Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(2): 134-138
Received: 12-01-2017
Accepted: 13-02-2017

Farhina Rahman Laboni
Department of Pharmacy, World University of Bangladesh, Dhaka, Bangladesh

Tasmin Sultana
Department of Pharmacy, World University of Bangladesh, Dhaka, Bangladesh

Samia Kamal
Department of Pharmacy, World University of Bangladesh, Dhaka, Bangladesh

Samira Karim
Department of Pharmacy, World University of Bangladesh, Dhaka, Bangladesh

Sajan Das
Department of Pharmacy, University of Asia Pacific, Dhaka, Bangladesh

Md. Harun-Or-Rashid
Department of Pharmacy, World University of Bangladesh, Dhaka, Bangladesh

Mohammad Shahriar
Department of Pharmacy, University of Asia Pacific, Dhaka, Bangladesh

Correspondence
Mohammad Shahriar
Associate Professor, Department of Pharmacy, University of Asia Pacific, Dhaka, Bangladesh

Biological investigations of the ethanol extract of the aerial part (leaf) of *Coccinia grandis* L

Farhina Rahman Laboni, Tasmin Sultana, Samia Kamal, Samira Karim, Sajan Das, Md. Harun-Or-Rashid and Mohammad Shahriar

Abstract
In this present study, the leaf extracts of *Coccinia grandis* L., were subjected to evaluation of the phytochemical screenings, thrombolytic, membrane stabilizing, antioxidant, antimicrobial and cytotoxic activity. The ethanol extract showed 43.71% clot lysis as compared to 65.16% clot lysis produced by standard streptokinase. *In vitro* membrane stabilizing activity for hypotonic solution and heat induced haemolysis the ethanol extract inhibited 79.53% and 90.30% haemolysis of RBCs as compared to 71.90% and 77.20% produced by acetyl salicylic acid respectively. *In vitro* antioxidant activity revealed that the ethanol extracts of *C. grandis* exhibited significant antioxidant activity with IC_{50} value of 1.15 µg/ml in comparison with the standard 5.80 µg/ml. Leave extracts of *Coccinia grandis* L. revealed moderate antimicrobial activity against some gram positive and gram negative microorganisms. Leave extracts showed strong inhibitory effect on brine shrimp lethality with LC_{50} at 24.20 µg/ml.

Keywords: *Coccinia grandis,* phytochemical screenings, thrombolytic, membrane stabilizing, antioxidant, antimicrobial and cytotoxic activity

1. Introduction
Throughout history, plants have been used as a major medicinal source, with interest in herbal formulations increasing globally over the past decade. In addition, extracts of natural products provide a useful source of bioactive compounds which can be developed as drugs directly or provide novel structure templates. A large proportion of drugs in clinical use are produced by the synthesis of natural products and/or their derivatives, and new plant-derived medicines are continually being discovered as the herbal remedies are cost effective, having minimum toxicity with reduced health hazards and easily available in market as compared to synthetic medicines [1]. There are more than 500 medicinal plants growing in our country, however, the inventory is not complete, and many plants with medicinal value are yet to be determined.
Coccinia grandis (L.), is a perennial climber with single tendrils and glabrous leaves having 5 lobes and are 6.5–8.5 cm long and 7-8 cm wide and belongs to the family of Cucurbitaceae in the genus Coccinia locally known as Telakucha in Bangladesh. Female and male flowers emerge at the axils on the petiole, and have 3 stamens. *C. grandis* is an indigenous plant to Bangladesh including India, the Philippines, Cambodia, China, Indonesia, Malaysia, Myanmar, Thailand, Vietnam, eastern Papua New Guinea, Australia and the Northern Territories.
This plant has also been used in traditional medicine for the treatment of diabetics [2-5], skin diseases [6], jaundice, biliary disorders, coughs, spleen disorders, respiratory problems, mucus, leprosy, acne, diabetes, mucus in stool, goiter, antidote to poison [7], scabies [8], hypertension [4], abscess, lack of appetite, vomiting [3], dysentery, burns [9].
It has been reported to have antioxidant, antimutagenic, antimicrobial, antiulcer, hepatoprotective, expactorants and analgesic activities [10], anthelmintic activity [11], cytotoxic activity [12-13], anti-dibetic activity [14], mast cell-stabilizing, anti-anaphylactic, and antihistaminic potential [15], antimitotic, anti-inflamatory activities [12, 10].
In the present study, the organic soluble materials of the ethanol extract (aerial part) of *C. grandis* was evaluated for phytochemical screenings, thrombolytic, membrane stabilizing, antioxidant, antimicrobial and cytotoxic activity.

