Activities of *Youngia japonica* Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Authr MSM designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author MHK managed the literature searches. Author IJB guided about protocols of thesis writing. Author MLN performed the statistical analysis and Author MAM managed the analyses of the study. Author IH conducted the research activities. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: *Youngia japonica* (YJA), belonging to the family asteraceae, exhibits strong antiallergic, antioxidant and antitumor activities. The present study was carried out to assess the antioxidant potentials, analgesic, anti-inflammatory and CNS depressant activities of different fractions of YJA plant extracts.
Study Design: For the purpose of this experiment the different plant extracts were subjected for an *in-vitro and in-vivo* study.
Place and Duration of Study: The study was carried out on March 2015 in the Department of Pharmacy, Southeast University, Dhaka, Bangladesh.
Methods: The antioxidant capacity of ethyl acetate (EA), pet ether (PET) and chloroform(CLF)

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extracts of YJA were investigated for free radical scavenging activity using DPPH and hydroxyl radical scavenging assay. Total antioxidant activity and total phenolic content of different extracts were determined spectroscopically. Analgesic activity was evaluated by using acetic acid induced writhing, formalin test and eddy's hot plate method. Extracts of YJA were also investigated for anti-inflammatory activity using carrageenan induced hind paw edema model. The CNS depressant activity was evaluated by hole cross test.

Results: In DPPH scavenging assay, CLF exhibited the highest DPPH scavenging activity (IC_{50} 9.70 µg/ml). In case of hydroxyl radical scavenging assay, EA extracts showed the most significant activity (IC_{50} 15.09 µg/ml). This result was in line up with the total phenolic content where EA extracts possessed the highest amount of it (43.92 mg of GAE / gm. of dried extract). Moreover, the highest total antioxidant activity was also found in EA fraction (109.30 GAE/gm of dried sample) that rationalizes the previous outcome. All fractions significantly ($p<0.01$) reduced the writhing and the number of licking in a dose dependent manner (100 and 200 mg/kg). The Extracts also showed significant ($p<0.001$) inhibition of carrageenan induced paw edema. A statistically significant ($p<0.001$) decrease in locomotor activity was also observed.

Conclusion: The result demonstrates that the YJA has appreciable antioxidant, analgesic, anti-inflammatory and CNS depressant activities.

Keywords: Youngia japonica; antioxidant; analgesic; anti-inflammatory; CNS activity.

1. INTRODUCTION

Youngia japonica (Commonly known as oriental false hawksbeard) belonging to the family of asteraceae, is a weed species distributed worldwide especially temperate zones. YJA is used as a Chinese traditional medicine (native plant) and generally it is used for reducing pyrexia, detoxification and atopy. It contains guaiane-type sesquiterpene and other constituents that show strong antiallergic, antioxidant, and antitumor activities [1]. The plant is antitussive and febrifuge. It is also used in the treatment of boils and snakebites [2]. Taraxasteryl acetate, n-docosanol, β-sitosterol and stigmasterol, retinol, β-daucosterol, docosanoic acid, apigenin compounds are found in this plant [3]. Eleven sesquiterpenoids have been isolated from the whole plants of YJA [4]. Recently, researchers have tried to separate nontoxic antioxidants from edible plants to avoid autoxidation and lipid peroxidation with the try to find to replace synthetic antioxidants [5]. Plant extracts containing high amounts of bioactive compounds especially antioxidants, have the prospective of being used in food, agriculture, nutraceuticals, cosmetics and pharmaceutical products [6]. So far researcher's knowledge, previously no reports have been found on analgesic, anti-inflammatory and CNS depressant activities of this plant.

Present study was aimed to investigate the antioxidant properties along with total phenolic contents, analgesic, anti-inflammatory and CNS depressant activities of different fractions based on polarities of YJA whole plant.

2. METHODS

2.1 Plant Materials

The whole plants of YJA were collected from the adjoining area Dhaka district, Bangladesh during March 2015. The plant material was taxonomically identified by the National Herbarium of Bangladesh and a voucher specimen number DACB-41233 was deposited in that institution for future references.

2.2 Preparation of Plant Extracts

The whole plant materials were collected and washed thoroughly with distilled water and shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve size 40, and stored in a tight container. The total dried powder material was obtained 600 gm. It was divided into three portions equally and each was refluxed with ethyl acetate (EA), pet ether (PET) and chloroform (CLF) solvent for thrice. The extracts were filtered and concentrated by means of a rotary evaporator. The dried extracts were stored at room temperature until further use.

2.3 Determination of Antioxidant Activity of Different Extracts of YJA Plant

2.3.1 DPPH free radical scavenging assay

The antioxidant potential of the extracts was determined on the basis of its scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The DPPH assay was

carried out as per the procedure outlined by Fresin [7]. 0.1 ml of different fraction of each extracts, at various concentrations was added to 3 ml of a 0.004% methanol solution of DPPH and was allowed to stand for 30 min for the reaction occur. Thirty minutes later, the absorbance was measured at 517 nm using spectrophotometer (Shimadzu uv-1800, Japan). The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation: % inhibition= $[(A_B–A_S) / A_B] \times 100$.

