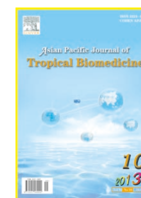
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(13)60158-6 © 2013 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Anti-inflammatory and antioxidant activities of ethanolic extract of aerial parts of *Vernonia patula* (Dryand.) Merr.

Arpona Hira[1*], Shubhra Kanti Dey[1], Md. Sariful Islam Howlader[2], Arif Ahmed[1], Hemayet Hossain[3], Ismet Ara Jahan[3]

[1]*Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh*

[2]*Department of Pharmacy, World University of Bangladesh, Dhaka-1205, Bangladesh*

[3]*BCSIR Laboratories, Dhaka, Bangladesh Council of Scientific and Industrial Research, Dr. Qudrat-E-Khuda Road, Dhaka-1205, Bangladesh*

PEER REVIEW

Peer reviewer

Subrata Kumar Biswas, Assistant Professor, Department of Pharmacy, BGC Trust University Bangladesh, Chandanaish, Chittagong, Bangladesh.
Tel: +88-01753717041
Fax: +88-0312550224
E-mail: subratapharmacy@yahoo.com

Comments

This is a well-organized and an interesting study in which the authors investigated anti-inflammatory and antioxidant activities of ethanolic leaf extract of *V. patula*. This study is well designed and carefully done and the findings are interesting and meaningful. I found no fault whatsoever with the methods, data analysis, or conclusions. Consideration of these points, I believe, this manuscript leads to an improved research paper that better illustrates the key concepts and conclusions.
Details on Page 804

ABSTRACT

Objective: To investigate the inflammatory and antioxidant activities of ethanolic extract of aerial part of *Vernonia patula* (Dryand.) Merr (EAV).
Methods: The anti-inflammatory activity of EAV was studied using carrageenan and histamine-induced rat paw edema test at different doses (100, 200 and 400 mg/kg body weight). DPPH free radical scavenging, nitric oxide scavenging, reducing power and Fe^{2+} ion chelating ability were used for determining antioxidant activities.
Results: The EAV, at the dose of 400 mg/kg, showed a significant anti-inflammatory activity ($P<0.01$) both in the carrageenan and histamine-induced oedema test models in rats, showing 62.86% and 64.42% reduction in the paw volume comparable to that produced by the standard drug indomethacin (67.26% and 66.01%) at 5 h respectively. In DPPH free radical scavenging test, IC_{50} value for EAV was found fairly significant 36.59 μg/mL when compared to the IC_{50} value of the reference standards ascorbic acid 8.97 μg/mL. The IC_{50} values of the extract and ascorbic acid were 47.72 and 12.39 μg/mL, respectively in nitric oxide scavenging assay. The IC_{50} value of the EAV (33.59 μg/mL) as percentage of Fe^{2+} ion chelating ability was also found significant compared to that of EDTA (9.16 μg/mL). The maximum absorbance for reducing power assay was found to be 1.928 at 100 μg/mL when compared to 2.449 for standard ascorbic acid. The total phenolic content was 198.81 mg/g of gallic acid equivalent. Acute toxicity test showed that the plant might be safe for pharmacological uses up to a dose level of 3 200 mg/kg of body weight in rats.
Conclusions: Therefore, the obtained results suggest the acute anti-inflammatory and antioxidant activities of the EAV and thus provide the scientific basis for the traditional uses of this plant part as a remedy for inflammations.

KEYWORDS
Vernonia patula, Anti-inflammatory, DPPH free-radical scavenging, Nitric oxide radical scavenging, Reducing power, Acute toxicity

1. Introduction

Vernonia patula (Dryand.) Merr. (*V. patula*) [Bengali name: Kukshim, Tribal name: Loo Hu (Murong), Hung Fui (Marma), English name: Ash-coloured Fleabane, Purple Fleabane] belongs to family Asteraceae. It is widely distributed in Bangladesh which is an erect branching herb, genarelly 1 m long and 20-70 cm tall stems ribbed. Two terpenoids (α-amyrin acetate, α-amyrin) and one steroid (β-sitosterol) were isolated from from the aerial part of *V. patula*[1]. Four triterpenoids were isolated and identified as bauerenyl acetate, friedelin, epifriedelanol, 20(30)-taraxastene-3-beta 21-alpha-diol[2]. Four flavones, luteolin, tricin, luteolin 4′-O-β-D-glucoside and luteolin 7-O-β-D-glucoside, four caffeoyl-quinates, 3,4-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, ethyl 3,4-dicaffeoyl-quinate and chlorogenic acid, as well as esculetin, and two catechols, caffeic acid and protocatechuic acid were isolated from the

*Corresponding author: Arpona Hira, Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh.
Tel: +8801719306183
E-mail: arponahira@gmail.com
Foundation Project: Supported by the Ministry of Science and Information & Communication Technology (Grant No. MOSICT/Sha-9/PRC-24/2009-2010/(Part-1)/BS-72/192, date-03/12/2009), Government of Bangladesh.

Article history:
Received 15 Jul 2013
Received in revised form 26 Jul, 2nd revised 30 Jul, 3rd revised 4 Aug 2013
Accepted 20 Sep 2013
Available online 28 Oct 2013

aerial parts of *V. patula*[3].

Since no literature is currently available to substantiate anti-inflammatory and antioxidant properties of the ethanolic extract of aerial part of *V. patula* (EAV), the present study was designed to provide scientific evidence for its use as a traditional folk remedy by investigating the anti-inflammatory and antioxidant potential of the EAV that also confirm its use in folk remedy for inflammation, pain and other pathological conditions where free radicals are implicated.