2. Materials and Methods
2.1 Collection, Identification and Processing of Plant Samples
The leaves of *C. grandis* were collected from Dhaka, Bangladesh and then plant sample was submitted to the National Herbarium of Bangladesh, Mirpur-1, Dhaka for its identification and the voucher specimen is DACB- 39526.

Leaves were sun dried for seven days in order to remove the moisture contents and then ground into coarse powder using high capacity grinding machine (Jaipan designer mixer grinder, jaipan, India) which was then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

2.2 Extraction Procedure
The powdered plant parts (30 gm) were successively extracted in a soxhlet extractor at elevated temperature using 500 ml of distilled ethanol (40-60) ºC. After drying all extracts were labeled and kept in refrigerator at 4 °C for future investigation.

2.3 Preliminary Phytochemical Screening
Ethanol extract was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents by using standard protocols [16].

2.4 Streptokinase (SK)
Commercially available lyophilized alteplase (Streptokinase) vial (Popular pharmaceutical Ltd.) of 15, 00,000 I.U, was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolytic activity evaluation.

2.5 Blood Sample
Blood (n=6) was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 1ml of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

2.6 Thrombolytic Activity
The thrombolytic activity of all extracts was evaluated by the method developed by Prasad *et al*., (2006**)** [17] and slightly modified by Sharif *et al*., (2014) [18] using streptokinase (SK) as the standard.

2.7 Membrane Stabilizing Activity
The membrane stabilization by hypotonic solution and heat-induced haemolysis method was used to assess anti-inflammatory activity of the plant extracts by following standard protocol [19]. The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane [20-21]. The membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced and heat-induced human erythrocyte haemolysis. To prepare the erythrocyte suspension, whole blood was obtained from healthy human volunteer and was taken in syringes (containing anticoagulant 3.1% Nacitrate). The blood was centrifuged and blood cells were washed three times with solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) through centrifugation for 10 min at 3000 gm.

2.7.1 Hypotonic Solution Induced Haemolysis
The test sample consisted of stock erythrocyte (RBC) suspension (0.5mL) mixed with 5mL of hypotonic solution (50mM NaCl) in 10mM sodium phosphate buffered saline (pH 7.4) containing either the extract (1.0mg/mL) or acetyl salicylic acid (ASA) (0.1mg/mL). The control sample consisted of 0.5mL of RBCs mixed with hypotonic-buffered saline alone. The mixture was incubated for 10min at room temperature, centrifuged for 10min at 3000g and the absorbance of the supernatant was measured at 540nm.
The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation:

$$\% \text{ inhibition of haemolysis} = 100 \times (OD_1\text{-}OD_2)/OD_1$$

Where, OD_1= optical density of hypotonic-buffered saline solution alone (control)
OD_2= optical density of test sample in hypotonic solution

2.7.2 Heat Induced Haemolysis
Isotonic buffer containing aliquots (5ml) of the different extractives were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 56°C for 30min in a water bath, while the other pair was maintained at (0-5) °C in an ice bath. The reaction mixture was centrifuged for 5min at 2500g and the absorbance of the supernatant was measured at 560 nm. The percentage inhibition or acceleration of hemolysis in tests and was calculated according to the equation:

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

Where,
OD_1= optical density of unheated test sample
OD_2= optical density of heated test sample
OD_3= optical density of heated control sample