Where, A_B is the absorbance of the control reaction (containing all reagents except the test compound and A_S is the absorbance of the test compound. Ascorbic acid was used as positive control. The extracts concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against extract concentration.

2.3.2 Hydroxyl radical scavenging method

Hydroxyl radical scavenging activity was measured by the ability of the extract to scavenge the hydroxyl radicals generated by the Fe^{3+}ascorbate-EDTA-H_2O_2 system (Fenton reaction) [8]. 1 ml of reaction mixture of different extracts were made by adding 100 µl of 2-deoxy-D-ribose ((28 mM in 20 mM KH_2PO_4 buffer, pH 7.4), 500 µl of the fractions at various concentrations (6.5-100 µg/ml) in buffer, 200 µl of 1.04 mM EDTA and 200 µM $FeCl_3$ (1:1v/v), 100 µl of 1.0 mM hydrogen peroxide (H_2O_2) and 100 µl of 1.0 mM ascorbic acid. After incubation for 1 h at 37°C, 1 ml of 1% thiobarbituric acid (TBA) and 1 ml of 2.8% Trichloroacetic acid (TCA) were added to the test tubes and were incubated at 100°C for 20 min. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution containing deoxyribose and buffer. Catechin was used as a positive control. The percentage (%) inhibition activity was calculated from the following equation $\% I = \{(A_o – A_1)/A_o\} X 100$, Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the test sample

2.3.3 Determination of total antioxidant capacity

Total antioxidant capacity of different extracts of *Youngia japonica* was determined by the method of Prieto et al. [9]. An aliquot of 0.3 ml of different fractions of sample solution were combined in a test tube with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The test tubes were incubated at 95°C for 90 minutes. After the sample had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank.

2.3.4 Determination of Total phenolic content

Total phenolic content was determined using Folin-Ciocalteu reagent described by Singleton et al. [10]. A dilute extract of each fraction of plant extracts (o.5ml) or gallic acid (standared phenolic compound) was mixed with 2.5 ml of Folin-Ciocalteu reagent (1: 10 diluted with distilled water) and 7.5% of 2.5 ml sodium carbonate. The mixture was allowed to stand for 20 min and the total phenolic content were determined by spectrophotometer at a wavelength of 760 nm. The standard curve was prepared using 6.25, 12.5, 25, 50, 100, 200 µg/ml solution of gallic acid. Total phenolic contents are expressed in terms of gallic acid equivalent (mg/ g) which is a common standard. The total content of phenolic compounds in plant extracts of different fractionates in gallic acid equivalents (GAE) was calculated by the following formula

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

Where,

C = total content of phenolic compounds, mg/g plant extract, in GAE;
c = the concentration of gallic acid established from the calibration curve, mg/ml;
V = the volume of extract, ml;
m = the weight of different pure plant extracts, gm.

2.4 *In Vivo* Analgesic Activity

2.4.1 Albino mice

Swiss albino mice (25-30 g) were used for assessing biological activity. These were maintained under standard laboratory conditions and had free access to food and water *ad libitum.* The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting of five animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Southeast university, Dhaka, Bangladesh.

2.4.2 Acetic acid-induced writhing test

Acetic acid-induced writhing model in mice was used to observe the analgesic activity [11]. In this method, acetic acid is administered intra-peritoneally to the experimental animals to create pain sensation. As a positive control, Diclofenac sodium 10 mg/kg body weight was used. The plant extract was administered orally in two different doses (100 and 200 mg/kg body weight) to the Swiss Albino mice. The animals were divided into eight groups with five mice in each group. Group I animals received vehicle (Saline water), animals of Group II received Diclofenac-Na at 10 mg/kg body weight while animals of Group III, IV ,V, VI,VII,VIII were treated with 100 and 200 mg/kg body weight (p.o.) of EA,CLF and PET extracts of YJA respectively. Test samples and vehicle were administered orally 30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1 ml/10 g) but Diclofenac sodium was administered 15 minutes prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse of all groups was observed individually for counting the number of writhing they made in 30 minutes beginning just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while Diclofenac sodium (10 mg/kg) was used as a reference substance (positive control).

> The percent inhibition (% analgesic activity) was calculated by the equation {(A-B) /A} × 100

Where, A= Average number of writhing of the control group; B= Average number of writhing of the test group.

2.4.3 Formalin test

The antinociceptive activity of the YJA was determined using the formalin test described by Achinta et al. [12]. The animals were also divided into eight groups with five mice in each group. Group I that is Control group animals received vehicle (Saline water), animals of Group II received Diclofenac-Na at 10 mg/kg body weight while animals of Group III, IV ,V, VI,VII,VIII were treated with 100 and 200 mg/kg body weight (p.o.) of EA, CLF and PET extracts of YJA respectively. Twenty micro liters (20 µl) of 1% formalin was injected intradermally on the plantar surface of the hind paw of each mouse one hour after administration of the test extract and also the controls. The time in seconds spent in paw licking as an index of painful response was determined at 0 – 10 min (Early) and 15– 30 min (late phase) after formalin injection. This represent, neurogenic and inflammatory responses, respectively. The total time spent licking or biting the injured paw (pain behavior) was measured with a stop watch. The data was presented as Mean ± S.E.M of time(s) spent in pain behaviour. The mean of time (s) spent in pain behavior for the each extract was compared with that of the control.

