Rahman MM *et al.* / *International Journal of Phytopharmacology*. 5(5), 2014, 383-389.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

EVALUATION OF ANTIOXIDANT, ANALGESIC AND CYTOTOXIC ACTIVITIES OF THE AERIAL PART OF *CASSIA SOPHERA* L. (CAESALPINIACEAE)

Murshid GMM, Barman AK and Rahman MM*

Pharmacy Discipline, Life Science School, Khulna University, Khulna 9208, Bangladesh.

ABSTRACT

Cassia sophera L. is used in tropical countries of Asia by local healers traditionally for the management of wide range of ailments including diarrhea, skin diseases, jaundice and diabetes. Considering the widespread use in traditional medicine the present study was undertaken to explore the possible antioxidant, analgesic and cytotoxic activities of the methanolic extract of *C. sophera* L. Total phenolic content (TPC) and total tannin content (TTC) were determined using Folin Chiocalteu's reagent while the total flavonoid content (TFC) was measured by colorimetric method. The antioxidant activity of the crude methanolic extract was evaluated using DPPH radical scavenging and ferric reducing power assays. The acetic acid induced writhing inhibition method was employed to screen the analgesic activity. The general toxicity was assessed by brine shrimp lethality bioassay. Total phenolic content (TPC) and total flavonoid content (TFC) were found to be 135 mg GAE/g and 144 mg QE/g of dried plant extract, respectively. Total tannin content (TTC) was obtained 10.2 mg GAE/g of the dried extract. The extract showed DPPH radical scavenging activity at IC_{50} value of 33.49 µg/mL. The reducing power was found comparable to standard ascorbic acid. In acetic acid-induced writhing inhibition test the extract exhibited significant inhibition of writhing property (34.3% and 64.3% at the doses of 250 & 500 mg/kg, respectively). The extract showed cytotoxicity against brine shrimp lethality bioassay (LD_{50}=23.682 µg/mL). The extract exhibited preliminary evidences for having its antioxidant, analgesic and cytotoxic activities; however further investigation is suggested to isolate active compound(s) responsible for these pharmacological activities.

Key words: Antioxidant activity, Analgesic activity, *Cassia sophera*, Caesalpiniaceae, Cytotoxicity, DPPH assay.

INTRODUCTION

Various plants are traditionally used to cure different ailments and still now, huge number of world's population is dependent on herbal medicine due to the high cost of modern medicine (Aminabee & Lakshmana Rao, 2012). Moreover, plants can be used as valuable sources of bioactive compounds and may serve as lead compound for new drug, thus play an important role in developing new therapeutic agents (Ajaiyeoba *et al.*, 2006). *Cassia sophera* L. is a glabrous shrub about 3 m. in height belonging to Caesalpiniaceae family and commonly known as 'Kalkasunda' in Bangladesh. It is commonly found in forests, on roadsides and waste lands throughout Bangladesh, India, Nepal and most tropical countries. Traditionally the plant juice is used to cure diarrhea, apositia, skin diseases, ascites, piles, epilepsy, jaundice, diaphoresis, fever, palpitation and articular pain (Tagboto & Townson, 2001). It is also used in acute bronchitis, asthma, pityriasis, psoriasis, convulsions of children, osteoarthritis and allergic rhinitis. The plant has blood purifier, digestive, carminative, diaphoretic (Aminabee & Lakshmana Rao, 2012) and spasmolytic properties. Root bark and seeds are often used in ringworm, constipation and diabetes (Ghani, 2003). Some anthraquinones have been isolated from the plant such as 1,8-dihydroxy-2 methylanthraquinone-3neohesperidoside ,1,2,7-trihydroxy-6,8-dimethoxy-3-ethylanthraquinone, 1,2,6-trihydroxy-7,8-dimethoxy-3-anthraquinone, 1,8-