2. Materials and methods

2.1. Collection and identification of plant material

The plant selected for present work was the aerial parts of *V. patula*, which was collected from Chittagong, Bangladesh in June, 2011. The samples were identified by Sarder Nasir Uddin, Senior Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. A voucher specimen (DACB: 35107) has been deposited in the herbarium for further reference.

2.2. Preparation of ethanolic extract

The collected aerial part of plant was separated from undesirable materials and then was washed with water and shed-dried for one week. The dried plant materials were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powdered sample was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 350 g of powered material was taken in a clean, flat-bottomed glass container and soaked in 1500 mL of ethanol. The container along with its contents was sealed and kept for a period of 10 d with occasional shaking or stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. It was then filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate was concentrated by using rotary vacuum evaporator (R-210, Buchi, Switzerland) and dried. It rendered a 33 g of gummy concentrate (13.43%) and was designated as crude ethanol extract.

2.3. Test animals

For the screening of *in vivo* anti-inflammatory activity, male rats of Wister strain weighing 175-202 g were used. The animals were housed under standard Laboratory (at Pharmacology Laboratory of BCSIR, Chittagong) conditions maintained at (25±1) °C and under 12/12 h light-dark cycle and fed with balanced diet and water *ad libitum*. All experimental protocols were in compliance with Bangladesh Council of Scientific and Industrial Research (BCSIR) ethics committee on research in animals as well as internationally accepted principles for laboratory animal use and care.

2.4. Chemicals and drugs

1,1-Diphenyl-2-picryl hydrazyl (DPPH), L-ascorbic acid, gallic acid, Folin-Ciocalteu phenol reagent, ferrozine, Griess reagent, histamine phosphate and indomethacin were obtained from Sigma Chemical Co. (St. Louis, USA). Trichloroacetic acid (TCA), phosphate buffer (pH 6.6), potassium ferricyanide [$K_3Fe(CN)_6$], ferrous chloride ($FeCl_2$), ferric chloride ($FeCl_3$), sodium nitroprusside, sodium phosphate, EDTA, Tween 80, ammonium molybdate and sodium carbonate were of analytical grade and purchased from Merck (Darmstadt, Germany).

2.5. Test for different chemical groups

The EAV was tested for its different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins[4].

2.6. Total phenolic content determination

The total phenolic content of the extract was determined by the modified Folin-Ciocaltu method[5]. Briefly, 1 mL of each extract (1 mg/mL) was mixed with 5 mL Folin-Ciocaltu reagent (1:10 v/v distilled water) and 4 mL (75 g/L) of sodium carbonate. The mixture was vortexed for 15 seconds and allowed to stand for 30 min at 40 °C for color development. The absorbance was read at 765 nm with a spectrophotometer. Total phenolic content was determined as mg of gallic acid equivalent per gram using the equation obtained from a standard gallic acid calibration curve $y=6.2548x-0.0925$, $R^2=0.9962$.

2.7. Anti-inflammatory activity

2.7.1. Carrageenan-induced oedema test

Carrageenan induced rat hind paw edema was used as the animal model of acute inflammation according to the method of Lanhers *et al*[6]. In this experiment, the rats were divided into five groups of five animals each. Group I (control) received 1% Tween 80 in normal saline (10 mL/kg). Group II (positive control) received 10 mg/kg body weight of indomethacin orally. Group III, IV and V received 100, 200 and 400 mg/kg body weight of the EAV orally respectively. Acute inflammation was induced in all the four groups by sub plantar injection of 0.1 mL of its suspension of carrageenan with 1% Tween 80 in normal saline in the right paw of the rats 1 h after the oral administration of the tested materials. The paw volume was measured with a micrometer screw gause at 1, 2, 3, 4 and 5 h after the administration of the drug and the extract. The percentage inhibition of inflammatory effect of the extract was calculated using the following expression:

Inhibition of inflammation (%)=$[(V_c-V_t)/V_c]\times100$

Where V_c is the average degree of inflammation by the control group and V_t is the average degree of inflammation by the test group.

2.7.2. Histamine-induced oedema test

Using the method of Perianayagam *et al.*[7], the paw oedema was produced by sub-plantar administration of 0.1% freshly prepared solution of histamine into the right hind paw of the rats. In this experiment, 25 rats were divided into five groups of five animals each. Group I (control) received

1% Tween 80 in normal saline (10 mL/kg). Group II (positive control) received 10 mg/kg body weight of indomethacin orally. Group III, IV and V received 100, 200 and 400 mg/kg body weight of the EAV orally respectively. Acute inflammation was induced in all the five groups by sub plantar injection of 0.1 mL of histamine with 1% Tween 80 in normal saline in the right hind paw of the rats 1 h after the oral administration of the tested materials. The paw volume was measured with a micrometer screw gause at 1, 2, 3, 4 and 5 h after the administration of the drug and the extract. The percentage inhibition of inflammatory effect of the extract was calculated using the same formula for carrageenan-induced paw oedema.

