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Research Article
Phytochemical Screening and Pharmacological Activities of the Ethanolic Stem Extract of *Cleome gynandra*

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Abstract

Objective: This study aimed to identify the phytochemical compounds of the ethanolic stem extract of *Cleome gynandra* (CGEE) and to determine the antioxidant, membrane stability and analgesic activities of CGEE. **Materials and Methods:** Phytochemical analysis was conducted qualitatively. Content of total phenolic compounds and tannins was determined using Folin Ciocalteau reagent. Antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl assay, membrane stabilizing activity was tested using hypotonic solution and heat-induced hemolysis and analgesic activities were evaluated using the acetic acid-induced writhing test in mice. **Results:** CGEE is rich in tannins, flavonoids, steroids, glycosides, acidic compounds and proteins. The total phenolic and tannin contents were 237.92 mg gallic acid equivalents (GAE)/100 g and 21.07 mg GAE/100 g of dried plant extract, respectively. CGEE also displayed promising DPPH free radical scavenging activity, with an IC_{50} value of 9.62 μg mL^{-1}. In the hypotonic solution-induced hemolysis test, the extract showed 43.67, 42.85 and 38.66% inhibition at 0.5, 1.0 and 2.0 mg mL^{-1}, respectively, whereas the standard resulted in 30.57% inhibition. In the heat-induced hemolysis test, 1 mg mL^{-1} of extract resulted in 84.43% inhibition of hemolysis. Furthermore, in the analgesic activity test, CGEE doses of 250 and 500 mg kg^{-1} body weight resulted in good inhibition of the writhing reflex (40.87 and 53.92%, respectively), while the standard drug (diclofenac sodium) at 25 mg kg^{-1} body weight resulted in inhibition of 64.35%. **Conclusion:** Results of this study suggest that the stem extract of *Cleome gynandra* possesses antioxidant, membrane stabilizing and analgesic activities.

Key words: *Cleome gynandra*, antioxidants, stem extract, membrane stabilizing, oxidative stress

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Oxidative stress is a factor that leads to the onset of numerous chronic diseases, such as hypertension, diabetes, cancer and neurodegenerative disorders[1,2]. In healthy individuals, the production of free radicals is balanced by the antioxidative defense system. Imbalance occurs when equilibrium favors free radical generation due to the depletion of antioxidant levels, thus causing oxidative stress[3]. The inflammatory reaction is part of the body's protective response to foreign organisms, including viruses, dust particles and pathogens[4]. Studies have shown that inflammatory reactions are positively related to the presence of free radicals and establishment of the oxidative stress condition. During the inflammatory reaction, free radicals and reactive oxygen species (ROS) such as non-radical hydrogen peroxide, nitrite oxide, superoxide and hydroxyl are overproduced. The excess free radicals and ROS can cause injury to the tissue by damaging macromolecules and inducing lipid peroxidation of membranes[5], thereby leading to the onset and progression of various inflammatory associated diseases. Tissues, consequently, require antioxidants to neutralize and decompose these free radicals and ROS. Antioxidants protect cells from damage caused by these free radicals by inhibiting or delaying cellular damage, mainly through free radical scavenging properties[6]. Membrane stabilization is the process of maintaining the integrity of biological membranes, such as those of erythrocytes and lysosomal membranes, against osmotic and heat-induced lysis[7]. Stabilization of lysosomal membranes is important for limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophils, such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release[8]. Therefore, plants with antioxidant and membrane stabilizing properties should offer more significant protection against inflammatory induced diseases than plants without these traits.

Currently, numerous scientific studies are focused on discovering natural drugs from medicinal plants due to the promising therapeutic effects of phytochemicals and their less severe side effects. *Cleome gynandra* is a genus of flowering plant in the family Cleomaceae. The genus includes about 170 species of herbaceous annual or perennial plants and shrubs[9]. Nutritional analyses have found that *C. gynandra* is high in micronutrients, including β-carotene, folic acid, ascorbic acid, vitamin E, oxalic acid, iron and calcium. For centuries, *C. gynandra* has been incorporated in Ayurvedic medicine to treat various illnesses, such as gulma (tumor, irregularity or diverticulosis), krmiroga (worm infection), asthila (prostate enlargement), kandu (pruritus) and karnaroga (ear infections)[10]. Recent scientific studies have also reported the pharmacological activities of different parts of this plant and they include antioxidant[11,12], antidiabetic[13], anticarcinogenic and anti-inflammatory[14] properties. These activities are attributable to the high concentrations of bioactive compounds present, namely flavonoids, tannins, glucosinolates and iridoids[15].

