International Journal of Pharmacy

Journal Homepage: http://www.pharmascholars.com

Original Article **CODEN: IJPNL6**

Evaluation of antioxidants, membrane stabilizing, cytotoxic and anthelmintic activity with phytochemical screening of *Chromolaena odorata*: A medicinal shrub

Md. Tanvir Haider Tanna[1], Aninda Kumar Nath[2], Mohammad Nurul Amin[3], Md. Ibrahim[3], Manjurul Islam Chowdhury[1], Md. Emdadul Hasan Mukul[4], Md. Saif Uddin Rashed[5], Asma Kabir[3], Monsur Ahmed[1], Mohammad Salim Hossain[1*]

[1]Department of Pharmacy, Noakhali Science and Technology University, Noakhali-3814, Bangladesh
[2]Department of Pharmacy, BGC Trust University Bangladesh, Chittagong, Bangladesh
[3]Department of Pharmacy, Atish Dipankar University of Science and Technology, Dhaka-1213, Bangladesh
[4]Department of Pharmacy, Khwaja Yunus Ali University, Sirajganj-6751, Bangladesh
[5]Department of Statistics, Jahangir Nagar University, Savar-1342, Bangladesh

***Corresponding author e-mail:** pharmasalim@yahoo.com

Received on: 14-10-2015; Revised on: 05-11-2015; Accepted on: 21-12-2015

ABSTRACT
The present study was an attempt to evaluate the antioxidant, membrane stabilizing, anthelmintic and cytotoxic properties of crude methanolic extract of *C. odorata* and different fractions of the crude extract. The phytochemical screening revealed the potent presence of alkaloids, phytosterols, tannins, terpenes, fats and fixed oils. Determination of total phenolic contents and DPPH methods were evaluated for antioxidant activity. The total phenolic content (mg of GAE / gm) of Crude Methanol Extract (CME), Petroleum Ether Soluble Fraction (PESF), Ethyl Acetate Soluble Fraction (EASF) and Chloroform Soluble Fraction (CSF) were 71.08±0.38, 52.08±1.01, 86.33±0.38 and 54.25±0.90, respectively. In case of DPPH method, the IC_{50} values were 19.48±0.49 μg/ml, 22.44±0.63μg/ml, 99.05±0.81μg/ml, 166.96±0.51 μg/ml, 317.10±0.99 μg/ml for standard (BHT: tert-butyl-1-hydroxytoluene) and the four extracts, respectively. Inhibition of haemolysis by standard acetyl salicylic acid (ASA) (70.82% and 75.91%), CME (20.65% and 53.53%), PESF (35.59% and 36.28%), EASF (34.74% and 30.18%), and CSF (30.01% and 32.26%) for hypotonic and heated solution respectively, were evaluated. For the anthelmintic test, paralysis and death time for standard (albendazole) 10mg/ml and crude extracts (10, 20, 30, 40, 50) mg/ml are 56.20±0.20 and 77.4±0.24, 30.4±0.75 and 60.8±1.03, 24.6±0.32 and 50.2±0.37, 20.6±0.40 and 40.8±0.51, 16.4±0.68 and 32.4±1.29, 10.2±0.40 and 10.2±0.40 minutes respectively. Moreover, the LC_{50} values were 0.839μg/ml, 10.245μg/ml, 8.98μg/m, 8.28μg/ml and 9.298μg/ml for standard group (vincristin sulphate), CME, PESF, EASF and CSF respectively in brine shrimp lethality bioassay. From the study it was revealed that all the extracts were abundant of various phytochemicals and showed various biological activities.

Key words: Phytochemical, Antioxidant, Membrane stabilizing, Cytotoxic, Anthelmintic.

INTRODUCTION
Antioxidants prevent oxidation of other molecules by counteracting reactive oxygen species (ROS). Antioxidants that are obtained from natural sources such as plants are more beneficial than synthetic antioxidants [1, 2]. Synthetic antioxidants may possess adverse effects on human body [3]. ROS are responsible for oxidative stress which mediates many acute and chronic diseases. A proper balance between oxidation and anti-oxidation is essential for maintaining a sound biological system [1]. Besides, a stabilized membrane is required to retard oxidative damage and associated inflammatory effects caused

by ROS produced within the body [4]. Now a day, serious side effects are available in many anti-tumor drugs. Hence, if any remarkable cytotoxic effect exerting herbal chemical can be obtained which is relatively cheap and locally available then it will be so fruitful in the cancer treatment [2]. A common macro parasitic disease throughout the world including Bangladesh is helminthiasis. Generally, parasitic worms are responsible for this disorder. About two billion people in the world are affected by parasitic worm infection due to insufficient control measures, as declared by World Health Organization (WHO) [5].