2.4.4 Hot plate method

Experimental animals were divided into eight groups designated as group-I, group-II, group-III group-IV, group-V, group-VI, group-VII and group-VIII consisting of five mice in each group for control, positive control and test sample group of EA, CLF and PET extracts respectively. Each group received a particular treatment i.e. control (saline water, 10 ml/kg, p.o.), positive control (Diclofenac sodium 10 mg/kg, p.o.) and the test sample (100 mg/kg, p.o. & 200 mg/kg, p.o. respectively). One hour after the respective treatment, animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5°C. The latency to lick the paw or jump from the hot plate was noted as the reaction time. The reaction time was measured at 0, 1, 2, 3, 4 and 5 hour. Each mouse of all groups was observed individually for counting the number of licking or jumping they made at 0, 1, 2, 3, 4 and 5 hours after the respective treatment [13]. A cut off period of 15 s [14] was observed to avoid damage to the paw.

2.5 Anti-Inflammatory Activity

2.5.1 Carrageenan induced paw oedema test

Swiss albino mice (25-30 g) were divided into eight groups of four animals each. The test groups received 100 and 200 mg/kg body weight, p.o. of EA, CLF and PET extracts respectively. The reference group received Indomethacin (10 mg/kg body weight, p.o.) while the control group received 1 ml/kg body weight normal saline. After 30 min, 0.1 ml, 1% carrageenan suspension in normal saline was injected into the subplanatar tissue of the right hind paw. The paw volume was

measured at 1, 2, 3 and 4 h after carrageenan injection using a micrometer screw gauge. The percentage inhibition of the inflammation was calculated from the formula:

$$\% \text{ inhibition} = (1-D_t/D_o) \times 100$$

Where, D_o was the average inflammation (hind paw edema) of the control group of mice at a given time, D_t was the average inflammation of the drug treated (i.e., extract or reference indomethacin) mice at the same time [12].

2.6 CNS Depressant Activity

2.6.1 Hole cross test

The method used was done as described by Takagi et al. [15]. The apparatus was a cage of 30 cm×20 cm×14 cm with a steel partition fixed in the middle, dividing the cage into two chambers. A hole of 3.5 cm diameter was made at a height of 7.5 cm in the center of the cage. Animals were randomly divided into control, positive control and test groups containing 5 mice each. The control group received vehicle (0.9% Saline in water at the dose of 10 ml/kg p.o.) whereas the test group received extract (at the doses of 100 and 200 mg/kg p.o.) and standard group received diazepam at the dose of 1 mg/kg body weight orally. Spontaneous movement of the animals through the hole from one chamber to the other was counted for 3 minutes on 0, 30, 60, 90 and 120 minutes after intra-peritoneally injection of EA, CLF and PET extracts of YJA respectively.

2.7 Statistical Analysis

All values were expressed as the mean ± SEM of three replicate experiments. The analysis was performed by using SPSS statistical package for WINDOWS (version 16.0; SPSS Inc, Chicago). All *in vivo* data are subjected to ANOVA followed by Dunnett's test and $p<0.05$, $p<0.01$, $p<0.001$ were considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Result of Antioxidant Activity of Different Extracts of YJA Plant

3.1.1 DPPH radical scavenging assay

The antioxidant activities of EA, CLF and PET extracts of YJA were compared with ascorbic acid. The IC_{50} value of PET, CLF and EA fractions were 93.24, 9.70 and 13.26, respectively. The obtained IC_{50} values were represented in Fig. 1.