Despite extensive literature on the pharmacological activities of *C. gynandra*, to date there is no information available about the possible pharmacological effect of the stem extract of *C. gynandra*. Previous studies of this plant focused mainly on the leaves. Therefore, the goal of this study was to assess the antioxidant, analgesic and membrane stabilizing activities of the ethanolic stem extract of *C. gynandra* (CGEE). Results of this study will provide an evidence-based validation on the folkloric use of this plant.

MATERIALS AND METHODS

Chemicals: Sodium carbonate and Folin-Ciocalteu (FC) reagents were obtained from Merck (Darmstadt, Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium dichromate, gallic acid (GA), ascorbic acid (AA), aluminium chloride, sulphuric acid, nitric acid, sodium hydroxide, ethylenediaminetetraacetic acid (EDTA), diclofenac sodium, sodium nitrous, Tween-80 and ferrous chloride were purchased from the Sigma Chemical Co., St. Louis, MO, USA.

Plant materials: *Cleome gynandra* was collected from Bandarban, Bangladesh in July 2018. The plants were washed and dried under direct sunlight for one week. The plant was identified by the taxonomist at the Bangladesh National Herbarium, Mirpur-1, Dhaka-1216 (voucher specimen no: DACB-47043).

Preparation of the extract: Two hundred grams of dried stem were ground into a fine powder using a grinder machine. The powder was then extracted with 1500 mL of ethanol. Thereafter, the mixture was filtered using cotton followed by Whatman (No. 1) filter paper. The obtained filtrate was evaporated to dryness in open air to yield the crude ethanolic extract, which was denoted as CGEE. It was kept at 4°C until used for further analysis.

Qualitative phytochemical screening: The following reagents were used to quantitatively screen for the presence of the phytochemicals: Reducing sugar was identified using

Fehling's solution and Benedict's reagent; alkaloids with Mayer's and Dragendroff's reagent; saponins with distilled water; glycosides with sodium hydroxide solution; steroids with sulphuric acid; tannins with ferric chloride and potassium dichromate; and gum content with Molish reagent[16].

Measurement of total phenolic content: The FC technique was used to estimate the phenolic content in CGEE[17]. For this test, an aliquot of CGEE or a positive control was mixed with 2 mL of FC reagent (1:10, v/v) and 2 mL of sodium carbonate (75 g L^{-1}). The tubes were shaken for 15 sec and incubated for 20 min at room temperature for colour development. Absorbance was recorded at 750 nm using an UV spectrophotometer. The total phenolic contents were expressed as mg of gallic acid equivalents (GAE) per 100 g of the dried extract.

Measurement of total tannin content: Total tannin content was also measured using FC reagent[18]. To estimate the total tannin content, 0.1 mL of CGEE or a positive control was mixed with 7.5 mL of distilled water and 0.5 mL of FC reagent. The mixture was thoroughly mixed and kept for 5 min. Next, 1 mL of 35% sodium carbonate was added, followed by dilution with 10 mL of distilled water. The mixture was incubated at room temperature for 30 min. Absorbance was recorded at 725 nm using the UV spectrophotometer. The total tannin contents were expressed as mg of GAE per 100 g of the dried extract.

DPPH Scavenging assay: The antioxidant activity of the extract was estimated using the DPPH free radical scavenging assay[19]. DPPH solution at the concentration of 0.04% (w/v) was prepared in ethanol. A volume of 1 mL of CGEE at different concentrations was mixed with 3 mL of DPPH solution. The mixture was shaken thoroughly and placed aside in the dark for the 30 min reaction period at room temperature. After incubation, the absorbance of the mixture was recorded at 517 nm using the UV spectrophotometer. The following equation was used to calculate the DPPH free radical scavenging percentage[20]:

$$\text{DPPH free radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 is the absorbance of the control (DPPH solution without sample) and A_1 is the absorbance of the plant extract/positive control. Ascorbic acid was used as the positive control. The percentage of scavenging activity was then plotted against log concentration and a graph for the half-maximal inhibitory concentration (IC_{50}) was created.

