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Antioxidant and analgesic activities of ethanol leaf extract of *Brownea coccinea*

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ABSTRACT

The current study aimed to investigate the analgesic and antioxidant properties of ethanol extract of *Brownea coccinea* leaves (EEBL). The initial phytochemical study revealed the presence of tannins, flavonoids, saponins, gums, terpenoids, steroids and reducing sugars. The antioxidant property was checked by using DPPH free radical scavenging method which was conducted at doses of 25, 50, 100, 200, 400 and 800 µg/ml. Antioxidant activity was also tested by reducing power capacity (Fe^{3+} to Fe^{2+}) at concentrations of 25, 50, 100 and 200 µg/ml. All the results illustrated the presence of antioxidant property of *B. coccinea*. The analgesic activity was tested in mice at doses of 250 and 500 mg/kg by Hot Plate Method and Acetic Acid Induced Writhing Method. The analgesic analysis was conducted on Swiss Albino mice and the results clearly exhibited significant ($p < 0.001$ to 0.05) activity.

Keywords: *Brownea coccinea*, Fabaceae, phytochemical, antioxidant, analgesic, EEBL.

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INTRODUCTION

Plants are used as natural remedies by mankind from the very beginning of civilization (Seidl, 2002) and a large number of modern drugs that are used today had been obtained from natural sources on the basis of their use in folk culture. An estimated 80% of the population in developing countries depends on medicinal plants to meet their primary healthcare needs (Hostettmann and Marston, 2002).

One of the major contributing factors for developing degenerative diseases like atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer etc, is oxidative stress (Ames et al., 1993). Oxidative process results in the formation of free radicals such as superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$) and non-free-radical species like H_2O_2 and singled oxygen (Huda Faujan et al., 2009). Antioxidants have the ability of curbing free radical chain reaction. Plants are one of the best sources for natural antioxidants and consumption of plants rich in antioxidants have been shown to reduce the risk of degenerative diseases like Alzheimer's, cancer and diabetes (Bae et al., 2009; Parejo et al., 2002). Plants contain a large variety of free radical scavenging molecules such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, and tannins), nitrogen compounds (e.g. alkaloids, amines), vitamins and other endogenous metabolites rich in antioxidant activity (Cai et al., 2003; Zheng and Wang, 2001).

Brownea coccinea which is commonly known as Scarlet Flame Bean, Mountain Rose and Copper Hoop belongs to the subfamily Caesalpinioideae of the family Fabaceae and is a native plant of Tropical South America (Klitgaard, 1991; Hanelt, 2001). The plant is used in Guyana for treating gynecological disorders like dysmenorrhea and menorrhagia (De Filipps et al., 2004; Roth and Lindorf, 2013). In Bangladesh, the plant is known as Supti and is distributed in regions of Chittagong and Sylhet. The Chakma tribe of Bangladesh uses the root and leaves of this plant for treating gynecological problems (Roy et al., 2008). Based upon the folkloric use

of this plant the present study had been designed to investigate the antioxidant and analgesic activities of ethanol extract of *B. coccinea* leaves.

MATERIALS AND METHODS

Plant materials

The leaves of *B. coccinea* were collected from Bangladesh National Herbarium, Dhaka, Bangladesh during the month of May 2013 and were identified by the experts of Bangladesh National Herbarium (BNH), Dhaka. (Accession number: 38374).

Preparation of crude extracts

The leaves of the selected plant were washed with water, separated from undesirable materials. They were then dried at room temperature (24 to 26°C) for two days. The fully dried leaves were then grinded to coarse powder and were stored in zipper bag in refrigerator at +4°C for two weeks. After sieving, 400 g of powered material obtained was taken in a clean, flat-bottomed glass container and soaked in 600 ml of 95% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper. The filtrate (Ethanol extract) obtained was evaporated by Rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and at 68°C temperature. It rendered a gummy concentrate of dark bottle green color. The gummy concentrate was designated as crude ethanol extract of *B. coccinea* leaves (EEBL). Then the crude extract was dried in freeze drier and preserved at +4°C for two weeks.

Phytochemical test

The phytochemical screening of EEBL was carried out to identify the presence of tannins, flavonoids, saponins, gums, steroids, alkaloids, reducing sugars and terpenoids by known methods (Harborne, 1998; Sazada et al., 2009). The screening of the extract was performed using the following reagents and chemicals: Alkaloids with Wagner reagent, flavonoids with the use of concentrated HCl, tannins with 10% Ferric Chloride, and saponins with ability to produce suds. Gum was tested using Molish reagents and concentrated sulfuric acid, steroids with sulfuric acid, reducing sugar with the use of α-napthol and hydrochloric acid and terpenoids with chloroform and concentrated sulfuric acid. These phytochemicals are assumed to be responsible for biological activities of plants. Therefore before going for studying biological activities, phytochemical screening is required in order to ascertain the presence of phytochemicals from which we can make an initial assessment regarding activities.