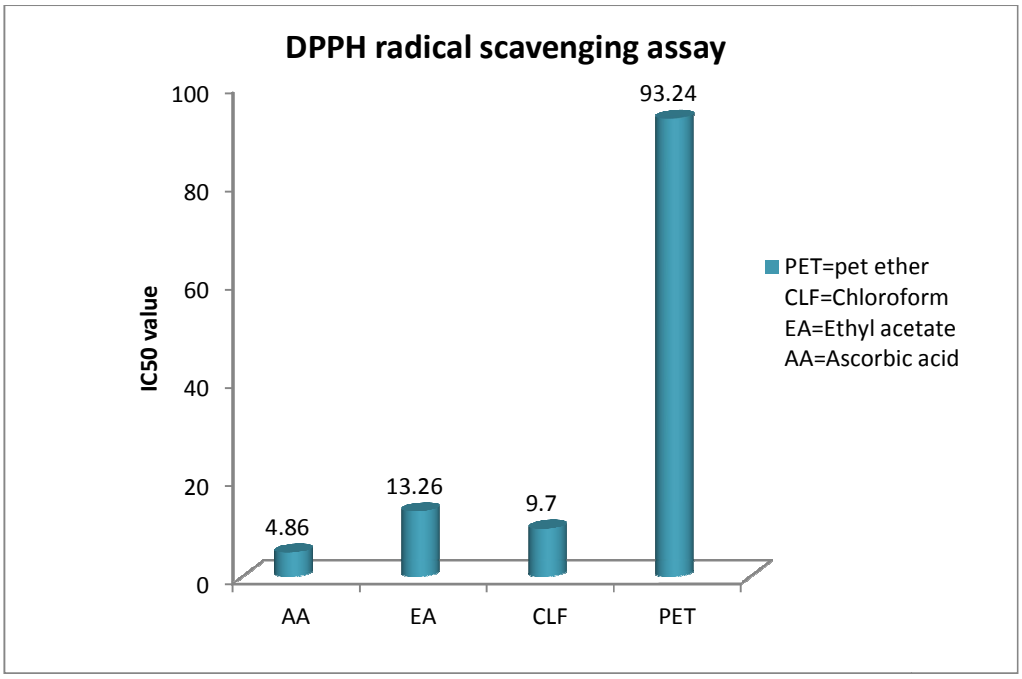

Fig. 1. IC $_{50}$ value of AA, EA, CLF and PET extracts of YJA

3.1.2 Hydroxyl radical scavenging activity

All fractions of YJA had hydroxyl radical scavenging effects at all concentrations in a dose dependant manner. The scavenging effect of the EA fractions (IC_{50} value of 15.09 µg/ml) was closer to that standard of Catechin with IC_{50} value of 14.85 µg/ml). Hydroxyl radical scavenging activity of different extracts of YJA are presented in Fig 2 and IC_{50} value are given in Fig. 3.

3.1.3 Determination of total phenolic content

Total Phenolic Content of different extracts of YJA are shown in Fig. 4.

3.1.4 Determination of total antioxidant activity

Total Antioxidant Capacity of EA, CLF and PET extracts of YJA are represented in Fig. 5.

3.2 Analgesic Activity

3.2.1 Acetic acid induced writhing method

Analgesic activity of EA, CLF and PET extracts of YJA by acetic acid induced writhing method are represented in Table 1.