Membrane stabilization assays

Hypotonic solution-induced hemolysis: The membrane stability activity of the extract was evaluated using hypotonic solution-induced hemolysis. The test sample consisted of a stock erythrocyte suspension (0.50 mL), 4.5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffer saline (pH 7.4) and either CGEE (2.0 mg mL^{-1}) or acetyl salicylic acid (0.1 mg mL^{-1}). The mixtures were incubated for 10 min at room temperature. After incubation, the mixtures were centrifuged for 10 min at 3000 g and the supernatant was collected. The absorbance of the supernatant was measured at 540 nm using the UV spectrophotometer. The percentage inhibition of either hemolysis or membrane stabilization was calculated using the following equation[18]:

$$\text{Inhibition of hemolysis (\%)} = \frac{(OD_1 - OD_2)}{OD_1} \times 100$$

where, OD_1 is the optical density of the hypotonic buffered saline solution alone (control) and OD_2 is the optical density of the test sample in hypotonic solution.

Heat-induced hemolysis: For this analysis, 5 mL of isotonic buffer containing 1.0 mg mL^{-1} of different concentrations of CGEE were added to two sets of centrifuge tubes[21]. The vehicle control group was prepared with the same amount of extract. Erythrocyte suspension (30 mL) was added to each tube and inverted gently. One set of tubes was incubated at 54°C for 20 min in a water bath and the other set of tubes was placed into an ice bath at 0-5°C. The mixtures then were centrifuged at 1300 g for 3 min. The supernatant was collected and the absorbance was recorded at 540 nm using the UV spectrophotometer. The percentage of inhibition or acceleration of hemolysis was calculated using the following equation[18]:

$$\text{Inhibition of hemolysis (\%)} = \frac{1 - OD_2 - OD_1}{OD_3 - OD_1} \times 100$$

where, OD_1 is the optical density of the unheated test sample, OD_2 is the optical density of the heated test sample and OD_3 is the optical density of the heated control sample.

Analgesic activity assay

Acetic acid-induced writhing technique: Twenty Swiss albino mice (18-22 g) were used in this study. Animals were divided into four groups of five and fasted for 2 h before commencement of the test. The control group (Group I) was treated with 1% Tween-80 solution dissolved in water

(10 mL kg^{-1}). The positive control group (Group II) was treated with diclofenac sodium at a dose of 25 mg kg^{-1} body weight. Group IIIs and IV were treated with CGEE at doses of 250 and 500 mg kg^{-1} body weight, respectively. Thirty minutes after the treatment, 0.7% acetic acid was injected intraperitoneally. Five minutes after the injection of acetic acid, the number of abdominal constrictions (writhing) within the 15 min observation period was recorded[22].

Statistical analysis: Mean±standard error of the mean (SEM) was used to present the data. All parameters were evaluated for their significance level by correlation and regression analysis and t-tests ($p<0.05$) also were used. Microsoft Excel 2016 was used for both statistical analysis and graphical exhibition.

RESULTS

Phytochemical screening of CGEE: Phytochemical screening of CGEE indicated the presence of reducing sugar, combined reducing sugar, tannins, flavonoids, glycosides, proteins and steroids (Table 1).

Total phenol and tannin content of CGEE: The total amounts of phenol and tannin in CGEE were calculated from the linear regression equation of gallic acid standard calibration curves ($y = 0.113x - 0.201$, $R_2 = 0.8626$ and $y = 0.014x - 0.010$, $R_2 = 0.947$, respectively) and expressed in GAE. Total phenol and total tannin contents in CGEE were 237.92±0.0129 mg GAE/100 g and 21.07±0.0004 mg GAE/100 g of CGEE.

DPPH free radical scavenging activity of CGEE: Antioxidant activity of CGEE was evaluated using the DPPH free radical scavenging method. The IC_{50} values of CGEE and ascorbic acid were 9.62 and 5.51 µg mL^{-1}, respectively (Fig. 1).