Test animal

Swiss-albino mice aged 4 to 5 weeks with average weight of 22 to 30 g were used for the analgesic activity study. The mice were collected from the animal house of Department of Pharmaceutical Sciences of North South University and were housed in cages under standard environmental conditions of room temperature 24 ± 1°C and 55 to 65% relative humidity. The animals were provided with standard laboratory food and distilled water *ad libitum* and maintained at natural day night cycle.

Antioxidant activity of plant extracts

DPPH free radical scavenging assay

The antioxidant activity of EEBL was assessed according to the method established by Brand-William et al. (1995) with slight modifications. The extracts (25, 50, 100, 200, 400 and 800 µg/ml) were prepared in 90% ethanol. The positive control was ascorbic acid and was prepared at concentration of 25, 50, 100, 200, 400 and 800 µg/ml. DPPH solution was prepared in ethanol and 5 ml of the solution was mixed with the same volume of extract and the positive control ascorbic acid solution. After incubating the solutions for 30 min, the absorbance was read at 517 nm using a UV Spectrophotometer. The percentage of radical scavenging by the sample was determined by comparing it with that of the ascorbic acid control group.

Reducing power capacity

The reducing power capacity of EEBL was carried out according to the method determined by Oyaizu (1986). 1.0 ml of the extract solution of concentrations 25, 50, 100 and 200 µg/ml was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 min. After incubation, 2.5 ml of trichloroacetic acid was added to the mixture followed by centrifugation at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride. The absorbance of the resulting solution was measured at 700 nm against a blank by using UV Spectrophotometer. An increase in the absorbance of the reaction mixture indicates increasing reducing power.

Analgesic activity of plant extracts

Hot plate test method

The hot-plate test employed for measurement of analgesic activity was done in accordance to the method previously described by Lanhers et al. (1992) and which was modified by Mahomed and Ojewole (2004). Mice were divided into four groups- control, positive control and test groups consisting of five mice in each group. The mice of each group were placed in the beaker (on the hot plate) in order to obtain its response to electrical heat induced pain stimulus. Licking of the paws or jumping out of the beaker was taken as an indicator of the animal's response to heat-induced pain stimulus. The time for each mouse to lick its paws or jump out of the beaker was taken as reaction time. Before treatment, the reaction time was taken once. Each of the test mice were thereafter treated with distilled water, Diclofenac (10 mg/kg of body weight) and EEBL at the doses of 250 mg/kg and 500 mg/kg of body weight orally. The temperature of the hot plate was maintained at 54 ± 1°C. The test sample, control and standard drug were given 30 min prior to the beginning of the experiment. The mice were observed before and at 60, 120, 180 and 240 min after administration. The reaction time was recorded when the mice licked their fore or hind paws or jumped prior to and 0, 60, 120, 180 and 240 min after oral administration of the samples. Percent analgesic score was calculated as:

$(PAS) = T_b\text{-}T_a/T_b \times 100$

Where, T_b = Reaction time (in second) before drug administration; T_a = Reaction time (in seconds) after drug administration.

Acetic acid induced writhing test method

The analgesic activity of the plant extract was evaluated by the

method described by Koster et al. (1959). The mice were divided into 4 groups (control, standard and test groups) consisting of 5 mice in each group. The control group received normal saline solution (10 ml/kg), standard group received Diclofenac sodium (10 mg/kg) and the test group received the plant extract at doses of 250 and 500 mg/kg of body weight. After 30 min, 0.7% v/v acetic acid solution at 10 ml/kg body weight was administered intraperitoneally to all the mice in the different groups. The mice were kept individually under glass jar for observation. Each mouse of all groups were observed individually for counting the number of writhing they made in 10 min commencing just 5 min after the intraperitoneal administration of acetic acid solution.

Statistical analysis

The data are expressed as the mean ± SEM and were evaluated by one – way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Dunnett's test are well known and widely used in multiple comparison procedure for simultaneously comparing all active treatments with a control when sampling from a distribution where the normality assumption is reasonable. It is very commonly used in medical experiments, for example comparing blood count measurements on three groups of animals, one of which served as a control while the other two were treated with two different drugs. In our study, the results obtained were compared with the vehicle control group. The P value <0.05, $p<0.01$ and $p<0.001$ were considered as statistically significant. All statistical tests were carried out using SPSS statistical software.