2.8 Antioxidant Activity
2.8.1 DPPH Free Radical Scavenging Assay
The free radical scavenging capacity of the extracts was determined using DPPH [22-23]. 1 ml of plant extract or standard of different diluted (6.25 µg/ml to 800 µg/ml) concentration solutions was taken in test tube and freshly prepared 2 ml of 0.004% DPPH solution was added in each test tube to make the final volume 3 ml. The mixture was incubated at room temperature for 30 minutes; the absorbance was read at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as standard. Control sample was prepared containing the same volume without any extract and standard. The absorbance was read at 517 nm using a UV-VIS spectrophotometer. Ethanol was used as blank. Percent of inhibition of the DPPH free radical was measured by using the following equation:

$$\% \text{ inhibition} = (1\text{-}A_1/A_0) \times 100\%$$

Here,
A_1 = Absorbance of the extract or standard
A_0 = Absorbance of the control.

2.9 Antibacterial Activity
The antimicrobial screening, which is the first stage of antimicrobial drug discovery, was performed by the disc diffusion method against gram positive and gram negative bacteria (Table 5) collected as pure cultures from the department of microbiology, Medinova Medical Services Limited, Bangladesh. Standard disc of Ciprofloxacin (5 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control,

respectively. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm [24].

2.10 Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay technique was applied for the determination of general toxic properties of the plant extracts [25-26]. Dimethylsulfoxide (DMSO) solutions of the samples were applied against Artemia salina in a 1-day *in vivo* assay. For the experiment, 1 mg of each extracts was added with 5 ml of sea water. Concentration was found to be 200 µg/ml. Then 50 µl DMSO was added to these and sample was prepared. Then the solution was serial diluted to 100, 50, 25, 12.5, 6.25, 3.125, 1.563 µg/ml with sea water. Then 2.5 ml of plant extract solution was added to 2.5 ml seawater containing 10 nauplii. Vincristine Sulphate (VS) was used as positive control.

2.11 Statistical Analysis

All data are presented as a mean ±Standard deviation. IC_{50} values for scavenging of free radicals by the extracts were calculated from the dose - response curve by using Microsoft Excel 2010.

3. Results and Discussions

3.1 Phytochemical Screening

In primary phytochemical screening, leaves extract of *C. grandis* was found to contain alkaloids, carbohydrates, saponins, glycosides, flavonoids, phenols, tannins, proteins and amino acids (Table 1). The occurrence of different secondary metabolites suggests a wide range of biological application of that plant.

Table 1: Analysis of phytochemicals in the chloroform extracts of *C. grandis*

Phytochemicals	Results
	Leaf extract
Alkaloids	+
Carbohydrates	+
Saponins	+
Glycosides	+
Flavonoids	+
Phenols	+
Tannins	+
Proteins	+
Amino acids	+
Deterpenes	-
Sterols	-

(+) Presence & (-) Absent

3.2 *In Vitro* Thrombolytic Activity

The ethanol extracts of *C. grandis* was also assessed for thrombolytic activity and the results are presented in table 2. Addition of 100 µl Streptokinase (30,000 I.U.), standard to the clots along with 90 minutes of incubation at 37°C, showed 65.16% clot lysis. Clots when treated with 100 µl sterile distilled water (control) showed only clot lysis 8.20%. The comparison of standard with control clearly demonstrated that clot dissolution does not occur when water was added to the clot as the mean difference in percentage of clot lysis between standard & control was found to be statistically significant. After treatment of clots with 100 µl ethanol extracts of *C. grandis*, 43.71% of clot lysis was obtained.

Table 2: % Clot lysis by ethanol extracts of *C. grandis* and standard

Samples	% of RBC lysis
Ethanol	43.71±0.05
Control	8.20±0.55
Streptokinase	65.16±1.08

3.3 Membrane Stabilizing Activity

The ethanol extracts of *C. grandis* at concentration 1.0mg/mL significantly protected the lysis of human erythrocyte membrane by hypotonic solution and heat induced haemolysis compared to the standard acetyl salicylic acid (0.10mg/ml). For hypotonic solution induced haemolysis, at a concentration of 1.0 mg/ml, the ethanol extract inhibited 79.53% haemolysis of RBCs as compared to 71.90% produced by acetyl salicylic acid (0.10 mg/ml). On the other hand, during heat induced condition ethanol extracts of *C. grandis* demonstrated 90.30% inhibition of haemolysis of RBCs, whereas acetyl salicylic acid inhibited 77.20% (Table 3). A possible explanation for the stabilizing activity of the extractives due to an increase in the surface area/volume ratio of the cells which could be brought about by an expansion of membrane or shrinkage of the cell and an interaction with membrane proteins [27].