Membrane stabilization activity of CGEE: Data in Table 2 show that CGEE inhibited lysis induced by the hypotonic solution, as indicated by the high percentage inhibition of hemolysis (3.66, 42.85 and 43.67%) recorded for doses of 2, 1 and 0.5 mg mL^{-1}, respectively. In the heat-induced hemolysis test, inhibition of hemolysis was 84.43% at the concentration of 1 mg mL^{-1} CGEE.

Analgesic effect of CGEE

Writhing test: Table 3 shows the effect of different concentrations of CGEE on the acetic acid-induced writhing reflex in mice. CGEE (250 and 500 mg kg^{-1}) and the positive control, diclofenac sodium (25 mg kg^{-1} body weight), significantly ($p<0.01$) inhibited abdominal writhing in mice compared to the control group. The writhing inhibition of CGEE occurred in a dose-dependent manner, as the percentage of inhibition increased when the concentration of CGEE increased.

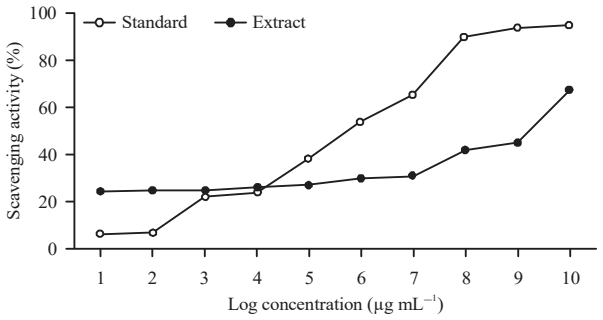

Fig. 1: DPPH scavenging activity of CGEE and ascorbic acid

Table 1: Qualitative phytochemical screening of ethanolic stem extract of *C. gynandra* (CGEE)

Phytochemical groups	CGEE
Reducing sugar	+
Combined reducing sugar	+
Tannins	+
Flavonoids	+
Saponins	-
Gums	-
Steroids	+
Alkaloids	+
Glycoside	+
Proteins	+
Acidic compounds	-

+: Presence, -: Absence

Table 2: Effect of different concentrations of CGEE on hypotonicity induced haemolysis of erythrocyte membrane

Treatments	Concentration	Inhibition of hemolysis (%)
Hypotonic medium (control)	50.0 mM	0.00
Standard acetyl (salicylic acid)	0.1 mg mL^{-1}	30.57
CGEE	2.0 mg mL^{-1}	38.66
	1.0 mg mL^{-1}	42.85
	0.5 mg mL^{-1}	43.67

DISCUSSION

In this study, phytochemical compounds of CGEE were evaluated using qualitative and quantitative assays. Pharmacological activities of CGEE, namely antioxidant, analgesic and membrane stability properties, were also studied. The results indicated that CGEE contains a reducing sugar, combined reducing sugar, tannins, flavonoids, alkaloids, glycosides, proteins and steroids but it lacks saponins, acidic

Table 3: Effect of different concentrations of CGEE on acetic acid-induced writhing reflex in mice

Animal groups	Mean of writhing	Writhing±SEM (%)	Writhing inhibition (%)	t-test (p-value)
Negative control (1% Tween-80)	4.60	100.00±2.12	0.00	
Diclofenac-Na (25 mg kg^{-1})	1.64	35.65±1.39	64.35	5.8320**
CGEE (250 mg kg^{-1})	2.72	59.13±1.39	40.87	4.4100**
CGEE (500 mg kg^{-1})	2.12	46.08±0.93	53.92	5.3600**

CGEE: *C. gynandra* ethanolic stem extract; **p<0.01, SEM: Standard error of mean

compounds and gums (Table 1). CGEE contained a good amount of total phenolic and total tannin contents that were comparable to the standards (Fig. 1).