RESULTS

Phytochemical analysis

The phytochemical screening of the ethanol extract of *B. coccinea* revealed the presence of tannin, flavonoid, saponin, gum, steroid and reducing sugars whereas alkaloid and terpenoid were not detected (Table 1).

Table 1. Phytochemical analysis of EEBL.

Phytochemicals	Results	Indication
Alkaloids	-	Absent
Flavonoids	+	Present
Tannins	+	Present
Saponins	+	Present
Terpenoids	-	Absent
Reducing Sugars	+	Present
Gums	+	Present
Steroids	+	Present

Antioxidant activity of plant extract

Free radical scavenging activity by DPPH

The free radical scavenging activity of EEBL is presented in Table 2. The % scavenging of DPPH radical was found to increase with increasing concentration of the ethanol extract of the leaves of *B. coccinea*. The most notable scavenging effect of the ethanol extract was 74.46% at a concentration of 800 µg/ml. The results were calculated as IC_{50} values, which denotes the concentration of sample required to scavenge 50% of DPPH free radicals. The IC_{50} value of ascorbic acid and ethanol extract of the leaves of *B. coccinea* was found 3.02 and 16.53 µg/ml, respectively.

Reducing power capacity

The reducing power capacity of the extract and the standard (Ascorbic Acid) is presented in Table 3.

Reducing power of the fractions was assessed using ferric to ferrous reduction activity as determined spectrophotometrically from the formation of Perl's Prussian blue color complex. The extracts were found to display moderate reducing power. Reducing power was found to increase with increasing concentration of the extracts in all cases but was not comparable to the standard.

Analgesic activity of plant extract

Hot plate method

In the present study of analgesic activity by hotplate method, EEBL showed a significant ($p < 0.01$) dose depended reduction of thermally induced pain stimulus at doses of 250 and 500 mg/kg respectively at various time intervals (Table 4). EEBL at the dose of 500 mg/kg showed significant ($p < 0.05$) activity from first till fourth hour after the drug administration. Positive control (Diclofenac) showed highly significant ($p < 0.001$) analgesic effect at the dose of 10 mg/kg during this timing interval.

Acetic acid-induced writhing method

In acetic acid induced writhing method, EEBL showed significant ($p < 0.001$) reduction in the number of writhings following intraperitoneal administration of acetic acid in a dose depended manner. EEBL at doses of 250 and 500 mg/kg inhibited writhing by 56 and 68%, respectively. However, for the standard drug Diclofenac the percentage inhibition of writhing was 84% (Table 5).

DISCUSSION

There are no scientific reports published on the antioxidant and analgesic activities of *B. coccinea*. The result of our study shows *B. coccinea* has significant antioxidant and analgesic properties. The result of the phytochemical investigation indicates the presence of

Table 2. Free radical scavenging activity study of EEBL by DPPH method.

Sample	Concentration (µg/ml)	Mean % DPPH scavenging activity ± SD	IC_{50} Value (µg/ml)
Ascorbic Acid	25	78.00 ± 0.576***	3.02
	50	79.24 ± 0.150***	
	100	81.00 ± 0.570***	
	200	81.90 ± 0.620***	
	400	83.32 ± 0.461***	
	800	84.54 ± 0.574*	
EEBL	25	32.23 ± 2.643**	16.53
	50	44.24 ± 3.717**	
	100	54.80 ± 2.245**	
	200	61.30 ± 1.360***	
	400	67.12 ± 2.601**	
	800	74.46 ± 4.549**	

Values in the table are expressed as mean ± SD, *P < 0.05, **P < 0.01, ***P < 0.001 were considered statistically significant in comparison with control.

Table 3. Reducing power capacity of EEBL.

Sample	Concentration (µg/ml)	Absorbance	Reducing capacity (Mean ± SD)	% Reducing capacity
Ascorbic acid	25	0.477	206.67 ± 4.16	210
	50	0.495	218.00 ± 3.00	221
	100	0.528	239.33 ± 4.04	243
	200	0.705	356.33 ± 5.68	358
EEBL	25	0.208	35.06 ± 5.04	35
	50	0.312	102.59 ± 4.21	103
	100	0.343	122.72 ± 6.44	123
	200	0.440	185.71 ± 49.31	186

SD = Standard Deviation, % = Percentage.