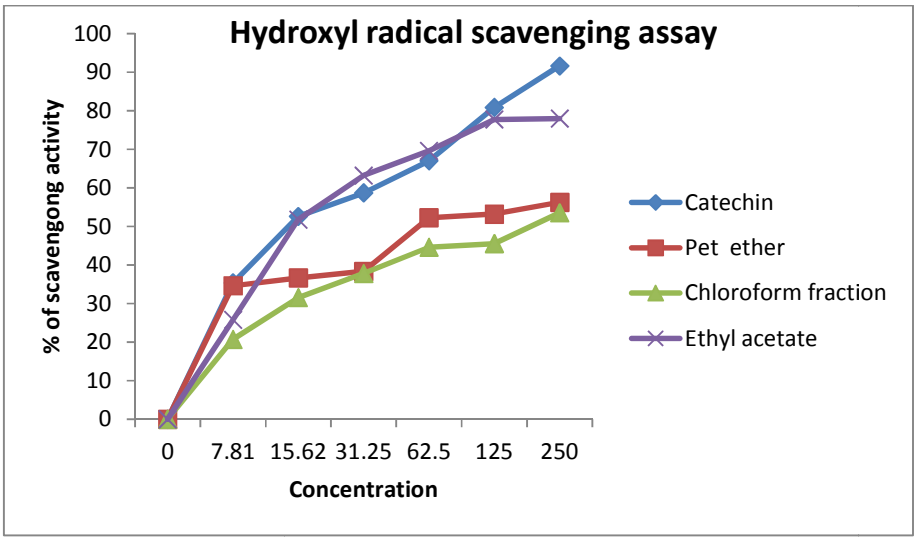

Fig. 2. Hydroxyl radical scavenging activity of EA, CLF and PET extracts of YJA

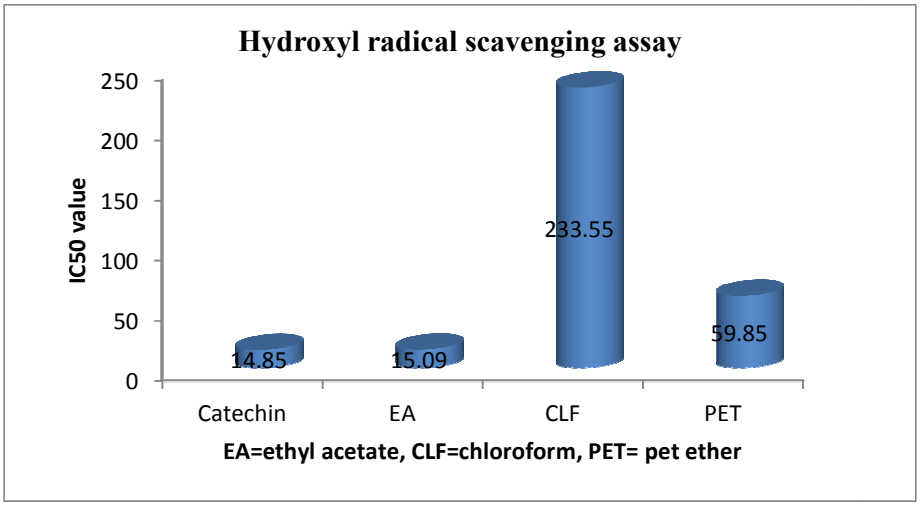

Fig. 3. IC_{50} value of AA, EA, CLF and PET extracts of YJA

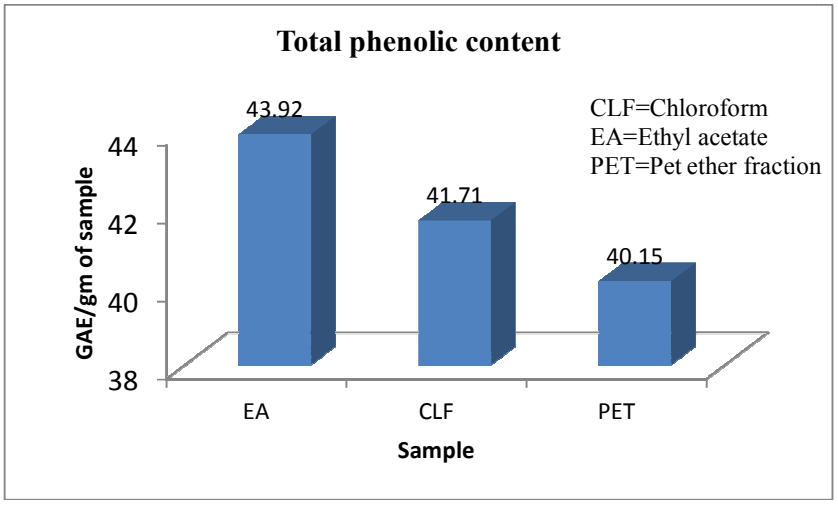

Fig. 4. Total Phenolic Content of EA, CLF and PET extracts of YJA

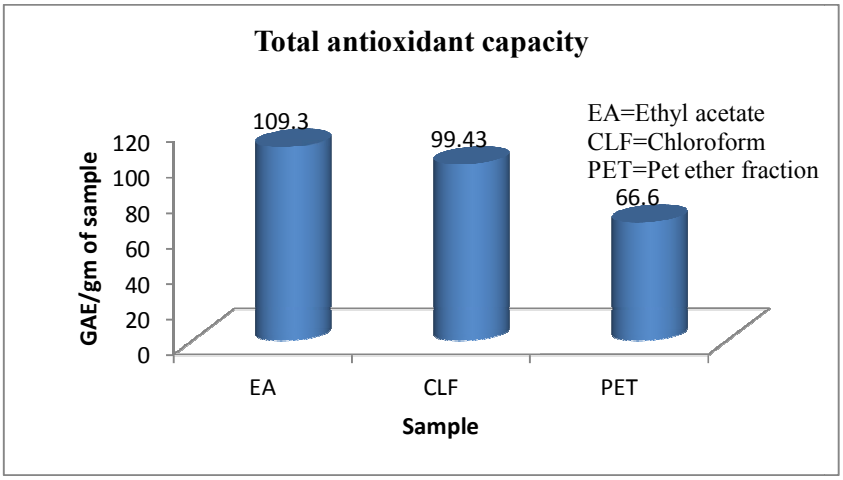

Fig. 5. Total Antioxidant Capacity of EA, CLF and PET extracts of YJA

Table 1. Evaluation of analgesic activity of EA, CLF and PET extracts of YJA by acetic acid induced writhing method

Groups	Treatment	Dose	Avg. no. of Writhing	% inhibition
			Early phase	
I	Control (Saline)	10 ml/kg	20.75±1.72	-
II	Diclofenac-Na	10 mg/kg	4 ± 0.90***	80.72
III	Ethyl Acetate Fraction	100	9.5 ±1.54**	54.21
IV		200	8.5 ±1.13**	59.03
V	Chloroform	100	7.75 ± 1.62**	62.65
VI		200	5.75 ± 2.207**	75.28
VII	Pet-ether Fraction	100	6.5 ±1.31**	68.67
VIII		200	5.75 ±1.43**	72.28

*Values are mean ± SEM, (n = 5), (*) indicates statistically significant compared to vehicle control group (*P<.05 and** P<.001 and *** P<.0001) using one way ANOVA followed by Dunnet test. Among these fractions the most potent activity was found in CLF fraction which showed highest % of inhibition (75.28) that was almost similar to standard Diclofenac-Na whose% of inhi bition was 80.72% and the % of inhibition of PET was (72. 28) and EA (59.03). From this result, it is clear that all the extractives of YJA contain considerable analgesic activity.*

3.2.2 Formalin test

Effects of EA, CLF and PET extracts of YJA in the hindpaw licking in the formalin test in mice (early phase) are shown in Table 2 and (Late phase) are shown in Table 3.