Corresponding Author

Rahman, MM
Email: dipti0103@yahoo.com

dihydroxy-3,6-dimethoxy-2-methyl-7-vinylanthraquinone and 1,3-dihydroxy-5,7,8-trimethoxy-2-methylanthra quin one along with sitosterol, chrysophanol and physcion (Aminabee & Lakshmana Rao, 2012). Identification of several bioactive components coupled with diversified traditional uses of *Cassia sophera* L made this plant a potential candidate for exploring more of its pharmacological activities. However, very few reports have been there to ascertain the scientific justification supporting the traditional uses of the plant. Here we have focused on the study of phyto-pharmacological activities of the aerial part of *Cassia sophera*. The present study, therefore, was designed to investigate probable antioxidant, analgesic and cytotoxic activities of the 'methanolic extract of *C. sophera* aerial part *[MECS]*' using different in-vitro and in-vivo models.

MATERIALS AND METHODS

Plant material

The plant *C. sophera* was collected from Bagerhat, Bangladesh during the month of October, 2011 and authenticated by the experts of Bangladesh National Herbarium, Mirpur, Dhaka. A voucher specimen of the plant part was also deposited there with the Accession no: DACB 36546 for future reference. The aerial part of the plant was separated from undesirable materials or plant parts and cut into small pieces with a sharp knife. The plant material was dried by shade drying for twenty days to avoid any phytochemical decomposition. The dried parts were grinded by a mechanical grinder to form coarse powder. To avoid the microbial attack the coarse powder was stored in an airtight container and in a cool, dark and dry place until analysis commenced.

Extraction

Coarse powdered plant material was subjected to cold extraction process. The powder was soaked with methanol for 5 days in regular shaking. After maceration, filtration was undertaken to get clear solution removing unwanted materials. The collected filtrate was evaporated in rotary evaporator at 50 °C. Crude extract (yield 2.71%) was stored in a refrigerator at 4 ºC.

Test animals

For *In vivo* pharmacological screening, Swiss albino mice with both genders (age 4-5 weeks, weight 18-25 g) were collected from the Animal Resource Department of the International Center for Diarrheal Disease Research, Bangladesh (ICDDR, B). The animals were kept in standard laboratory conditions (room temperature of 25±0.5 °C, and relative humidity of 55-60% with 12 hour light-dark cycle) and supplied with ICCDR, B prescribed rodent food and water *ad libitum*. For acclimatization, they were kept in the laboratory condition for 7 days before the commencement of experiment. The animal experimentation for the current work was performed with the prior approval by the Institutional Animal Ethical Committee.

Chemicals and reagents

Used chemicals and reagents for the present study include: ascorbic acid, ferric chloride, sodium carbonate, sodium monobasic phosphate, sodium dibasic phosphate, potassium ferricyanide, trichloroacetic acid, gallic acid, acetic acid and dimethyl sulfoxide -all were bought from Merck, Germany. Folin Chiocalteu's reagent and 2,-2-diphenyl-1-picrylhydrazyl (DPPH) were collected from Sigma Chemical Co. Ltd. (St. Louis, MO, USA) and tween-80 was purchased from Loba Chemie Pvt Ltd. of India. Standard diclofenac sodium and vincristine sulphate were purchased from Square Pharmaceuticals Ltd. of Bangladesh and Cipla Pharmaceuticals Ltd. of India respectively.

Preliminary Phytochemical Investigation

Phytochemical screening of the extract was carried out using various reagents through standard procedures for determination of the different phytoconstituents.