Nature plays a significant role in the treatment of almost all ailments of mankind by providing various remedies from its plants, animals and other sources [6,7]. Still 80% people in the world uses medicinal plants for various medical purposes, as reported by WHO [7]. It has been observed that phyto-constituents obtained from medicinal plants act as active and main compounds in the modern medicines [8]. It is believed that herbs and fruits containing phyto-constituents may protect human body from several types of disorders [9]. *Chromolaena odorata* (L) King and Robinson belongs to the family Asteraceae is usually known as “Siam weed”. It is a semi woody shrub [10]. It has been distributed in the tropical regions of Africa, Asia and other parts of the universe [11]. *Chromolaena odorata* (*C. odorata*) has been reported to possess antiprotozoal, antispasmodic, antibacterial, antihypertensive, diuretic, astringent, hepatotropic, antiinflammatory and antitrypanosomala ctivities. Besides, in developing countries, the extract of the fresh leaves of *C. odorata*are applied in the traditional herbal treatment for soft tissue wounds, burns and skin infections [11,12].

Now a day, in drug research, a systematic search is considered for significant bioactivities from medicinal plants. Therefore, the present study was designed to investigate the antioxidant, membrane stabilizing, cytotoxic and anthelmintic activity and also to identify the presence of phyto-constituents in crude methanolic extract of *C. odorata.*

METHODOLOGY

Plant materials: For the present study, *C. odorata* (whole plant) was collected from Maheshkhali island, Cox’s bazaar, in April, 2013. After collection the taxonomic identification of the plant was carried out with the help of taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (DACB Accession no. 38509). The voucher specimen was also deposited there for future reference.

Extraction and fractionation: For methanolic extraction 400 gm of air dried and powdered sample were immersed in 2500 ml of 80% methanol (Merck KGaA, Darmstadt, Germany) in clean, sterilized and flat-bottomed glass container. The container with its contents was sealed and kept for maceration for 15 days accompanying occasional shaking and stirring. At the end of 15th day, the whole mixture was filtered using filter cloth and Whatman® filter paper (Sargent-Welch, USA). The resultant filtrate was then allowed to evaporate in water bath maintained $45^{o}C$ to dryness and thus a dark green viscous extract was obtained (yield 24 gms). This is the Crude Methanol Extract (CME) of *C. odorata*. This concentrated CME was fractionated by the modified Kupchan partitioning protocol [13] and the resultant partitionates i.e., Petroleum Ether Soluble Fraction (PESF), Ethyl Acetate Soluble Fraction (EASF) and Chloroform Soluble Fraction (CSF) were used for the biological screenings.

Phytochemical screening: Small quantity of freshly prepared crude methanolic extract of *C. odorata* was qualitatively tested for the presence of phytochemicals such as alkaloids with Mayer’s and Hager’s reagent, Carbohydrates with Benedict’s test and Fehling’s test, glycosides with Legal’s test and Modified Borntrager’s test, phytosterols with Salkowski’s test and Libermann Burchard’s test, proteins with xanthoproteic test, flavonoids with alkaline reagent test and lead acetate test, tannins with gelatin test, saponins with Froth test and foam test and phenols with ferric chloride test, etc. [14].

Antioxidant activity: There are various well known methods, which are followed to determine the antioxidant properties. Among them, two complementary test methods namely total phenolic content determination and DPPH free radical scavenging assay methods were used for investigating the antioxidant activity of *C. odorata*.