3.2.3 Eddy's hot plate method

Evaluation of analgesic activity of EA, CLF and PET extracts of YJA by Eddy's hot plate method are shown in Table-4. Percentage of inhibition of EA, CLF and PET extracts of YJA by Eddy's hot plate method are represented in Table 5.

Table 2. Effects of EA, CLF and PET extracts of YJA in the hindpaw licking in the formalin test in mice (early phase)

Groups	Treatment	Dose	Early phase	% of protection
I	Control (Saline)	10 ml/kg	23.25 ± 1.759	-
II	Diclofenac-Na	10 mg/kg	4.75 ± 1.489***	79.56
III	Ethyl acetate fraction	100	10.00 ± 2.58**	56.98
IV		200	9.75 ± 2.45**	58.06
V	Chloroform fraction	100	13.75 ± 2.138**	40.86
VI		200	13.00 ± 1.861**	44.08
VII	Pet-ether Fraction	100	15.75 ± 1.94**	32.25
VIII		200	9.5 ± 2.05**	59.13

Table 3. Effects of EA, CLF and PET extracts of YJA in the hindpaw licking in the formalin test in mice (Late phase)

Groups	Treatment	Dose	Late phase	% of protection
I	Control (Saline)	10 ml/kg	18.00 ± 2.36	-
II	Diclofenac-Na	10 mg/kg	2.25 ± 0.97***	87.5
III	Ethyl acetate fraction	100	7.75 ± 2.30*	56.94
IV		200	5.55 ± 1.96**	69.44
V	Chloroformfraction	100	5.25 ± 1.81**	70.83
VI		200	3.5 ± 2.17**	80.55
VII	Pet-ether Fraction	100	8.75 ± 1.986**	51.38
VIII		200	3.00 ± 1.89**	83.33

*Values are mean ± SEM, (n = 5), (*) indicates statistically significant compared to vehicle control group (*P<.05 and** P<.001and *** P<.0001) using one way ANOVA followed by Dunnet test. Among these fractions the most potent activity was found in PET fraction which showed highest % of inhibition (83.33) after standard (Diclofenac-Na) in late phase. The % of inhibition of CLF was (80.55) and EA (69.44).From this result, it is clear that all the extractives of YJA contain analgesic activity*

Table 4. Evaluation of analgesic activity of EA, CLF and PET extracts of YJA by Eddy's hot plate method

Groups	Treatment	Dose	Basal Reaction time (Sec)			
		(mg/kg)	1h	2h	3h	4h
I	Control	10 ml/kg	9.33±1.747	7.33±0.75	5.0±1.0	4.66±1.58
II	Diclofenac -Na	10 mg/kg	3.66±1.44***	3.00±1.0***	2.0±1.0***	1.0±1.0***
III	EA fraction	100	7.00±1.41***	6.33±1.23***	2.66±1.07***	1.33±0.75***
IV		200	5.33±1.07***	5.1±1.00***	2.00±1.00***	1.5±1.00***
V	Chloroform fraction	100	6.66±0.75***	3.66±0.759***	1.66±0.75***	1.66±0.75***
VI		200	4.33±0.75***	3.00±1.00***	1.66±0.75***	1.00±1.00***
VII	Pet ether fraction	100	7±1.00***	6.33±0.759***	2.66±1.44***	2.00±1.00***
VIII		200	4.33±0.75***	4.66±1.07***	2.39±0.75***	1.33±0.65***

*Values are mean ± SEM, (n = 5), (*) indicates statistically significant compared to vehicle control group (*P<.05 and** P<.001and *** P<.0001) using one-way ANOVA followed by Dunnet's test.*

Table 5. % of inhibition of EA, CLF and PET extracts of YJA by Eddy's hot plate method

Groups	Treatment	Dose (mg/kg)	% of Inhibition			
			1h	2h	3h	4h
I	Control	10 ml/kg				
II	Diclofenac	10 mg/kg	60.77	59.07	60	78.54
III	EA fraction	100	24.97	14.05	46.8	62.23
IV		200	42.87	27.69	60	71.42
V	Chloroform	100	25	13.64	46.8	57.14
VI	fraction	200	53.52	36.36	52.2	71.45
VII	Pet ether	100	28.61	50.09	66.66	64.28
VIII	fraction	200	53.51	59.07	66.66	78.54

3.3 Anti Inflammatory Activity

3.3.1 Carrageenan induced paw edema test

Among these fractions the most potent activity was found in PET fraction show highest % of inhibition (64.05) after standard (Indomethacin). On the other hand, EA, CLF showed slight anti-inflammatory activity is measured by considering the % of inhibition.

From this result, it is clear that all the extracts of YJA contain a slightly anti-inflammatory activity. The activity of the standard and different extractives decreases in the following order Indomethacin > PET> EA> CLF. Effects of EA, CLF and PET extracts of YJA and % of inhibition of EA, CLF and PET extracts of YJA are shown in Table 6 and Table 7.