Determination of total phenolic content

Total phenolic content of the MECS was measured using Folin Ciocalteu's (FC) reagent as described earlier (Marinova *et al*., 2005). Gallic acid was used as the standard and different concentrations of gallic acid (400, 200, 100, 50, 25 and 12.5 mg/L) were prepared in ethanol. The extract was dissolved in ethanol using ultrasonic bath for 15 min to make proper solution of desired concentration (400 mg/L). 2 mL solution was centrifuged at 14000 rpm for 5 min and 1 mL supernatant was taken into 25 mL volumetric flask; 9 mL distilled water was added to it. One (1) mL FC reagent was diluted (1/10 in distilled water) and added to each volumetric flask and mixed properly with vigorous shaking. After 5 min, 10 mL Na_2CO_3 (7%) was added to individual flask and finally the volume was adjusted to 25 mL adding distilled water. The mixture was kept at room temperature for 60 min and absorbance was taken at 750 nm against blank. The blank was prepared through above procedure without addition of extract and standard. Standard curve was prepared by plotting absorbance versus respective concentrations of gallic acid. Total phenolic content was measured using standard curve and expressed as mg gallic acid equivalent (GAE)/g dried plant extract.

Determination of flavonoid content

Flavonoid content of plant extract following standard procedure (Chang *et al*., 2002). 1 mL from both the extract (100µg/mL) and standard quercetin (100, 80, 60, 40 and 20 µg/mL) were taken separately into 10 mL

volumetric flask. 4 mL distilled water followed by 0.3 mL $NaNO_2$ (5%) were added in each mixture. After 5 min, 0.3 mL $AlCl_3$ (10%) and 2 mL of 1M NaOH were sequentially added to the mixture and made the volume up to 10 mL with distilled water. The absorbance of the extract and standard was taken at 510 nm. Total flavonoid content of the MECS was expressed in terms of mg quercetin equivalent (QE)/g of dried plant mass.

Determination of tannin content

Total tannin content of the MECS extract was screened by Folin Ciocalteu method (Tamilselvi *et al.*, 2012). To find out the tannin content, 7.5 mL distilled water and 0.5 mL Folin Ciocalteu's reagent was added in 0.1 mL crude extract. 1 mL sodium carbonate (35%) was added to the mixture and the final volume was adjusted to 10 mL with water. Then the mixture was kept at room temperature for 30 min. Absorbance was taken at 725 nm against blank. Different concentrations of standard gallic acid (100, 80, 60, 40 and 20 µg/mL) were prepared to make the calibration curve. The result of total tannin content of MECS was expressed in terms of mg GAE/g of plant extract.

Determination of antioxidant activity:

DPPH scavenging activity test-Antioxidant activity of the MECS was assessed using stable 2,-2-diphenyl-1-picrylhydrazyl (DPPH) free radical according to the method described by Ayres GH (Ayres, 1949). For the analysis, stock solution was prepared by dissolving the extract in ethanol to obtain the desired final concentrations of 256, 128, 64, 32, 16, 8, 4, 2, 1 µg/mL using serial dilution. Then 1 mL solution from each concentration was taken into test tubes and mixed with 3 mL of 0.004% DPPH solution and kept in dark place for 30 min in room temperature to complete the reaction. Then absorbance was measured at 517 nm. Ascorbic acid was used as a standard. Scavenging activity of the plant extract was calculated in percentage using formula: % scavenging activity = $(1 - A_1/A_0) \times 100$, where A_0 is the absorbance of control and A_1 is the absorbance of sample or standard.

Ferric reducing antioxidant power (FRAP) assay

Reducing power of the MECS extract was estimated by a spectrophotometric method (Ferreira *et al.*, 2007). Different concentrations of extract (400, 200, 100, 50, 25, 12.5 µg/mL) were prepared in ethanol. From each concentrations, an aliquot (1 mL) of the extract was mixed with 2.5 mL phosphate buffer (200 mM, pH 6.6) and 2.5 mL potassium ferricyanide (1%) with continuous shaking. The mixture was incubated at 50°C for 20 min. After the incubation, 2.5 mL trichloroacetic acid (10%) was added to the mixture and centrifuged at 3000 rpm for 10 min, 2.5 mL supernatant, 2.5 mL distilled water and 0.5 mL ferric chloride (0.1%) were mixed with shaking. After 5 min, absorbance was estimated at 700 nm against blank. Ascorbic acid was used as a standard.