2.8. Antioxidant activities

2.8.1. DPPH free radical scavenging activity

The method of Chang *et al.* was used for performing the DPPH radical scavenging activity[8]. A stock solution of EAV (5 mg/mL) was prepared in respective solvent systems. A serial dilutions were the carried out to obtain concentrations of 5, 10, 20, 40, 60, 80, 100 μg/mL. An equal amount of sample solution was mixed with an equal amount of 0.1 mmol/L methanolic solution of DPPH. The mixture was vortex and allowed to stand at the dark at 25 °C for 30 min. After 30 min incubation, the absorbance of the mixture was read against a blank at 517 nm using a double beam Analykjena UV-vis spectrophotometer (Model 205, Jena, Germany). The radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as per the equation:

$$I(\%)=[(A_{blank}-A_{sample})/A_{blank}]\times100$$

Where A_{blank} is the absorbance of the control (containing all reagents except the test compound), and A_{sample} is the absorbance of the experimental sample with all reagents. IC_{50} value (the concentration of sample required to scavenge 50% DPPH free radical) was calculated from the plot of inhibition (%) against the concentration of the extract. All determination was carried out in triplicate and average of the results was noted. Ascorbic acid was used as standard for this study.

2.8.2. Nitric oxide (NO) scavenging activity

NO scavenging activity was measured spectrophotometrically[9]. Sodium nitroprusside (5 mmol) in phosphate buffered saline was mixed with different concentrations of the EAV (5-100 μg/mL) dissolved in methanol and incubated at 25 °C for 30 min. A control without the test compound but with an equivalent amount of methanol was taken. After 30 min, 1.5 mL of the incubation solution was removed and diluted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine dihydrochloride was measured at 546 nm. The NO radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as per the equation:

$$I(\%)=[(A_{blank}-A_{sample})/A_{blank}]\times100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound with all reagents. IC_{50} value is the concentration of sample required to scavenge 50% NO free radical and was calculated from the plot of inhibition (%) against extract concentration. All the tests were carried out in triplicate and average of the absorptions was noted. Ascorbic acid was used as positive control standard for this study.

2.8.3. Ferrous ion chelating ability

The ferrous ions chelating activity of EAV and standards were investigated according to the method of Robu *et al*[10]. Briefly, different concentrations of the EAV (5-100 μg/mL) were added to 0.1 mL solution of 2 mmol/L ferrous chloride ($FeCl_2$). Then, the reaction was initiated by the addition of 0.2 mL of 5 mmol/L ferrozine and mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured at 562 nm in spectrophotometer, wherein the Fe^{2+} chelating ability of extracts was monitored by measuring the ferrous ion-ferrozine complex. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given in the below formula:

$$\text{Ferrous ions chelating ability }(\%)=[(A_0-A)/A_0]\times100$$

Where A_0 is the absorbance of the control solution (containing all reagents except extract); A is the absorbance in the presence of the sample of plant extracts. All the tests were carried out in triplicate and EDTA was used as standard.

2.8.4. Reducing power assay

The reducing power of EAV was determined according to method followed by Sikder *et al*[11]. Different concentrations of EAV (5-100 μg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. A 10% solution of trichloroacetic acid (2.5 mL) was added to the mixture, which was then centrifuged at 3 000 r/min for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance of the mixture was measured at 700 nm with spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing power. All the tests were carried out in triplicate and average of the absorptions was recorded. Ascorbic acid was used as the standard reference compound.

2.9. Acute toxicity test

The acute toxicity of EAV was determined in rats according to OECD guidelines with slight modifications[12]. Rats fasted for 16 h were randomly divided into groups of five rats per group. Graded doses of the extract (200, 400, 800, 1 600, and 3 200 mg/kg *p.o.*) were separately administered to the rats in each of the groups by means of bulbed steel needle. All the animals were then allowed free access to food and water and observed over a period of 72 h for signs of acute toxicity. The number of deaths within this period was recorded.

2.10. Statistical analysis

Data were presented as mean±SD. Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons using SPSS Data Editor for Windows, Version 11.5.0 (SPSS Inc., USA). The results obtained were compared with the control group. P<0.05 was considered to be statistically significant.

3. Results

3.1. Chemical group test

Results of different chemical tests on the EAV showed the presence of reducing sugars, saponins, glycosides, flavonoids and tannins.

3.2. Anti-inflammatory activity

3.2.1. Carrageenan-induced paw oedema

The anti-inflammation effect of the EAV using carrageenan induced oedema tests is expressed in Table 1. In this test, the positive control (indomethacin) significantly (P<0.05; P<0.01) decreased the paw edema at 1 h to 5 h after carrageenan injection compared to saline with inhibition 53.37% to 67.26%. A maximum oedema paw volume of (1.45 ±0.23) mm was observed in the control rats, 5 h after the carrageenan injection. Rats with the extract at 400 mg/kg body weight significantly decreased (P<0.05; P<0.01) the carrageenan-induced oedema paw volume from 1 h to 5 h compared to the standard drug indomethacin at a dose of 10 mg/kg body weight. The inhibition percentage of the oedema paw volume by the 400 mg/kg body weight of the extract was also found statistically significant when it was compared with the indomethacin treated animals at 1, 2, 3, 4 and 5 h. The highest reduction in the paw volume by the 400 mg/kg body weight was 62.86%, which was comparable to that of the indomethacin (67.26%) at 5 h.

3.2.2. Histamine-induced paw oedema

Table 2 shows the anti-inflammation effect of the EAV using histamine-induced paw oedema tests. In the histamine-induced oedema test, a maximum oedema paw volume of (1.52±0.27) mm was observed in the control rats, 5 h after the histamine injection. Rats pre-treated with the extract at 400 mg/kg body weight significantly decreased (P<0.05; P<0.01) the histamine-induced oedema paw volume from 1 h to 5 h compared to the standard drug indomethacin at a dose of 10 mg/kg body weight. The percentage inhibition of the oedema paw volume by the 400 mg/kg body weight of the extract was also statistically significant (P<0.05; P<0.01) compared favorably with the indomethacin treated animals at 1, 2, 3, 4 and 5 h. The maximum reduction in the paw volume by the 400 mg/kg body weight was 64.42% compared to the indomethacin (66.01%) at 5 h.