Table 3: Effect of ethanol extracts of *C. grandis* on hypotonic solution and heat induced of erythrocyte membrane

Samples	% Inhibition of haemolysis	
	Hypotonic solution	Heat induced
Ethanol	79.53±1.63	90.30±0.37
Acetyl salicylic acid	71.90±0.42	77.20±0.23

3.4 Antioxidant Activity

3.4.1 DPPH Free Radical Scavenging Assay

The IC_{50} value of ethanol extracts of *C. grandis* is presented in table 4. The ethanol extracts of *C. grandis* exhibited significant antioxidant activity with IC_{50} value of 1.15 µg/ml in comparison with Ascorbic acid with IC_{50} value of 5.80 µg/ml as a reference standard. The phenomenon in this study is acceptable since ascorbic acid has the highest inhibition percentage inhibits free radical activity. Kunle and Egharevba, (2009) [28] suggested to consider the presence of flavonoids in a plant as indication of its antioxidant, antiallergic, antiinflammatory, antimicrobial and anticancer properties.

Table 4: IC_{50} values of ethanol extracts of *C. grandis* and standard in DPPH free radical scavenging assay

Samples	IC_{50} Value (µg/ml)
Ethanol	1.15±0.59
Ascorbic acid	5.80±0.21

3.5 Determination of Antibacterial Activity

Ethanol extract of *C. grandis* (leaf) showed a wide range of antibacterial activity against *Bacillus cereus, Staphylococcus aureus, Sarcina lutea, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella boydii, Shigella dysenteriae,* and *Vibrio parahemolyticus* at the concentration of 400µg/ml although *Bacillus megaterium and Bacillus subtillis* showed resistance at the same concentration whereas the range of zone of inhibition was within 9-16 mm (Table 5). Maximum zone of inhibition was observed against *Staphylococcus aureus* (16 mm).

In agreement with this finding, Dewanjee *et al*., (2007) [29] reported that antibacterial activity against *Staphylococcus aureus, Sarcina luteus, Bacillus pumilus, Escherichia coli, Shigella dysenteriae, Shigella soneii, Shigella flexneri, Shigella boydii, Pseudomonas aeruginosa, Vibrio cholerae* showed maximum efficiency in the methanol extract.

Table 5: Antibacterial activity by disc diffusion assay

Bacterial isolates	Zone of inhibition in diameter (mm)	
	Leaf extract	Ciprofloxacin
Gram positive acteria		
Bacillus cereus	13	33
Bacillus megaterium	-	37
Bacillus subtilis	-	32
Staphylococcus aureus	16	37
Sarcina lutea	9	34
Gram negative acteria		
Escherichia coli	11	37
Pseudomonas uriginosa	10	31
Salmonella typhi	12	36
Shigella boydii	12	33
Shigella dysenteriae	10	38
Vibrio parahemolyticus	11	37

3.6 Brine Shrimp Lethality Bioassay

In the brine shrimp lethality bioassay the LC_{50} value of the test samples after 24 hours was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration. The best-fit line was obtained from the curve data by means of regression analysis. Vincristine sulfate was used as positive control and the LC_{50} was found to be 0.451 µg/ml. The LC_{50} value of ethanol extracts was found to be 13.69 µg/ml (Table 6). However, varying degree of lethality to Artemia saline was observed with exposure to different dose levels of the test samples ranging from 0-100µg/ml.

In agreement with this finding, Alamgir *et al*., (2014) [12] reported that leaf extract showed strong inhibitory effect on brine shrimp lethality with LC_{50} at 24.20 µg/ml. The cytotoxicity of plant material would indicate the presence of antitumour compounds in plant extract [30]. Crude extracts resulting in LC_{50} values less than 250 µg/ml could be considered significantly active and potential for further investigation [31].