Total phenolic content determination: The amount of total phenolics in extracts was determined with the Folin-Ciocalteu reagent [8]. Here, gallic acid was used as a standard and the amount of total phenolics were expressed as mg/g of gallic acid equivalents (GAE). Concentration of 6.25, 12.5, 25, 50, and 100 μg/ml of gallic acid and concentration of 2 μg/ml of plant extract were prepared in methanol and 0.5 ml of each sample were placed into test tubes and mixed carefully with 2.5 ml of a 10- fold dilute Folin-Ciocalteu reagent and 2 ml of 7.5% sodium

carbonate. The test tubes were covered with para-film and allowed to stand for almost 30 min at room temperature before the absorbance was read at 760 nm spectrophotometrically (UV-1800, Shimadzu, Japan). All determinations were performed nicely in triplicate [8]. Thus, total phenolic content was determined as mg of gallic acid equivalent per gram using the equation obtained from a standard gallic acid calibration curve.

DPPH scavenging activity: The free radical scavenging activities (antioxidant capacity) of the plant extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams [15]. 2.0 ml of ethanol solution of the extracts at different concentration were mixed with 3.0 ml of a DPPH ethanol solution (20µg/ml). The antioxidant potential was assayed from the bleaching of purple colored ethanol solution of DPPH radical by the plant extract as compared to that of *tert*-butyl-1-hydroxytoluene (BHT) by UV spectrophotometer.

Membrane stabilizing activity: The membrane stabilizing activity of the extracts was assessed by using hypotonic solution and heat-induced hemolysis of human erythrocyte [4].

Preparation of erythrocyte suspension: To prepare the erythrocyte suspension, 2 ml of blood was obtained using syringes from male volunteers under standard condition. EDTA was used to prevent clotting. The blood was centrifuged for 10 minutes at 3000 g using centrifugal machine. Then, it was washed three times using isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4). Thus the suspension finally collected was the stock erythrocyte (RBC) suspension.

Hypotonic Solution- Induced Haemolysis: The experiments were carried out with hypotonic solution. The test sample which consisted of stock erythrocyte (RBC) suspension (0.50 mL) with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing either the different methanolic extract (1.0 mg/mL) or Acetyl Salicylic Acid (0.10 mg/mL). The Acetyl Salicylic Acid was used as a reference standard. The control sample which consisted of 0.5 ml of RBCs was mixed with hypotonic-buffered saline alone. The mixtures were incubated for 10 min at room temperature, centrifuged for 10 min at 3000 g and the absorbance of the supernated was measured at 540 nm using Shimadzu UV spectrophotometer [4]. The percentage inhibition of either haemolysis or membrane stabilization was calculated using the following equation:

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

Where,

OD_1 = Optical density of hypotonic-buffered saline solution alone (control) and

OD_2 = Optical density of test sample in hypotonic solution.

Heat Induced Hemolysis: Aliquots (5 ml) of the isotonic buffer, containing 1.0 mg/ml of different extracts of the plant were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension (30 mL) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54˚C for 20 min in a water bath. The other pair was maintained at (0-5)˚C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 g and the absorbance of the supernatant was measured at 540 nm using UV spectrometer [4]. The percentage inhibition or acceleration of hemolysis in tests and was calculated using the following equation:

% inhibition of haemolysis = $100 \times [1- (OD_2 - OD_1)/(OD_3 - OD_1)]$

Where,

OD1 =test sample unheated,

OD2 = test sample heated and, OD3 = control sample heated

Cytotoxic activity: The cytotoxic property of the extract was determined using brine shrimp lethality test [16]. The investigation was done on *artemia salina* (brine shrimp). *Artemis Salina leach (*brine shrimp eggs) collected from pet shops was used as the test organism. One spoon of cyst was hatched for about 48 hrs in saline water, prepared by dissolving 20 g pure NaCl and 18 g normal edible NaCl into 1 L water. The hatched cyst in turn became living nauplii. Different concentrations of the extract were prepared using dimethyl sulfoxide (DMSO) as solvent. For the test, different concentrations of plant extract prepared were added to test tubes, each containing 10 shrimps in saline water. Here, vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get a primary concentration of 40µg/ml from which serial dilutions were made using DMSO to get 20µg/ml, 10µg/ml, 5µg/ml, 2.5µg/ml, 1.25µg/ml, 0.625µg/ml, 0.3125 µg/ml, 0.15625µg/ml and 0.78125µg/ml solution from the extract. Then the positive control solutions were added to the pre-marked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups.