Evaluation of analgesic activity

The analgesic activity test was carried out using acetic acid induced writhing method according to the procedure described previously (Whittle BA 1964) with slight modification. Experimental mice were divided into 4 groups denoted as Group I, Group II, Group III and Group IV consisting of six mice in each groups. Methanolic extract of *C. sophera* was dissolved in distilled water with 1% tween-80. Using a feeding needle the extract was supplied to the test groups (Group III and Group IV) at the doses of 250 and 500 mg/kg body weight whereas diclofenac sodium was administered to the positive control group (Group II) at the dose of 25 mg/kg body weight. Then distilled water (10 mL/kg, p.o.) was given to control group (Group I). After thirty minutes, 0.6% acetic acid was injected to each mouse intraperitoneally to induce writhing or abdominal contraction. Five minutes later, the number of writhes for individual mouse was counted for ten minutes (Meir *et al.*, 1995). The number of writhes counted for test and standard groups was compared with control group. Percent inhibition was calculated using the formula: Percent inhibition = $(W_0-W_1)/W_0 \times 100$, where W_0 is the number of writhes of control group, and W_1 is the number of writhes of test or standard group.

Brine shrimp lethality bioassay

Cytotoxicity of the extract was evaluated using brine shrimp lethality bioassay (Meyer BN *et al.*, 1982). In brine shrimp lethality bioassay, the eggs of *Artemia salina* were hatched at 25-30ºC for 24 h in artificial sea water to get nauplii (shrimp larvae). To make artificial sea water, 20 g NaCl and 18 g table salt was dissolved in 1L of distilled water. Extract was dissolved in dimethylsulfoxide (DMSO) and different concentrations (320, 160, 80, 40, 20, 10, 5 µg/mL) were obtained with serial dilution using artificial sea water. DMSO concentration did not exceed 10 µL/mL any test tube. Then ten alive naupliis were added in each test tube containing five milliliter simulated sea water. Vincristine sulphate was used as positive control at the concentrations of 5, 2.5, 1.25, 0.625 and 0.312 µg/mL. After an incubation period of 24 h at room temperature, the number of viable naupliis was counted using a magnifying glass.

Statistical analysis

Experimental results were expressed as mean ± standard error of mean (SEM). Dunnett's test was carried out to evaluate the statistical significance followed by ANOVA (one-way analysis variance). Statistical analysis

was conducted in Prism 6.0 (GraphPad software Inc., San Diego, CA). P values were taken to evaluate statistical significance. The LD_{50} value was determined using LdP line software (http//:www.ehabsoft.com/ldpline/).

RESULTS

Preliminary Phytochemical Investigation

The results of phytochemical screening of the MECS are summarized in Table 1.

Total phenolic content

The absorbance values obtained in the test using different concentrations of gallic acid were plotted to produce a standard calibration curve with the equation, y=0.001x+0.520 (R^2= 0.990). Total phenolic content was found to be 135 mg GAE/g of the dried extract.

Total flavonoid content

Different concentrations of quercetin were used and absorbance was measured in the test. A standard curve was also obtained with the equation, y=0.003x+0.005 where R^2=0.989. Total flavonoid content of the MECS was found to be 144 mg QE/g of dried extract.

Total tannin content

Total tannin content of the MECS was found to be 10.2 mg GAE/g of dried plant extract. Standard curve of gallic acid was prepared (standard curve equation: y=0.001x+0.005, R^2=0.993).

DPPH scavenging activity test

Antioxidant activity of MECS was evaluated by DPPH scavenging activity test. Scavenging activity was linearly increased at low concentration and saturation was attained at higher concentration for both MECS and standard. The values were plotted as log concentration versus % inhibition on graph showing a linear relation (Figure 1). The extract revealed IC_{50} value of 33.49 µg/mL while the standard ascorbic acid displayed IC_{50} value of 9.27 µg/mL.