Table 6: LC_{50} values of ethanol extracts of *C. grandis* and standard

Extract	LC_{50} Value (µg/ml)
Ethanol	13.69±0.41
Vincristine sulfate	0.451

4. Conclusion

All the conducted experiments in the present study are based on crude extract and are considered to be preliminary and more sophisticate research is necessary to reach a concrete conclusion about the findings of the present study. It can be concluded from the above findings, the plant *C. grandis* have significant thrombolytic, membrane stabilizing, antioxidant, antimicrobial and cytotoxic activity. The plant has strong antioxidant activity. So, further scientific studies are necessary to elucidate detailed mechanism of action and isolate the responsible active principles.

5. References

1. Khandaker S, Das S, Opo FADM, Akhter R, Shahriar M. *In vivo* pharmacological investigations of the crude extracts of *Calamus viminalis* (L.). Journal of Pharmacognosy and Phytochemistry. 2016; 5(3):263-269.
2. Rahmatullah M, Azam MNK, Khatun Z, Seraj S, Islam F, Rahman MA *et al*. Medicinal plants used for treatment of diabetes by the Marakh Sect of the Garo tribe living in Mymensingh District, Bangladesh. African Journal of Traditional, Complementary and Alternative medicines. 2012; 9(3):380-385.
3. Rahmatullah M, Hasan A, Parvin W, Moniruzzaman M, Khatun A, Khatun Z *et al*. Medicinal plants and formulations used by the soren clan of the Santal tribe in Rajshahi District, Bangladesh for treatment of various ailments. African Journal of Traditional, Complementary and Alternative medicines. 2012; 9(3):350-359.
4. Rahmatullah M, Noman A, Hossan MS, Harun-Or-Rashid M, Rahman T, Chowdhury MH *et al*. A survey of medicinal plants in two areas of Dinajpur district, Bangladesh including plants which can be used as functional foods. American-Eurasian Journal of Sustainable Agriculture. 2009; 3(4):862-876.
5. Jarald E, Joshi SB, Jain DC. Diabetes and herbal medicines. Iranian Journal of Pharmacology & Therapeutics. 2008; 7(1):97-106.
6. Muthu C, Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. Journal of Ethnobiology and Ethnomedicine. 2006; 2:43.
7. Rahmatullah M, Mollik MAH, Islam MK, Islam MR, Jahan FI, Khatun Z *et al*. A survey of medicinal and functional food plants used by the folk medicinal practitioners of three villages in Sreepur Upazilla, Magura District, Bangladesh. American-Eurasian Journal of Sustainable Agriculture. 2010; 4(3):363-373.
8. Bapuji JL, Ratnam SV. Traditional Uses of Some Medicinal Plants by tribals of Gangaraju Madugula Mandal of Visakhapatnam District, Andhra Pradesh. Ethnobotanical Leaflets. 2009; 13:388-398.
9. Hasan MM, Annay MEA, Sintaha M, Khaleque HN, Noor FA, Nahar A *et al*. A survey of medicinal plant usage by folk medicinal practitioners in seven villages of Ishwardi Upazilla, Pabna District, Bangladesh. American-Eurasian Journal of Sustainable Agriculture. 2010; 4(3):326-333.
10. Yadav G, Mishra A, Tiwari A. Medical properties of ivy gourd (*Cephalandra indica*): A review. International Journal of Pharmaceutical Research and Development. 2010; 2(9):92-98.
11. Dewanjee S, Maiti A, Kundu M, Mandal SC. Evaluation of anthelmintic activity of crude extracts of *Diospyros peregrina*, *Coccinia grandis* and *Schima wallichii*. Dhaka University Journal of Pharmaceutical Sciences. 2007; 6(2):121-123.
12. Alamgir ANM, Rahman M, Rahman A. Phytochemical characteristics, antimitotic, cytotoxic and anti-inflamatory activities of *Coccinia grandis* (L.) J. Voigt. Journal of Pharmacognosy and Phytochemistry. 2014; 3(1):222-225.
13. Orech FO, Akenga T, Ochora J, Friis H, Aagaard-Hansen J. Potential toxicity of some traditional leafy vegetables consumed in Nyang'oma Division, Western Kenya. African Journal of Food and Nutritional Sciences. 2005; 5(1):1-13.
14. Sudha P, Zinjarde SS, Bhargava SY, Kumar AR. Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. BMC Complementary and Alternative Medicine. 2011; 11:5.
15. Taur DJ, Patil RY. Mast cell stabilizing, anti-anaphylactic and antihistaminic activity of *Coccinia grandis* fruits in asthma. Chinese Journal of Natural Medicines. 2011; 9(5):359-362.
16. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review.