3.3. Antioxidant activities

EAV was screened for evaluation of its possible antioxidant activities. Four complementary test systems, namely DPPH free radical scavenging, NO scavenging activity, reducing power and ferrous ion chelating ability were followed for this analysis.

3.3.1. DPPH free radical scavenging activity

DPPH free radical scavenging activity of the EAV was found to be increased with the increase of concentration of the

Table 1

Effects of EAV and indomethacin on carrageenan-induced oedema paw volume in Wistar rats.

Treatment	Dose (mg/kg)	Right hind paw volume (% Inhibition)				
		1 h	2 h	3 h	4 h	5 h
Vehicle	10 (mL/kg)	0.98±0.11	1.08±0.16	1.28±0.18	1.37±0.20	1.45±0.23
Indomethacin	10	0.44±0.16 (53.37)**	0.48±0.14 (56.25)**	0.52±0.16 (59.24)**	0.50±0.18 (63.90)**	0.47±0.19 (67.26)**
EAV	100	0.78±0.04 (20.48)*	0.78±0.12 (28.68)*	0.89±0.15 (29.78)*	0.92±0.16 (32.45)*	0.88±0.17 (38.92)*
EAV	200	0.74±0.11 (24.54)*	0.69±0.14 (36.02)*	0.72±0.16 (43.88)*	0.75±0.17 (45.56)*	0.71±0.20 (51.31)*
EAV	400	0.62±0.18 (37.53)*	0.58±0.12 (46.69)*	0.56±0.14 (56.11)**	0.54±0.15 (60.70)**	0.54±0.15 (62.86)**

Each value is presented as the mean±SEM (n=5). EVP: Ethanol extract of aerial part of *V. patula*. *indicates P<0.05 compared with control group (Dunnett's test); ** indicates P<0.01 compared with control group (Dunnett's test).

Table 2

Effect of EAV and indomethacin on histamine-induced oedema paw volume in wistar rats.

Treatment	Dose (mg/kg)	Right hind paw volume (% Inhibition)				
		1 h	2 h	3 h	4 h	5 h
Vehicle	10 (mL/kg)	1.10±0.17	1.29±0.17	1.35±0.19	1.41±0.22	1.52±0.27
Indomethacin	10	0.54±0.15 (51.18)**	0.59±0.14 (53.80)**	0.58±0.15 (57.22)**	0.56±0.17 (60.79**	0.52±0.21 (66.01)**
EAV	100	0.76±0.12 (30.92)*	0.99±0.08 (22.63)*	0.90±0.18 (33.92)*	0.91±0.19 (35.97)*	0.82±0.21 (45.45)*
EAV	200	0.74±0.11 (32.73)*	0.77±0.17 (39.84)*	0.80±0.22 (41.29)*	0.80±0.17 (43.02)*	0.77±0.19 (49.40)*
EAV	400	0.60±0.19 (46.11)*	0.62±0.17 (51.94)**	0.62±0.16 (52.27)**	0.60±0.16 (57.69)**	0.54±0.18 (64.42)**

Each value is presented as the mean±SEM (n=5). EAV: Ethanol extract of aerial part of *V. patula*. *indicates P<0.05 compared with control group (Dunnett's test); ** indicates P<0.01 compared with control group (Dunnett's test).

extract (Table 3). The extract exhibited (87.92±0.27)% radical inhibitions at 100 μg/mL whereas at the same concentration the standards ascorbic acid exhibited (95.67±0.19)% inhibitions respectively. IC_{50} value of the EAV was found to be very fairly significant [(36.59±0.21) μg/mL] compared to the IC_{50} value of the reference compounds ascorbic acid [(8.97±0.15) μg/mL] respectively.

Table 3

DPPH radical scavenging activity of the EAV and standard at different concentration.

Concentration	% Inhibition of EAV and standards	
(μg/mL)	EAV	Ascorbic acid
5	8.69±0.15	18.63±0.13
10	13.28±0.19	57.71±0.19
20	23.65±0.20	66.24±0.19
40	57.46±0.22	75.75±0.15
60	65.17±0.15	89.97±0.22
80	85.18±0.28	94.55±0.17
100	87.92±0.27	95.67±0.19
IC_{50}	36.59±0.21	8.97±0.15

Values are expressed as mean±SD (*n*=3). EAV: Ethanol extract of aerial part of *V. patula*.

3.3.2. NO scavenging assay

The scavenging of NO by the EAV was also increased in dose dependent manner. A significant decrease in the NO radical due to the scavenging ability of the extract and ascorbic acid. The ethanol extract showed maximum scavenging activity of (83.06±0.29)% at 100 μg/mL, where as ascorbic acid at the same concentration exhibited (90.58±0.54)% inhibition (Table 4). The IC_{50} value for ethanolic extract was found fairly significant (47.72±0.32) μg/mL while compared to the IC_{50} value of the reference standard ascorbic acid (12.39±0.28) μg/mL.

Table 4

Nitric oxide radical scavenging activity of the EAV and standard at different concentration.