Reducing power assay

Reducing power of the MECS was found to be accelerated with concentration. The reducing ability was comparable with standard ascorbic acid. Higher absorbance in a certain concentration ascertains better reducing power (Figure 2).

Evaluation of analgesic activity

The MECS showed significant (P<0.001) analgesic activity at a dose-dependent manner. At doses of 250 and 500 mg/kg body weight MECS caused writhing inhibition of 34.3% and 61.3%, respectively whereas standard diclofenac sodium (25 mg/kg) showed 78.6% writhing inhibition (Table 2).

Brine shrimp lethality bioassay

Probit analysis software (LdP Line software, USA) was used to determine LD_{50} for both sample and positive control. LD_{50} was found to be 23 µg/mL for MECS whereas vincristine sulfate showed 0.45 µg/mL and the percentage mortality of the nauplii caused by them is presented in Fig 3(a) and Fig 3(b).

Fig 1. DPPH scavenging activity of MECS

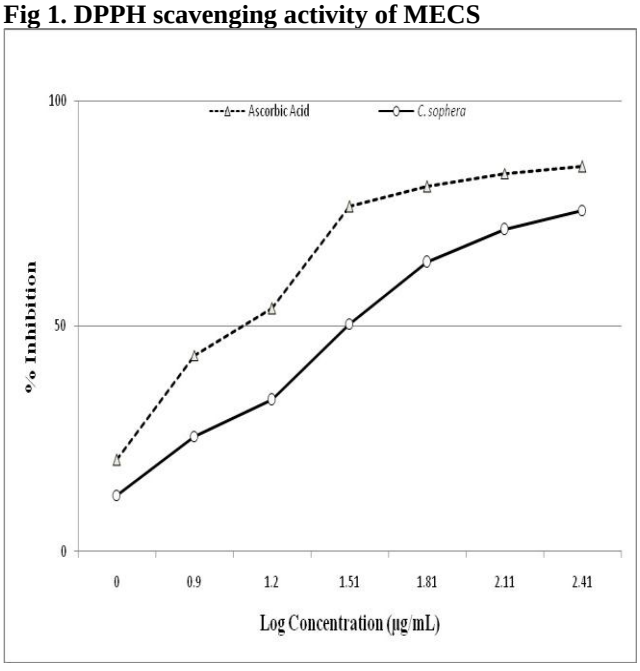

Figure 2. Reducing power of MECS; higher absorbance from a certain concentration confirms better reducing power of the extract.

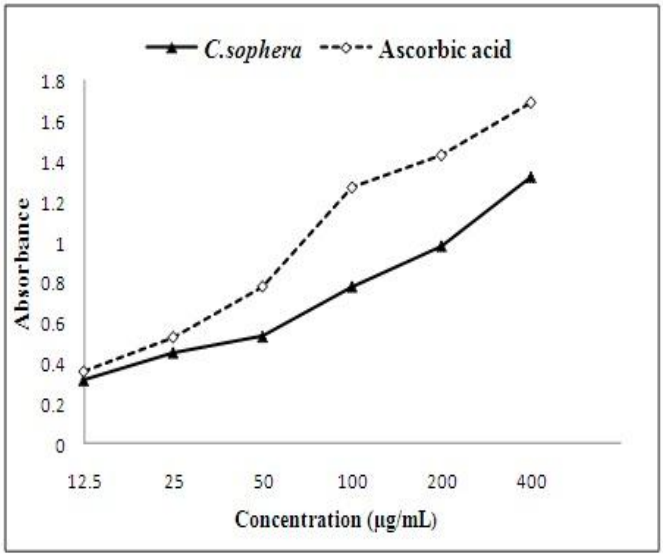

Fig 3. Graphical representation of brine shrimp lethality bioassay and LC50 for the MECS (a) and vincristine (b) by Ldp line software