Table 4. Analgesic activity study of EEBL by hot plate method.

Group	Reaction time at different time intervals (in seconds)				
	0 min	60 min	120 min	180 min	240 min
Control	10.70 ± 1.893	8.00 ± 1.820	6.58 ± 1.432	5.52 ± 1.227	5.00 ± 0.989
Standard	9.14 ± 1.171	12.60 ± 2.113**	14.16 ± 2.406***	15.96 ± 1.512***	12.48 ± 1.561***
EEBL (250 mg/kg)	9.07 ± 0.535	12.02 ± 0.570**	13.64 ± 0.404***	14.74 ± 0.583***	11.94 ± 0.282***
EEBL (500 mg/kg)	8.39 ± 0.587	11.46 ± 0.758*	12.74 ± 0.505***	14.36 ± 0.592***	11.26 ± 0.704***

Values in the table are expressed as mean ± SEM, *P < 0.05, **P < 0.01, ***P < 0.001 were considered statistically significant in comparison with control.

tannins, flavonoids, saponins, gum, steroids and reducing sugars in the extract. Previous studies had shown the presence of flavonoids (Vinson et al., 1995; Ramesh et al., 1998) and tannins (Kumar et al., 2010; Vanu et al., 2006) are associated with antioxidant and analgesic activities. The antioxidant and analgesic activity observed can be attributed to the presence of these phytochemicals.

To determine antioxidant activity of *B. coccinea*, two different methods were employed: free radical scavenging activity by DPPH method and reducing power capacity. In the current study the antioxidant activity of ethanol extract of *B. coccinea* in terms of its ability to scavenge DPPH radical was conducted and compared to

Table 5. Analgesic activity study of EEBL by acetic acid induced writhing method.

Group	Number of writhings	Percentage inhibition
Control	26.60 ± 1.07	
Standard	4.00 ± 0.31***	84%
EEBL (250 mg/kg)	11.80 ± 2.70***	56%
EEBL (500 mg/kg)	8.60 ± 1.46***	68%

Values in the table are expressed as mean ± SEM, (n=5); ***P < 0.001 was considered statistically significant in comparison with control.

the reference standard- ascorbic acid. When an antioxidant donates an electron to the DPPH, there is a change in color of the DPPH solution from purple to yellow which can be determined by changes in absorbance at 517 nm (Kale et al., 2011). The DPPH radical scavenging activity of the extract increased along with an increase in the extract concentration. The result of the radical scavenging was expressed in terms of half-inhibition concentration (IC_{50}), which denotes the concentration required to scavenge 50% of DPPH radicals (Molyneux, 2004). The reducing power capacity is based on the concept of the ability of an antioxidant to convert Fe^{3+} to Fe^{2+} which subsequently reacts with ferric chloride to form ferric ferrous complex having an absorption maxima at 700 nm (Senevirathne et al., 2006). From our study it was found that the extract possesses moderate reducing power. In this study, ascorbic acid was used as reference standard. The reducing power was found to increase in a dose dependent manner; however the reducing power of the extract was lower than that of ascorbic acid.

The hot plate test is based on the principle of inducing pain by means of thermal stimuli (Mandegary et al., 2004). The hot plate test was designed to study nociception that had been centrally mediated (Parkhouse and Pleuvry, 1979). Our extract has the ability to extend the reaction latency to the pain induced by thermal stimuli it can be suggested that it possess centrally acting analgesic activity. To investigate the presence of peripheral analgesic activity, the acetic acid induced writhing method was used. Intraperitoneal administration of acetic acid induces an increase in the level of prostaglandins like PGE2 and PGF2α (Derardt et al., 1980) and lipooxygenase products (Sulaiman et al., 2008) within the peritoneal cavity. The extract may exert peripheral analgesic activity possibly by inhibiting the increase in prostaglandin level or by exerting its action on the visceral receptors that are sensitive to acetic acid (Magaji et al., 2008). Dysmenorrhea is a painful disorder of women which is initiated by an increased release of prostaglandins like PGF2α from the endometrium during the menstrual phase (Dawood, 2006). The plant is used for remedial purposes for gynecological pain associated conditions possibly by blocking the release of endogenous prostaglandins in the endometrium.

Conclusion

The results from our study shows that ethanol leaf extract of *Brownea coccinea* have significant antioxidant and analgesic properties. Further investigations are required to find the active component of the extract and to confirm the mechanism of action for the development of a potent antioxidant and analgesic agent.

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