Table 6. Effects of EA, CLF and PET extracts of YJA on carrageenan induced paw edema test

Group	Treatment	Dose	Oedema diameter (mm)			
			1h	2h	3h	4h
I	Control (Saline)	10 ml/kg	4.70±0.11	4.40± 0.09	4.17±0.11	3.75±0.14
II	Indomethacin	10 mg	2.25 ±0.14***	1.98±0.09***	1.32±0.14***	1.05±0.06***
III	EA Fraction	100	3.25±0.48***	2.90±0.42***	2.55±0.37***	2.10±0.35***
IV		200	2.60±0.22***	2.13±0.21***	1.93±0.25***	1.58±0.22***
V	Chloroform	100	3.35±0.25***	2.88±0.13***	2.47±0.22***	2.13±0.15***
VI	Fraction	200	2.45±0.15***	1.98±0.10***	1.7±0.1***	1.75±0.12***
VII	Pet-ether	100	2.75±0.48***	2.28±0.13***	1.73±0.11***	1.68±0.12***
VIII	Fraction	200	2.58±0.05***	2.18±0.09***	1.95±0.13***	1.35±0.15***

*Values are mean ± SEM, (n = 5), (*) indicates statistically significant compared to vehicle control group (*P<.05 and** P<.001and *** P<.0001) using one way ANOVA followed by Dunnet test.*

Table 7. Tables are shown of %Inhibition of EA, CLF and PET extracts of YJA on carrageenan induced paw edema test

Group	Treatment	Dose	Inhibition (%)			
			1h	2h	3h	4h
I	Control (Saline)	10 ml/kg				
II	Indomethacin	10 mg	52.12	55.11	68.26	72.00
III	EA Fraction	100	30.85	34.09	38.92	44.00
IV		200	44.68	51.70	53.89	58.00
V	Chloroform Fraction	100	28.72	34.66	40.71	43.33
VI		200	47.87	55.11	59.28	53.33
VII	PET ether Fraction	100	41.49	48.30	58.68	55.33
VIII		200	45.21	50.57	53.29	64.00

3.4 CNS Depressant Activity

3.4.1 Hole cross test

Effect of EA, CLF and PET extracts of YJA on Hole cross test in mice are represented in Table 8. At maximum wavelength at 517 nm, The DPPH free radical can easily accept an electron or hydrogen from antioxidant molecules to develop into a stable diamagnetic molecule [16]. Due to the DPPH radical's ability to bind hydrogen, it is considered to have a radical scavenging property. Discoloration occurs due to the decreasing quantity of DPPH radicals in the environment. The discoloration of the DPPH therefore reflects the radical scavenging activity of the analyzed extracts [17-18]. Based on the data obtained from this study, DPPH radical scavenging activity of YJA extract of CLF fraction (IC_{50} 9.7 µg/ml) was similar to that standard ascorbic acid ((IC_{50} 4.86 µg/ml).

Total hydroxyl radical antioxidant activity of YJA extracts was measured and compared with standard reference compound catechin. The results of EA fractions are reported as about equivalents to catechin gives the activity for destroying the hydroxyl radicals. PET and CLF fractions were less effective than standard.

Phenolic compounds have redox properties, which let them to act as antioxidants [19]. Free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Among the fractions the highest phenolic content was found in EA (43.92 mg of GAE / gm. of dried extract) and then CLF (41.71 mg of GAE / gm. of dried extract) and PET (40.18 mg of GAE / gm. of dried extract). Comparing with the phenolic content of each extracts of YJA, it was observed that EA contains considerable amount of phenolic compounds than others.

The total antioxidant capacity (TAC) was based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of green phosphate/Mo(V)complex at acid pH. It evaluates both water-soluble and fat-soluble antioxidants. Among the different extracts, EA fraction showed the highest total antioxidant activity (109.30 GAE/gm of dried sample).

The study investigated the antinociceptive activity of YJA extracts in traditional pharmacological models of pain. The findings of this study indicate that YJA extracts possesses the peripheral and central antinociceptive activity at different doses in mice. In acetic acid induced writhing test, after oral administration of YJA extracts, a dose dependent antinociceptive effect was observed (Table 1). From the table it has been observed that, all fractions showed significant antinociceptive effect. However, CLF (75.28%) and PET fractions (72.28%) exhibited better activity. Writhing test in mice is generally used for measuring peripheral analgesic activity. [20] In general, endogenous substances such as serotonin histamine, prostaglandins (PGs), bradykinins, IL-1β, IL-8,TNF-α and substance P are liberated by intra peritoneal administration of acetic acid and these mediators are responsible for pain.

These mediators stimulate primary afferent nociceptors entering dorsal horn of the central nervous system [21] and is thought to contribute to increased blood-brain barrier (BBB) permeabilization or interruption [22]. Moreover, acetic acid enhance vasodilation and vascular fluid permeability [23].