Internationale Pharmaceutica Sciencia, 2011; 1:103-104.
17. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. Thrombosis Journal. 2006; 4:14.
18. Sharif S, Ahmed T, Haque MA, Bhuiyan MA, Shahriar M. Phytochemical screenings, thrombolytic activity, membrane stabilizing activity and cytotoxic properties of *Polygonum hydropiper*. Research Journal of Medicinal Plant. 2014; 8(2):92-98.
19. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Membrane stabilizing activity - a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia. 1999; 70:251-257.
20. Omale J, Okafor PN. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata.* African Journal of Biotechnology. 2008; 7:3129-3133.
21. Shahriar M, Khair NZ, Sheikh Z, Chowdhury SF, Kamruzzaman M, Bakhtiar MSI *et al*. Characterization of phytoconstituents and potential bioactivity of *Annona reticulata* L. leaf extract. Journal of Pharmacognosy and Phytochemistry. 2016; 5(1):42-45.
22. Ahmed T, Akter R, Sharif S, Shahriar M, Bhuiyan MA. *In vitro* antioxidant activities and *In vivo* anti-nociceptive and neuropharmacological activities of *Mimosa pudica.* International Journal of Pharmacy. 2014; 4(2):70-78.
23. Shafa F, Shahriar M, Opo FADM, Akhter R, Hossain MMH, Choudhury N. Characterization of phytoconstituents, *in vitro* antioxidant activity and pharmacological investigation of the root extract of *Typhonium trilobatum.* International Journal of Pharmaceutical Sciences and Research. 2016; 7(4):1694-1704.
24. Bauer AW, Kirby WMM, Sherries JC, Tuck M. Antibiotic susceptibility testing by a standardized disc diffusion method. American Journal of Clinical Pathology. 1966; 45:493-496.
25. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen JB, Nicholsand DE, Mclaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Medica. 1982; 45(5):31-34.
26. Shahriar M, Khair NZ, Sheikh Z, Chowdhury SF, Kamruzzaman M, Bakhtiar MSI *et al*. Phytochemical analysis, cytotoxic and *in vitro* antioxidant activity of *Erythrina variegate* bark. European Journal of Medicinal Plants. 2016; 11(3):1-5.
27. Bakhtiar MSI, Akhter R, Narjish SN, Chisty SJ, Shahriar M, Bhuiyan MA. *In vitro* and *in vivo* biological investigations of the whole plant extracts of *Chrozophora prostrata* (Dalz.). International Journal of Pharmacy. 2015; 5(4):1188-1195.
28. Kunle OF, Egharevba HO. Preliminary studies on *Vernonia ambigua*, phytochemistry and antimicrobial screening of whole plant. Ethnobotanical Leaflets. 2009; 13:1216-1221.
29. Dewanjee S, Kundu M, Maiti A, Majumdar R, Majumdar A, Mandal SC. *In Vitro* Evaluation of Antimicrobial activity of crude extract from plants *Diospyros peregrina*, *Coccinia grandis* and *Swietenia macrophylla.* Tropical Journal of Pharmaceutical Research. 2007; 6 (3):773-778.
30. Martin-Cordero G, Saenz MT, Ayuso MJ. Cytotoxic activity of *Retama sphaerocarpa*. Fitoterapia. 1995; 16:495-498.
31. Rieser MJ, Gu ZM, Fang XP, Zeng L, Wood KV, McLaughlin JL. Five novel mono-tetrahydrofuran ring acetogenins from the seeds of *Annona muricata*. Journal of Natural Products. 1996; 59:100-108.