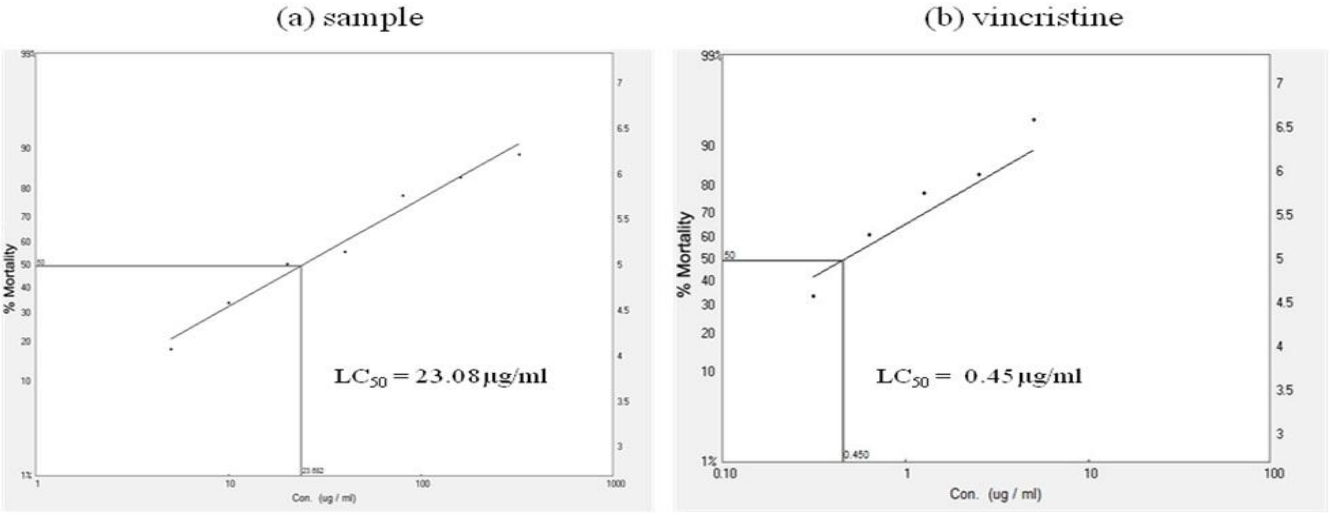

Table 1. Results of preliminary phytochemical investigation of the MECS

Phytochemical groups	Result
Reducing sugars	+
Alkaloids	+
Glycosides	-
Flavonoids	+
Tannins	+
Gums	+
Steroids	+

Annotation: (+): indicates the presence of phytoconstituents and (-): indicates the absence of phytoconstituents.

Table 2. Effect of MECS on acetic acid induced writhing in mice

Treatment n = 6	Dose (mg/kg body weight)	No. of writhes	% Inhibition
Control	---	35.6 ± 0.7	---
Diclofenac sodium	25	7.6± 0.9***	78.6
MESC	250	23.4 ± 1.5**	34.3
	500	13.8± 1.05***	61.3

Results are expressed as mean ± SEM; SEM=Standard error for mean; **P<0.01 while ***P<0.001 (Student's t-test).

DISCUSSION AND CONCLUSION

Free radicals are known to play a definite role in a wide variety of pathological manifestations in the biological system. Antioxidants fight against free radicals and protect us from various diseases such as hypertension, cancer, atherogenesis, Parkinson's disease and alzheimer's disease *etc.* (Sahoo *et al.,* 2013). Antioxidants exert their action either by scavenging the reactive oxygen species (ROS) or protecting the antioxidant defense mechanism (Khatoon *et al.*, 2013). Antioxidants are known to prevent decomposition of the ROS as well as nullify free radicals in lipids and improve the quality and stability of food (Javanmardi *et al.*, 2003; Li *et al.*, 2009). Antioxidant activity of MECS extract was tested at different concentrations by using DPPH free radical scavenging power assay and reducing power assay. The extract revealed potential free radical scavenging activity in the DPPH assay (Figure 1).