The formalin test is a widely used model of constant nociception [24,25]. The test shows a biphasic response. The first phase begins immediately after the formalin injection represents neurogenic pain and is caused by direct action on the local sensory C-fibers, resulting in the release of calcitonin gene-related peptide (CGRP) and substance P [26,27]. The second phase (15–30 min after injection) is associated with inflammatory pain of the peripheral tissues due to the release of inflammatory mediators, such as prostaglandins and nitric oxide, and is responsive to non-steroidal anti-inflammatory drugs (NSAIDs) [25-26, 28-29].

The present results showed that the number of paw licking was significantly reduced by different fractions of YJA in both neurogenic and inflammatory pain responses ($p < 0.01$) in a dose dependant manner (Table 2 and 3). EA extract shows better protection than CLF and PET fractions. However, the effect of all extracts was more emphasized in the late phase. Centrally acting analgesic drugs inhibit both the phases of formalin test, while peripherally acting analgesics restrict only the late phase responses [30]. The first phase finding of formalin test confirms the central antinociceptive effect of YJA that we have observed in the hot plate tests. The late phase response as the antinociceptive effect observed in formalin test is due to this inhibition of the inflammatory mediators [31].

Table 8. Effect of EA, CLF and PET extracts of YJA on hole cross test in mice

Group	Treatment	Dose	Number of movements				
			0 min	30 min	60 min	90 min	120 min
I	Control (Saline)	10 ml/kg	8.75 ± 1.84	8.50 ±2.25	8.00±0.70	6.50 ±1.04	6.25± 2.39
II	Diazepum	10	5.25±2.39***	4.00 ±1.22***	3.50±0.28***	3.25±1.65***	2.75±1.33***
III	EA	100	4.50±2.21***	4.00 ±1.47***	2.8 ±1.03***	2.5 ±1.19***	2.00 ±2.00***
IV	fraction	200	2.0 ±1.08***	1.75 ±0.49***	1.50±0.95***	2.0±0.70***	1.50 ±0.64***
V	CLF	100	3.50±1.19***	4.50 ±2.17***	3.5± 0.95***	3.0± 0.70***	1.50± 0.5***
VI	Fraction	200	3.00±0.91***	4.00 ±1.34***	3.0± 1.78***	1.5 ±0.95***	1.25 ±0.49***
VII	PET	100	5.3 ±0.75***	4.25±0.75***	2.25±1.1***	1.5±0.5***	2.25±0.75***
VIII	Fraction	200	5.0±1.91***	3.50±1.19***	2.0±0.4***	1.25±0.25***	2.0±0.81***

*Values are mean ± SEM, (n = 5), (*) indicates statistically significant compared to vehicle control group (*P<0.05 and** P<0.001 and *** P<0.0001) using one-way ANOVA followed by Dunnet test.*

The hot plate test is an easy and reliable method that is capable of evoking supra spinal responses [32]. This method certifies the involvement of central opioid receptors in the mechanism of pain suppression [33] and is selective for μ-agonists. In Hot plate method, It has been observed that all extracts exhibited prominent same analgesic activity at 200 mg/kg. The findings suggest that YJA plant contain active principles that act both centrally and peripherally but do better in peripherally.

The present study also investigated the anti-inflammatory activity of YJA extracts *in* experimental animal models. Carrageenan-induced paw edema in mice as an *in vivo* model of inflammation has been frequently used. Carrageenan induced paw edema is a useful replica in assessing the contribution of mediators involved in vascular changes associated with acute inflammation. Edema formation in the carrageenan-induced paw edema model is a biphasic response. In the early hyperemia, 0-2 hrs after carrageenan injection, there is a release of histamine, serotonin, and bradykinin in affecting vascular permeability. The inflammatory edema reached its maximum level at the third hour and after that it started declining. In our study, test extracts of different solvent system in both doses and indomethacin showed anti-inflammatory effects in carrageenan-induced paw edema. In the experiment, pet ether extracts showed good activity.

In CNS depression activity, on Hole cross method, EA fraction has good activity compare to other fraction that further confirm that YJA extracts have such compound that can cross the BBB and can act centrally. It may possible that the mechanism of anxiolytic action of YJA extract could be due to the binding of any of the phyto-constituents to the GABAA-BZD complex. In support of this, it has been found that flavones bind with high affinity BZD site of the GABAA receptor [34]. The results were also dose dependent and statistically significant.

4. CONCLUSION

Literature review find that YJA contain flavonoids, quinic acid, terpenoids, glycosides like compounds [35]. Flavonoids have the capability to inhibit ecosanoid biosynthesis such as prostaglandin [36]. It can be suggest that YJA showed significant and dose dependant analgesic, anti-inflammatory and CNS depressant activity due to the presence of flavonoid, phenolic and terpenoid like compounds. However, further investigations are required to understand the mechanisms of action of YJA and to identify the active constituents that may be used as a lead compound foe new drug development.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All the experimental mice were treated following the Ethical principles and guidelines for scientific experiments on animals (1995) formulated by the Swiss Academy of Medical Sciences and the Swiss academy of sciences. The institutional Animal Ethical Committee (SEU /IAEC /18-01) of Southeast University Bangladesh approved all experimental rules.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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