Concentration	% NO inhibition of the EAV and standard	
(μg/mL)	EAV	Ascorbic acid
5	4.96±0.33	30.37±0.40
10	17.56±0.48	48.48±0.39
20	36.73±0.24	60.81±0.40
40	44.98±0.51	73.08±0.38
60	58.75±0.28	83.72±0.39
80	79.15±0.33	88.75±0.24
100	83.06±0.29	90.58±0.54
IC_{50}	47.72±0.32	12.39±0.28

Values are expressed as mean± (*n*=3). EAV: Ethanol extract of aerial part of *V. patula*.

3.3.3. Fe^{2+} ion chelating ability

Fe^{2+} ion chelating ability of EAV is shown in Table 5. The extract showed (77.11±0.16)% Fe^{2+} ion chelating ability at 100 μg/mL where as the standard EDTA showed (97.09±0.17)% at the same concentration. The IC_{50} value of the EAV was also found significant (33.59±0.19) μg/mL while compared to the IC_{50} value of the reference standard EDTA (9.16±0.16) μg/mL.

Table 5

Fe^{2+} ion chelating ability of EAV and EDTA (Standard) at different concentration.

Concentration	% Chelating ability of EAV and standard	
(μg/mL)	EAV	Na_2EDTA (Standard)
5	19.98±0.20	22.19±0.13
10	30.42±0.23	35.64±0.20
20	43.40±0.23	55.71±0.19
40	53.77±0.16	63.46±0.16
60	67.02±0.13	74.94±0.23
80	71.66±0.21	85.83±0.21
100	77.11±0.16	97.09±0.17
IC_{50}	33.59±0.19	9.16±0.16

Values are expressed as mean±SD (*n*=3). EAV: Ethanol extract of aerial part of *V. patula*.

3.3.4. Reducing power assay

The reducing power of EAV was also determined using ascorbic acid as positive control (Table 6). The maximum absorbance for ethanolic crude extract was (1.928±0.009) at 100 μg/mL while compared to (2.449±0.003) for standard ascorbic acid.

Table 6

Reducing power assay of the EAV and standard at different concentration.

Concentration	Average absorbance at 700nm of EAV and standard	
(μg/mL)	EAV	Ascorbic acid
5	0.226±0.003	0.516±0.004
10	0.423±0.002	0.870±0.006
20	0.625±0.006	1.332±0.007
40	0.920±0.005	1.533±0.005
60	1.274±0.007	1.944±0.009
80	1.531±0.006	2.139±0.008
100	1.928±0.009	2.449±0.003

Values are expressed as mean±SD (*n*=3). EAV=Ethanol extract of aerial part of *V. patula*.

3.3.5. Total phenolic content

The average absorbance at 765 nm was 1.151±0.570 and the amount of total phenolic content was calculated as quite high in EAV [(198.81±12.66) mg of gallic acid equivalent/g of dry extract].

3.4. Acute toxicity test

In acute toxicity study, oral administration of graded doses (200, 400, 800, 1600 and 3200 mg/kg *p.o.*) of the EAV to rats did not produce any significant changes in behaviour, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the observation period. No mortality or any toxic reaction was recorded in any group after 72 h of administering the extract to the animals. EAV was safe up to a dose level of 3200 mg/kg of body weight in rats.

4. Discussion

The anti-inflammatory effect of EAV was evaluated in

carrageenan and histamine induced paw oedema, which are widely used for the screening of anti-inflammatory compounds and have frequently been used to assess the antioedematogenic effect of medicinal plants. In rats, the inflammatory response induced by carrageenan is characterized by a biphasic response with marked oedema formation resulting from the rapid production of several inflammatory mediators such as histamine, serotonin, and bradykinin (first-phase), which is subsequently sustained by the release of prostaglandins and NO (second-phase) with peak at 3 h, produced by inducible isoform of cyclooxygenase (COX-2) and nitric oxide synthase (iNOS), respectively[13]. In the present work, pre-treated oral administration of extract was effective in reducing the oedematogenic response evoked by carrageenan in rats between the 2nd and the 4th hours after the injection. This evidence allows us to suggest that anti-inflammatory actions of the extract are related to the inhibition of one or more intracellular signaling pathways involved in the effects of several inflammatory mediators.

Again, histamine is an important inflammation mediator, potent vasodilator substance and also increases the vascular permeability[14]. When histamine is subcutaneously injected into a rat, it forms a wheal around the injected place, due to increase of vascular permeability of the host capillary venules in the skin. Substances that antagonize the activity of histamine receptors reduce the area of the wheal formed. This result tends to suggest that the anti-inflammatory activity of the extract is possibly backed by its anti-histamine activity. The antihistaminic effect of the extract increased with the increase in the dose of the extract. Since the extract effectively suppressed the oedema produced by histamine, it showed that the extract exhibited anti-inflammatory actions by inhibiting the synthesis, release or action of inflammatory mediators such as histamine, serotonin, and prostaglandins. This study has shown that the EAV possesses a significant antioedematogenic effect ($P<0.01$) on paw oedema induced by carrageenan and histamine compared favorably with the standard drug (indomethacin) in treated rats.