Plants produce various secondary metabolites as their natural defense[23]. Among these secondary metabolites, few are toxic to animals and most of them possess various therapeutic properties. For example, glycosides and flavonoids have strong antidiabetic and antioxidant activities[24]. Different studies have proposed that various sorts of polyphenol compounds, such as phenolic acids, flavonoids and tannins, have numerous biological effects, including antioxidant activity[25,26]. In agreement with these statements, our results showed that CGEE possessed antioxidant properties and acted by scavenging DPPH free radicals. Elmastas *et al.*[27] and Wang *et al.*[28], reported that phenolic compounds contain hydroxyl groups that may contribute to antioxidant activity and play a critical role in scavenging free radicals. Other studies have reported the positive correlation between the amount of phenolic contents and antioxidant activity of a plant[29,30]. Moreover, tannins are generally defined as naturally occurring polyphenol compounds of high molecular weight that can form a complex with proteins. Tannins are an important source of protein in animals but the amounts of tannins present vary and are very changeable and their effects on animals range from valuable to toxic to lethal[31].

Health risks can be prevented by consuming foods containing antioxidants and analgesics[32] which can have positive effects on cell structures such as the cell membrane. The robustness of a cell depends on the wholeness of its membranes. One way to test the effect of a compound on membrane stability is to expose red blood cells to a hypotonic or heated medium. Membrane stabilizers should protect the membrane against injury and elicit anti-inflammatory effects[33]. Compounds with membrane-stabilizing properties are well known for their ability to interfere with the initial phase of the inflammatory reaction that prevents the release of phospholipase, which stimulates the formation of inflammatory mediators[34]. Our results indicated that CGEE contains phenols and phenolic compounds inhibit prostaglandin cyclooxygenase as well as inflammatory mediators[35]. Results of our experiments showed that CGEE at a concentration of 1 mg mL^{-1} better prevented hypotonic solution-induced and heat-induced lysis of the human erythrocyte membrane compared to the standard acetyl salicylic acid (0.1 mg mL^{-1}). This finding suggests that CGEE may possess good membrane stabilizing activity.

The anti-nociceptive acetic acid-induced writhing reflex model was used to identify the analgesic activity of CGEE. Intraperitoneal injection of acetic acid creates a pain sensation[36] and the writhing reflex in animals by activating the chemo-sensitive nociceptors[37]. Acetic acid-induced twisting represents the pain sensation by triggering the localized inflammatory response. CGEE treatment resulted in significant inhibition of writhing compared to the standard drug diclofenac sodium (Table 3). The polar compounds present in the plant extract may explain the observed analgesic activity. The 500 mg kg^{-1} dose had the highest inhibition rate, the inhibitory effect of the positive control was greater and the inhibitory effects were statistically significant (control vs. CGEE 250 mg kg^{-1}, $p<0.01$ and control vs. CGEE 500 mg kg^{-1}, $p<0.05$). These results demonstrate that CGEE at the given doses significantly reduced the acetic acid-induced writhing reflex in mice.

In summary, we have demonstrated that CGEE has antioxidant, membrane stabilizing and analgesic properties. The results of this study provide a scientific basis for the utilization of this plant in folk medicine for the treatment of oxidative disorders. We can also use it to prevent cell lysis. This study, however, had some limitations. For example, all tests were conducted using an *in vitro* model. Further confirmation through *in vivo* models is needed to validate the findings. Additionally, in-depth study is required to elucidate the underlying mechanisms responsible for the antioxidant, anti-inflammatory and membrane stabilizing properties of CGEE.

CONCLUSION

This study reports that CGEE has potential antioxidant activity, free-radical scavenging activity, an analgesic effect and a membrane stabilizing effect. However, these preliminary results do not explain the actual mechanisms for the various pharmacological actions, thus more in-depth studies (including isolation and identification of active compounds

and in-depth pharmacological mechanistic assays) are required to elucidate the exact mechanisms of action of the extract and its active compound(s).