Phenolic compounds usually show significant antioxidant potential and act as free radical scavengers (Razali *et al.,* 2008). On the other hand, flavonoids are known as effective scavengers of most types of oxidizing molecules due to their hydrogen donating ability (Sandhar *et al.,* 2011). Tannins are aqueous polyphenols, also responsible for antioxidant activity (He *et al.,* 2011; Koleckar *et al.,* 2008). Considering all these aspects in our study we have determined total phenolic, flavonoid and tannin contents in our sample. MECS also showed substantial quantity of phenolic, flavonoid and tannin contents. These findings indicate the presence of bioactive compounds in aerial part of *C. sophera* having antioxidant

activity which in turn support the use of aerial part of *C. sophera* as a promising source of natural antioxidants.
MECS plant exhibited notable reducing power in concentration-dependent manner which proves the existence of some compounds that are capable of terminating chain reactions by reacting with free radicals and converting them into stable nonreactive form.

Recent studies have suggested that phenolic acids and flavonoids such as quercetin, rutin, and luteolin are responsible for analgesic activity by targeting prostaglandins (Arslan *et al.*, 2010; Deliorman *et al.*, 2007). Because of presence of phenolic and flavonoid compounds in our extract we carried out acetic acid induced writhing test in mice. MECS significantly lowered the number of writhing in a dose dependant manner. Here we hypothesize that there might be some active phenolic or flavonoid compound present in MECS that have analgesic activity. However, the mechanism is yet to be explored.

In the brine shrimp lethality bioassay, a gradual increase in percent mortality of the shrimp nauplii was exhibited in the experiment by both MECS and standard in concentration dependent manner. The result suggests that MECS might possess compounds having cytotoxic property.

Preliminary phytochemical analysis confirmed the presence of various classes of phytoconstituents, such as reducing sugar, saponin, alkaloid, flavonoid, tannin, gum and steroid. Presence of these phytochemical constituents may attribute to the observed activities of the plant extract. The present study provides a preliminary basis for the traditional use by exploring the antioxidant, analgesic and cytotoxic properties of *C. sophera*. We suggest for advanced investigation and isolation of biologically active constituents responsible for these activities.

Competing interest

We the authors of the present study declare that we have no competing of interests.

Author's contribution

Murshid GMM and Barman AK have jointly conducted this project. Rahman MM has made significant contribution in writing and editing the manuscript with Murshid and Barman. All took participation equally for data interpretation and statistical analysis. All authors read and agreed with the final manuscript.

Authors' information

All belongs to the Pharmacy Discipline, Life Science School, Khulna University, Khulna 9208, Bangladesh.

ACKNOWLEDGEMENTS

We are grateful to the authorities of Phytochemistry and Pharmacology Research Laboratory, Pharmacy Discipline, Life Science School, Khulna University, Khulna, Bangladesh for providing technical support to carry out the present pharmacological investigations successfully.

REFERENCES

Ajaiyeoba E, Falade M, Ogbole O, Okpako L, Akinboye D. In vivo antimalaria and cytotoxic properties of *Annona senegalensis* extract. *Af J Tradit Complementary Altern Med*, 3, 2006, 137-141.

Aminabee SK and Lakshmana Rao A. A plant review of *Cassia sophera* Linn. *Int J Pharm Chem Biol Sci*, 2, 2012, 408-414.

Arslan R, Bektas N, Ozturk Y. Antinociceptive activity of methanol extract of fruits of Capparis ovata in mice. *J Ethnopharmacol*, 131, 2010, 28–32.

Ayres, G. H. Evaluation of accuracy in photometric analysis. *Analytical Chemistry*, 21 (6), 1949, 652–657.

Chang C, Yang M, Wen H, Chem J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal*, 10, 2002, 178-182.

Deliorman OD, Hartevio˘glu A, Kupeli E, Yesilada E. *In vivo* anti-inflammatory and antinociceptive activity of the crude extract and fractions from *Rosa canina* L. fruits. *J Ethnopharmacol*, 112, 2007, 394-400.