Phytochemically, the EAV has been also reported to yield flavonoids and tannins. Flavonoids (or bioflavonoids) are naturally occurring compounds, considered to possess anti-inflammatory properties, both *in vitro* and *in vivo*[15]. Flavonoids also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediators of inflammation[16]. Especially the reported flavonoid luteolin from the aerial part of this plant[3] has been shown to inhibit the activity of cyclooxygenase and lipoxygenase and to reduce the levels of PGE_2 and the release and expression of the induced isoform cyclooxygenase-2 (COX-2)[17]. Tannins are important compounds known to be potent cyclooxygenase-1 inhibitors and with antiphlogistic activity[18]. Again it has been found that phenolic compounds exhibited a significant anti-inflammatory action on carrageenan-induced paw edema by inhibition of leukocyte migration, reduction of serum lysozyme levels, NO and PGE_2[19]. The mechanisms of anti-inflammatory activity may be related to the anti-phlogistic action of the tannins. Again, the isolated compound caffeic acid is also an essential constituent for the anti-inflammatory activity[3], since it inhibits the synthesis of arachidonic acid and suppresses the enzymatic activity of COX-1 and COX-2. In addition, caffeic acid inhibits the gene expression of COX-2 and the enzymatic activity of myeloperoxidase[20,21], ornithine decarboxylase, lipoxygenase, and tyrosine kinase[22]. Caffeic acid also presents immunosuppressive activity, inhibiting the early and late events of T cell activation and the consequent release of cytokines like IL-2[23]. Therefore, the acute anti-inflammatory activity of the EAV might be due to presence of these flavonoids, tannins *etc*.

Non-steroidal anti-inflammatory drugs (NSAID) like indomethacin used in this study are known to inhibit cyclooxygenase enzymes I and II which are implicated in the production of inflammation-mediating agent prostaglandin E_2 (PGE_2) from arachidonic acid[24]. Therefore, the pattern of anti-inflammatory activity exhibited by this extract was similar to that of indomethacin.

DPPH is a relatively stable free radical scavenger which converts the unpaired electrons to paired ones by hydrogen proton donation. Scavenging of DPPH radical in this study indicates the potency of the plant extracts in donating hydrogen proton to the lone pair electron of the radicals. Because the inhibition was more at a higher concentration in all the solvent extracts, it could be suggested that the plant extracts contain compounds capable of donating protons to the free radicals. The methods have proven the effectiveness of the extracts in a concentration-dependent manner[25]. The antioxidant activity observed in the DPPH radical scavenging assay may be as a result of the of phytoconstituents content in the plant extracts.

In vitro inhibition of NO radical is a measure of antioxidant activity of plant drugs. NO is a free radical which plays an important role in the pathogenesis of inflammation, *etc*. Scavenging of NO radical is based on the generation of NO from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent[26]. The absorbance of the chromophore is measured at 546 nm in the presence of the fractions. All the fractions of EAV decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. This may be due to the antioxidant principles in the fractions which compete with oxygen to react with NO• thereby inhibiting the generation of nitrite.

The transformation of Fe^{3+} into Fe^{2+} in the presence of various fractions was measured to determine the reducing power ability. The reducing ability of a compound generally depends on the presence of reductones (antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom[27]. The antioxidant principles present in the fractions of EAV caused the reduction of Fe^{3+}/ferricyanide complex to the ferrous form, and thus proved the reducing power ability.

The metal chelating ability of the fractions of EAV was measured by the formation of ferrous ion ferrozine complex. Ferrozine combines with ferrous ions forming a red coloured complex which absorbs at 562 nm[28]. It was reported that the chelating agents which form σ bond with a metal, are effective as secondary antioxidants, because they reduce the redox potential thereby stabilising the oxidised form of the metal ion[29]. The results of our study demonstrate that the fractions have an effective capacity for iron binding, suggesting its antioxidant potential. In addition, the metal

chelating ability of the fractions demonstrated that they reduce the concentration of the catalysing transition metal involved in the peroxidation of lipids.

The amount total phenolic compound was calculated as quite high in the ethanol extract of *V. patula* [(198.81±12.66) mg of gallic acid equivalent]. According to the results of this study, it can be revealed that the high inhibition value in the ethanol extract might be due to the high concentration of phenolic compounds present in the extract. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups[30]. It is known that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up to 1 g daily from a diet rich in fruits and vegetables[31]. Phenolic compounds from plants are known to be good natural antioxidants. However, the activity of synthetic antioxidants was often observed to be higher than that of natural antioxidants[32]. Various phytochemical components, especially polyphenols (such as flavonoids, tannins, phyenyl propanoids, phenolic acids *etc.*) are known to be responsible for the free radical scavenging and antioxidant activities of plants. It has been reported that saponins, tannins and certain flavonoids are potential free-radical scavengers, and their activity against the DPPH radical is closely associated with their chemical structure[33,34]. Tannins have also ferrous ions (Fe^{2+}) chelating activity[35]. Again, the previously isolated compounds from this plant luteolin, chlorogenic acid, and caffeic acid showed a concentration-dependent inhibitory activity in several models of oxidative stress[3,36]. Flavonoids are very potent NO radical scavengers[37]. So the antioxidant activity of the EAV might be due to presence of these phenolics, flavonoids, tannins *etc*.

Since the plant extract reduced significantly the formation of oedema induced by carrageenan and histamine, the EAV exhibited acute anti-inflammatory activity. The potential of the EAV as acute anti-inflammatory and antioxidant agents may be due to the presence of phyto constituents such as flavonoids, tannins, phenolics and might be responsible for its activity. Again, no mortality was recorded in the acute toxicity test; it showed that the plant extract might be safe for use in rats. Therefore, it can be revealed that the EAV possesses acute anti-inflammatory activity as well as antioxidant activities and justifies its use as a traditional folk remedy for inflammation, pain, boils *etc*. However, a more extensive study is necessary to determine the exact mechanism(s) of action of the extract and its active compound(s).