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REFERENCES

1. Fang, Y.Z., S. Yang and G. Wu, 2002. Free radicals, antioxidants and nutrition. Nutrition, 18: 872-879.
2. Uttara, B., A.V. Singh, P. Zamboni and R.T. Mahajan, 2009. Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Curr. Neuropharmacol., 7: 65-74.
3. Shyur, L.F., J.H. Tsung, J.H. Chen, C.Y. Chiu and C.P. Lo, 2005. Antioxidant properties of extracts from medicinal plants popularly used in Taiwan. Int. J. Applied Sci. Eng., 3: 195-202.
4. Chatterjee, S., 2016. Oxidative Stress, Inflammation and Disease. In: Oxidative Stress and Biomaterials. Dziubla, T. and D.A. Butterfield, Academic Press, United States, pp: 35-58.
5. Gillham, B., D. Papachristodoulou and J. Thomas, 1997. Free Radicals in Health and Disease. 7th Edn. but terworth Heinemann, Oxford..
6. Chakraborty, P., S. Kumar, D. Dutta and V. Gupta, 2009. Role of antioxidants in common health diseases. Res. J. Pharm. Technol., 2: 238-244.
7. Oyedapo, O.O., B.A. Akinpelu, K.F. Akinwunmi, M.O. Adeyinka and F.O. Sipeolu, 2010. Red blood cell membrane stabilizing potentials of extracts of *Lantana camara* and its fractions. Int. J. Plant Physiol. Biochem., 2: 46-51.
8. Vadivu, R. and K.S. Lakshmi, 2008. *In vitro* and *in vivo* anti-inflammatory activity of leaves of *Symplocos cochinchnensis* (Lour) *Moore* ssp. Laurina. Bangladesh J. Pharmacol., 3: 121-124.
9. Abd El-Kawy, M.A., S. El-Deib, Z. El-Khyat and Y.A. Mikhail, 2000. Chemical and biological studies of cleome droserifolia (Forssk.) Del. part I. Egypt. J. Biomed. Sci., 6: 204-215.
10. Government of India, Ministry of Ayush, 2016. The Ayurvedic Pharmacopoeia of India Part 1. Vol. 9, 1st Edn., Pharmacopoeia Commission for Indian Medicine and Homeopathy, New Delhi, India.
11. Anbazhagi, T., K. Kadavul, G. Suguna and A.J.A. Petrus, 2009. Studies on the pharmacognostical and *in vitro* antioxidant potential of *Cleome gynandra* Linn. leaves. Nat. Prod. Rad., 8: 151-157.
12. Moyo, M., S.O. Amoo, B. Ncube, A.R. Ndhlala, J.F. Finnie and J. van Staden, 2013. Phytochemical and antioxidant properties of unconventional leafy vegetables consumed in southern Africa. S. Afr. J. Bot., 84: 65-71.
13. Nicola, W.G., K.M. Ibrahim, T.H. Mikhail, R.B. Girgis and M.E. Khadr, 1996. Role of the hypoglycemic plant extract cleome droserifolia in improving glucose and lipid metabolism and its relation to insulin resistance in fatty liver. Boll. Chim. Farm., 135: 507-517.
14. Bala, A., P. Chetia, N. Dolai, B. Khandelwal and P.K. Haldar, 2014. Cat's whiskers flavonoid attenuated oxidative DNA damage and acute inflammation: Its importance in lymphocytes of patients with rheumatoid arthritis. Inflammopharmacology, 22: 55-61.
15. Yang, R.Y., S. Lin and G. Kuo, 2008. Content and distribution of flavonoids among 91 edible plant species. Asia Pac. J. Clin. Nutr., 17: 275-279.
16. Ghani, A., 2003. Medicinal plants of Bangladesh: Chemical Constituents and Uses, 2nd Edn., Asiatic Society of Bangladesh, Dhaka, Bangladesh, Pages: 460.
17. Hossain, A., F. Islam, M. Saifuzzaman, M.A.S. Saeed, M.K. Islam, G.M.M. Murshid and M.M. Rahman, 2016. Bioactivity of *Boehmeria macrophylla* (Urticaceae) leaf extract. Orien. Pharm. Exp. Med., 16: 233-241.
18. Islam, T., A. Das, K.B. Shill, P. Karmakar, S. Islam and M.M. Sattar, 2015. Evaluation of membrane stabilizing, anthelmintic, antioxidant activity with phytochemical screening of methanolic extract of Neolamarckiacadamba fruits. J. Med. Plant Res., 9: 151-158.
19. Sumi, S.A., M.A. Siraj, A. Hossain, M.S. Mia, S. Afrin and M.M. Rahman, 2016. Investigation of the key pharmacological activities of *Ficusracemosa* and analysis of its major bioactive polyphenols by HPLC-DAD. Evidence-Based Complement. Altern. Med., Vol. 2016. 10.1155/2016/3874516
20. Hossain, M.A. and M.D. Shah, 2015. A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant *Merremia borneensis*. Arab. J. Chem., 8: 66-71.
21. Shinde, U.A., A.S. Phadke, A.M. Nair, A.A. Mungantiwar, V.J. Dikshit and M.N. Saraf, 1999. Membrane stabilizing activity-a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia, 70: 251-257.
22. Ahmed, F., M.S. Selim, A.K. Das and M.S. Choudhuri, 2004. Anti-inflammatory and antinociceptive activities of *Lippianodiflora* Linn., Pharmazie, 59: 329-330.
23. Sahu, M.C. and R.N. Padhy, 2013. *In vitro* antibacterial potency of *Butea monosperma* Lam. against 12 clinically isolated multidrug resistant bacteria. Asian Pac. J. Trop. Dis., 3: 217-226.