Dhara AK, Suba V, Sen T, Pal S, Chaudhuri AKN. Preliminary studies on the anti-inflammatory and analgesic activity of the methanol fraction of the root extract of *Tragia involucrate* Linn. *J Ethnopharmacol*, 72, 2000, 265-268.

Ferreira ICFR, Baptista M, Vilas-Boas & Barros. Free radical scavenging capacity and reducing power of wild edible mashrooms from Northeast Portugal:Individual cap and stipe activity. *Food Chem*, 100, 2007, 1511-1516.

Ghani A. Medicinal plants of Bangladesh: chemical constituents and uses. 2nd ed. The Asiatic Society of Bangladesh. Dhaka, Bangladesh, 2003, p-387.

He Z, Fu M, Mao L. Total phenolic, condensed tannin and antioxidant activity of four *Carya* species from China. *Afr J Biotechnol*, 10, 2011, 10472-10477.

Javanmardi J, Stushnoff C, Locke E, Vivanco JM. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chem*, 83, 2003, 547-550.

Khatoon M, Islam E, Islam R, Rahman AA, Alam AHMK, Khondkar P, Rashid M, Parvin S. (2013). Estimation of total phenol and in vitro antioxidant activity of *Albizia procera* leaves. *BMC Res Notes*, 6, 2013, 121.

Koleckar V, Kubikova K, Rehakova Z, Kuca K, Jun D, Jahodar L, Opletal L. Condensed and hydrolysable tannins as antioxidants influencing the health. Mini-Rev. *Med Chem*, 8, 2008, 436-447.

Li HY, Hao ZB, Wang XL, Huang L, Li JP. Antioxidant activities of extracts and fractions from *Lysimachia foenum-graecum* Hance. *Bioresour Technol*, 100, 2009, 970-974.

Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *J Univ Chem Technol Metall*, 40, 2005, 255-260.

Meir S, Kanner J, Akiri B, Hadas SP. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *J Agric Food Chem*, 43, 1995, 1813-1817.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med*, 45, 1982, 31-34.

Paixao N, Perestrelo R, Marques JC, Camara JS. Relationship between antioxidant capacity and total phenolic content of red, rose and white wines. *Food Chem*, 105, 2007, 204-214.

Pervin R, Sabrin F, Billah MM, Islam KMD, Elmezughi J, Shilpi JA. Antibacterial and cytotoxic activity of *Meyna spinosa* Roxb. Stem. *Turk J Pharm Sci*, 9, 2012, 225-230.

Razali N, Razab R, Junit SM, Aziz AA. Radical scavenging and reducing properties of extracts of cashew shoots (*Anacardium* occidentale). *Food Chem*, 111, 2008, 38-44.

Sahoo S, Ghosh G, Das D, Nayak S. Phytochemical investigation and *in vitro* antioxidant activity of an indigenous medicinal plant *Alpinia nigra* B.L. Burtt. *Asian Pac J Trop Biomed*, 3, 2013, 871-876.

Sandhar HK, Kumar B, Prasher S, Tiwari P, Salhan M, Sharma P. A review of phytochemistry and pharmacology of flavonoids. *Internationale Pharmaceutica Sciencia*., 1, 2011, 25-41.

Tagboto S and Townson S. Antiparasitic properties of medicinal plants and other naturally occurring products. *Adv Parasitol*, 50, 2001, 199-295.

Tamilselvi N, Krishnamoorthy P, Dhamotharan R, Arumugam P, Sagadevan E. Analysis of total phenols, total tannins and screening of phytocomponents in *Indigofera aspalathoides* (Shivanar Vembu) Vahl EX DC. *J Chem Pharm Res*, 4, 2012, 3259-3262.

Whittle BA, The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics, *Br J Pharmacol Chemother*, 22, 1964, 246-253,

Wu KK. Aspirin and other cyclooxygenase inhibitors: new therapeutic insights. *Semin Thromb Hemostasis*, 3, 2003, 107-112.