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to Shahidul Islam, Jr Technician, CRD, BCSIR Laboratories, Dhaka for assisting the research. The work was supported by a special research grant from the Ministry of Science and Information & Communication Technology (Grant No. MOSICT/Sha-9/PRC-24/2009-2010/(Part-1)/BS-72/192, date-03/12/2009), Government of Bangladesh.

Comments

Background

V. patula (Dryand.) is widely used traditionally in the treatment of various pains, boils, and inflammation related health disorders in Bangladesh. However, no pharmacological evaluation has been done on *V. patula*, especially in aerial parts for its anti-inflammatory, and antioxidant activities.

Research frontiers

The most fashionable ideas of these studies was performed to evaluate anti-inflammatory activity by using carrageenan- and histamine-induced rat paw edema model and antioxidant effects by determining DPPH free radical scavenging, NO scavenging, reducing power, ferrous ion chelating ability, and total phenolic content.

Related reports

Luteolin, tricin, friedelin, caffeic acid, α-amyrin have been isolated from aerial parts of *V. patula* (Herrera *et al.*, 1980, Liang *et al.*, 2003, Lin *et al.*, 2002). No pharmacological evaluation has been done on *V. patula* aerial parts for its anti-inflammatory, and antioxidant activities.

Innovations and breakthroughs

There is no literature currently available to substantiate anti-inflammatory, and antioxidant properties of the aerial parts of *V. patula*. So the present study is innovative and creative that is held for the first time to screen its pharmacological activities.

Applications

From this manuscript, it can be revealed that the *V. patula* possesses acute anti-inflammatory activity as well as antioxidant activities and justifies its use as a traditional folk remedy for inflammation, pain, boils arthritis, and other pathological conditions where free radicals are implicated.

Peer review

This is a well-organized and an interesting study in which the authors investigated anti-inflammatory and antioxidant activities of ethanolic leaf extract of *V. patula*. This study is well designed and carefully done and the findings are interesting and meaningful. I found no fault whatsoever with the methods, data analysis, or conclusions. Consideration of these points, I believe, this manuscript leads to an improved research paper that better illustrates the key concepts and conclusions.

References

[1] Herrera CL, Chanco GL, Sison FM. Chemical and pharmacological studies on *Vernonia patula* (Dry.) Merr. and *Vernonia cinerea* (Linn.) Less. 4th Asian Symposium on Medicinal Plants and Spices; 1980 Sep 15-19; Bangkok, Thailand; 1980, p. 249.

[2] Liang QL, Min ZD. [Studies on the constituents from the herb of *Vernonia patula*]. *Zhongguo Zhong Yao Za Zhi* 2003; **28**(3):