Counting of Nauplii : After 24 hours, the vials were inspected by using a magnifying glass and the number of survived nauplii in each vial was counted consciously. From this result, the percent (%) of lethality of the brine shrimp nauplii was calculated nicely for each concentration.

Anthelmintic activity: The anthelmintic study was carried out by the method of Ajaiyeoba *et al.* [17] with minor modifications. Adult earthworms were used to study the anthelmintic activity because they are anatomically and physiologically resemble with the intestinal roundworm parasites of human being [16]. The earthworms belonging to species *pheritima posthuma* (annelida), about 3-5 cm in length and 0.1-0.2 cm in width weighing about 0.8-3.04 g, were collected from the moist soil of Noakhali Science and Technology University, Sonapur, Noakhali and identified by the Department of Fisheries and Marine Science (FIMS), Noakhali Science and Technology University (Voucher No. 229/2013). The crude methanolic extract of C. odorata was used as test samples. They were used to prepare different concentrations (10-80 mg/ml) separately. For the methanol extract different concentrations were prepared by weighing 100 mg, 200 mg, 400 mg, 600 mg and 800 mg extracts and dissolving them in 10 ml distilled water separately. 100 mg of piperazine citrate was measured by weighing machine and dissolved in 10 ml water to make a concentration of 10 mg/ml. A control group was established with distilled water to ensure that the test was a validate one. Earthworms were divided into twelve groups (each containing four earthworms) in petridish. Five groups were used for the five concentrations of methanolic extracts of the plant. One group was applied to reference standard and another to control group. Finally, the time of paralysis and death was determined consciously. Time for paralysis was noted when no movement of any sort could be observed except when the worms were vigorously shaken. Time for death of worms was taken after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (almost 50 °C) followed with fading away of their body colors [16].

RESULTS

Preliminary phytochemical screening: Phytochemical analysis of the crude methanolic extract of *C. odorata* revealed the presence of alkaloids, phytosterols, tannins, terpenes, fats and fixed oils.

Antioxidant activity

Total phenolic content determination: Based on the absorbance values of the extract solutions, the colorimetric analysis of the total phenolics of extract and fractions were determined and compared with that of standard solution (Fig. 1) of gallic acid equivalents. Total phenolic content of the samples were expressed as mg of GAE (gallic acid equivalent)/ gm of dry extract and are given in table 02. The amount of total phenolic content differed in different extracts. Among the four ethanolic extracts highest phenolic content was found in EASF (86.33±0.38mg of GAE / gm of extract) and the lowest was found in PESF (52.08±1.01mg of GAE / gm of extract). The total phenolic content of CME and CSF were 71.08±0.3801mg of GAE / gm of extract and 54.25±0.9001mg of GAE / gm of extract respectively.

DPPH free radical scaveging activity: In this investigation, the CME of *C. odorata* showed the highest free radical scavenging activity with IC_{50} value 22.44μg/ml. At the same time the PESF, EASF and CSF also exhibited strong antioxidant potential having IC_{50} value 99.05μg/ml, 166.96μg/ml and 317.10μg/ml respectively. Here, *tert* butyl- 1-hydroxytoluene (BHT) was used as reference standard.

Membrane stabilizing activity: The various extracts of *C. odorata* at concentration 1.0 mg/mL were tested to know the activity against lysis of human erythrocyte membrane induced by hypotonic solution, and heat as compared to the standard acetyl salicylic acid (ASA). Among all the fractions, CME showed maximum effect with a value of 20.65% and 53.53% inhibition of hemolysis caused by hypotonic and heated solution respectively. ASA (0.10 mg/mL) revealed 70.82% and 75.91% inhibition of hemolysis induced by heat and hypotonic solution correspondingly. The petroleum PESF inhibited 35.59% and 36.28%, EASF inhibited 34.74% and 30.18%, and CSF inhibited 30.01% and 32.26% haemolysis of RBC caused by hypotonic and heated solution respectively.

Cytotoxic activity: The present study observed that the mortality rate at the highest concentration (400μg/ml) for the CME, PESF, EASF and CSF is 100%, 100%, 100% and 100% respectively, while in case of standard drug (vincristine sulphate) it is 100% at the concentration 40 μg/ml.