24. Katsube, T., M. Yamasaki, K. Shiwaku, T. Ishijima, I. Matsumoto, K. Abe and Y. Yamasaki, 2010. Effect of flavonol glycoside in mulberry (*Morus alba* L.) leaf on glucose metabolism and oxidative stress in liver in diet induced obese mice. J. Sci. Food. Agric., 90: 2386-2392.
25. Prakash, O., R. Kumar, A. Mishra and R. Gupta, 2009. *Artocarpusheterophyllus* (Jackfruit): An overview. Pharmacogn. Rev., 3: 353-358.
26. Morshed, M.A., A. Uddin, R. Saifur, A. Barua and A. Haque, 2011. Evaluation of antimicrobial and cytotoxic properties of *Leucas aspera* and *Spilanthes paniculata*. Int. J. Biosci., 1: 7-16.
27. Elmastas, M., O. Isildak, I. Turkekul and N. Temur, 2007. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. J. Food Compos. Anal., 20: 337-345.
28. Wang, K.J., C.R. Yang and Y.J. Zhang, 2007. Phenolic antioxidants from Chinese toon (fresh young leaves and shoots of *Toonasinensis*). Food Chem., 101: 365-371.
29. Madaan, R., G. Bansal, S. Kumar and A. Sharma, 2011. Estimation of total phenols and flavonoids in extracts of actaea spicata roots and antioxidant activity studies. Indian J. Pharm. Sci., 73: 666-669.
30. Henriquez, C., S. Almonacid, I. Chiffelle, T. Valenzuela and M. Araya *et al*., 2010. Determination of antioxidant capacity, total phenolic content and mineral composition of different fruit tissue of five apple cultivars grown in chile. Chilean Agric. Res. J., 70: 523-536.
31. Yang, C.M. and J.B. Russell, 1992. Resistance of proline-containing peptides to ruminal degradation *In vitro*. Applied Environ. Microbiol., 58: 3954-3958.
32. Hossain, H., A.F.M. Shahid-Ud-Daula, I.A. Jahan, I. Nimmi, K. Hasan and M.M. Haq, 2012. Evaluation of antinociceptive and antioxidant potential from the leaves of *Spilanthes paniculata* growing in Bangladesh. Int. J. Pharm. Phytopharmacol. Res., 1: 178-186.
33. Umukoro, S. and R.B. Ashorobi, 2006. Evaluation of anti-inflammatory and membrane stabilizing property of aqueous leaf extract of *Momordica charantia* in rats. Afr. J. Biomed. Res., 9: 119-124.
34. Saffoon, N., R. Uddin, N. Subhan, H. Hossain, H.M. Reza and M.A. Alam, 2014. *In vitro* anti-oxidant activity and HPLC-DAD system based phenolic content analysis of codiaeum variegatum found in Bangladesh. Adv. Pharm. Bull., 4: 533-541.
35. Richter, L.A., J. Salazar and E. Rodriguez, 2003. A phytochemical analysis to suggest new applications of anti-inflammatory plants from the Dominican Republic. Emanations, 4: 24-30.
36. Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.
37. Onasanwo, S.A. and R.A. Elegbe, 2006. Anti-nociceptive and anti-inflammatory properties of the leaf extracts of *Hedranthera barteri* in rats and mice. Afr. J. Biomed. Res., 9: 109-117.