235-237. Chinese.
[3] Lin YL, Wang WY. Chemical constituents of *Vernonia patula*. *Chin Pharm J* 2002; **54**(3): 187-192.
[4] Evans WC. *Trease and Evan's pharmacognosy*. 3rd ed. Cambridge: University Press; 1989, p. 546-547.
[5] Badakhshan MP, Subramanion LJ, Lachimanan YL, Yeng C, Sreenivasan S. Antioxidant activity of methanol extracts of different parts of *Lantana camara*. *Asian Pac J Trop Biomed* 2012; **2**(12): 960-965.
[6] Jude EO, Anwanga EU, Samuel GF, Louis UA. Anti-inflammatory and analgesic activities of *Melanthera scandens*. *Asian Pac J Trop Biomed* 2012; **2**(2): 144-148.
[7] Perianayagam JB, Sharma SK, Pillai KK. Anti-inflammatory activity of *Trichodesma indicum* leaves extract in experimental animals. *J Ethnopharmacol* 2006; **104**: 410-414.
[8] Khadem A, Ayesha A, Nripendra NB. Analgesic, anti-inflammatory and anti-diarrheal activities of ethanolic leaf extract of *Typhonium trilobatum* L. *Asian Pac J Trop Biomed* 2012; **2**(9): 722-726.
[9] Francis MA, Andrew WV. Antioxidant activity, nitric oxide scavenging activity and phenolic contents of *Ocimum gratissimum* leaf extract. *J Med Plants Res* 2010; **4**(24): 2479-2487.
[10] Robu SV, aprotosoaie AC, Miron A, Cioanc O, stănescu U, Hăncianu M. *In vitro* antioxidant activity of ethanolic extracts from some *Lavandula* species cultivated in Romania. *Farmacia* 2012; **60**(3): 394-401.
[11] Sikder MA, Rahman MA, Islam MR, Kaisar MA, Rahman MS, Rashid MA. *In vitro* antioxidant, reducing power, free radical scavenging and membrane stabilizing activities of *Spilanthes calva*. *Bangladesh Pharm J* 2010; **13**(1): 63-67.
[12] Ejebe DE, Siminialayi IM, Emudainowho JO, Ofesi U, Morka L. Analgesic and anti-inflammatory activities of the ethanol extract of the leaves of *Helianthus annus* in Wistar rats. *Asian Pac J Trop Med* 2010; **3**(5): 341-347.
[13] Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W, et al. Pharmacological and biochemical demonstration of the role of cyclooxygenase-2 in inflammation and pain. *Proc Natl Acad Sci U S A* 1994; **91**: 12013-12017.
[14] Cuman RK, Bersani-Amadio CA, Fortes ZB. Influence of type 2 diabetes on the inflammatory response in rat. *Inflamm Res* 2001; **50**: 460-465.
[15] Vasudevan M, Gunman KK, Parle M. Antinociceptive and antiinflammatory effects of *Thespesia populnea* bark extract. *J Ethnopharmacol* 2007; **109**: 264-270.
[16] Sawadogo WR, Boly R, Lompo M, Some N, Lamien CE, Guissou IP et al. Anti-inflammatory, analgesic and antipyretic activities of *Dicliptera verticillata*. *Int J Pharmacol* 2006; **2**: 435-438.
[17] Shimoi K, Saka N, Kaji K, Nozawa R. Metabolic fate of luteolin and its functional activity on focal site. *Biofactors* 2000; **12**: 181-186.
[18] Pan MH, Lai CS, Ho CT. Anti-inflammatory activity of natural dietary flavonoids. *Food Funct* 2010; **1**: 15-31.
[19] Wu Y, Zhou C, Song L, Li X, Shi S, Mo J, et al. Effect of total phenolics from *Laggera alata* on acute and chronic inflammatory models. *J Ethnopharmacol* 2006; **108**: 243-250.
[20] Michaluart P, Masferrer JL, Carothers AM, Subbaramaiah K, Zweifel BS, Koboldt C, et al. Inhibitory effects of caffeic acid phenethyl ester on the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in a rat model of inflammation. *Cancer Res* 1999; **59**: 2347-2352.
[21] Frenkel K, Wei H, Bhimani R, Ye J, Zadunaisky JA, Huang MT, et al. Inhibition of tumor promoter mediated processes in mouse skin and bovine lens by caffeic acid phenethyl ester. *Cancer Res* 1993; **53**: 1255-1261.
[22] Rao CV, Desai D, Simi B, Kulkarni N, Amin S, Reddy BS. Inhibitory effect of caffeic acid esters on azoxymethane-induced biochemical changes and aberrant crypt foci formation in rat colon. *Cancer Res* 1993; **53**: 4182-4188.
[23] Márquez N, Sancho R, Macho A, Calzado MA, Fiebich BL, Muñoz E. Caffeic acid phenethyl ester inhibits T-cell activation by targeting both nuclear factor of activated T-cells and NF-kappaB transcription factors. *J Pharmacol Exp Ther* 2004; **308**: 993-1001.
[24] Dhara AK, Suba V, Sen T, Pal S, Chaudhuri AK. Preliminary studies on the anti-inflammatory and analgesic activity of the methanol fraction of the root extract of *Tragia involucrata* Linn. *J Ethnopharmacol* 2000; **72**: 265-268.
[25] Mondal SK, Chakroborty G, Gupta M, Mazumder UK. Hepatoprotective activity of *Diospyros malabarica* bark in carbon tetrachloride intoxicated rats. *Eur Bull Drug Res* 2005; **13**: 25-30.
[26] Marcocci L, Packer L, Droy-Lefaix MT, Sekaki A, Gardès-Albert M. Antioxidant action of *Ginkgo biloba* extracts EGb 761. *Methods Enzymol* 1994; **234**: 462-475.
[27] Meir S, Kanner J, Akiri B, Philosoph-Hadas S. Determination and involvement of aqueous reducing compounds in oxidative systems of various senescing leaves. *J Agric Food Chem* 1995; **43**: 1813-1819.
[28] Yamaguchi F, Ariga T, Yoshimara Y, Nakazawa H. Antioxidant and antiglycation of carcinol from *Garcina indica* fruit rind. *J Agric Food Chem* 2000; **48**: 180-185.
[29] Duh PD, Tu YY, Yen GC. Antioxidant activty of water extract of Harng Jyur (*Chrysanthemum morifolium* Ramat). *LWT-Food Sci Technol* 1999; **32**: 269-277.
[30] Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yasuhara T, et al. Effects of the interaction of tannins with co-existing substances. VI: Effects of tannins and related polyphenols on superoxide anion radical and on 1, 1-diphenyl-2-picrylhydrazyl radical. *Chem Pharm Bull* 1989; **37**: 2016-2021.
[31] Tanaka M, Kuie CW, Nagashima Y, Taguchi T. Application of antioxidative Maillard reaction products from histidine and glucose to sardine products. *Bull Jpn Soc Sci Fish* 1988; **54**: 1409-1414.
[32] Ningappa MB, Dinesha R, Srinivas L. Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (*Murraya koenigii* L.) extract. *Food Chem* 2008; **106**: 720-728.
[33] Ponou BK, Teponno RB, Ricciutelli M, Quassinti L, Bramucci M, Lupidi G, et al. Dimeric antioxidant and cytotoxic triterpenoid saponins from *Terminalia ivorensis* A. Chev. *Phytochemistry* 2010; **71**(17-18): 2108-2115.
[34] Yokozawa T, Chen CP, Dong E, Tanaka T, Nonaka GI, Nishioka I. Study on the inhibitory effect of tannins and flavonoids against the 1, 1-Diphenyl-2 picrylhydrazyl Radical. *Biochem Pharmacol* 1998; **56**(2): 213-222.
[35] Gülçin İ, Huyut Z, Elmastaş M, Aboul-Enein HY. Radical scavenging and antioxidant activity of tannic acid. *Arab J Chem* 2010; **3**: 43-53.
[36] Pérez-García F, Adzet T, Cañigueral S. Activity of artichoke leaf extract on reactive oxygen species in human leukocytes. *Free Radic Res* 2000; **33**(5): 661-665.
[37] Van Acker SA, Tromp MN, Haenen GR, van der Vijgh WJ, Bast A. Flavonoids as scavengers of nitric oxide radical. *Biochem Biophys Res Commun* 1995; **214**(3):755-759.