The 50% mortality rate for CME is observed at the concentration 12.5μg/ml. Furthermore, 50% mortality rate is observed for PESF when the concentration was 12.5μg/ml and for EASF and CSF it was 6.25μg/ml. In case of standard drug 50% mortality rate is showed at the concentration of 0.625μg/ml. At the lowest concentration (0.78125μg/ml) the CME,

PESF and CSF showed no mortality, while it was 10% for EASF and standard drug. The LC_{50} values of CME, PESF, EASF and CSF were 10.245, 8.98, 8.28 and 9.298 µg/ml respectively. The positive control vincristine sulphate showed LC_{50} at a concentration of 0.839µg/ml. From the results of the brine shrimp lethality bioassay it can be well predicted that CME, PESF, EASF and CSF possess mild cytotoxic properties.

Anthelmintic activity: Time recorded for paralysis and death of earthworm for crude methanolic extracts (CME) are given in the figure below. The paralysis and death time for standard (albendazole, 10mg/ml) and crude extracts (10, 20, 30, 40 and 50 mg/ml) are 56.20±0.20 and 77.4±0.24, 30.4±0.75 and 60.8±1.03, 24.6±0.32 and 50.2±0.37, 20.6±0.40 and 40.8±0.51, 16.4±0.68 and 32.4±1.29, 10.2±0.40 and 10.2±0.40 minutes respectively. From the results, it can be well predicted that crude methanolic extract of *C. odorata* possess moderate anthelmintic activity.

DISCUSSION

In many parts of the world, people still use medicinal plants in treating various ailments because such plants possess different types of phytoconstituents which exert a variety of pharmacological effects in human body [18]. In this study, phytochemical screening revealed the presence of several phytochemicals like alkaloids, carbohydrates, tannins, phenol, terpenes, fats and fixed oils. Phytochemicals like polyphenolic compounds (tannins, flavonoids and phenolic acids) are responsible for various biological actions including antioxidant activity [18]. In our study, the extract and its fractions showed strong antioxidant activity. Antioxidant action could be attributed to the presence of tannins. Antioxidant potentials of crude methanolic extract of *C. odorata* was observed to increase with the increasing concentration. Phenols are important phytoconstituents of medicinal plants which directly contribute to antioxidant effect [19]. In this investigation, total phenolic content was determined as moderate in the methanolic crude extract of *C. odorata.* Phenolic compounds are very important for the antioxidant and free radical scavenging activities of medicinal plants. Such compounds react as hydrogen donors to neutralize the free radicals [20,21]. Few researches reported that the higher the concentration of total phenolic content in a medicinal plant extract is, the higher is its antioxidant activity [22,23]. Moreover, plants free radical scavenging activity depends on the presence of phenolic components. Free radicals contribute to many disorders in human body including arthritis, ischemia, atherosclerosis, gastritis, cancer and injury of central nervous system [24]. DPPH assay is a common method for testing free radical scavenging property of a plant extract [25]. In this investigation, the extract and its fractions showed a higher radical scavenging property than the standard drug (Figure 02). Besides, the percentage of free radical inhibition was observed to increase with the increasing concentration of extract.

The vigour of cells in human body depends on the integrity of their membranes. Exposure of red blood cell (RBC) to hypotonic or heated medium results in lysis of its membrane through haemolysis and oxidation of haemoglobin. This haemolytic effect is related to excessive accumulation of liquid or fluid within the cell. Such injurious membrane will further render the cell more susceptible to secondary damage through free radical -induced lipid peroxidation [26]. Membrane stabilizing properties containing compounds interfere with the initial phase of inflammatory reactions, namely the inhibition of the release of phospholipases that may trigger the formation of different types of inflammatory mediators [26]. In this investigation, crude extract and its fractions showed moderate membrane stabilizing activity compared to the standard acetyl salicylic acid (Figure 03). This plant also showed mild cytotoxic activity (Figure 04). It is well known rhat bioactive compounds in a plant are responsible for exhibiting cytotoxic potency. Flavonoids show anti inflammatory, anti-cancer and anti-microbial activities. According to some previous studies, tannins may possess remarkable cytotoxic and anti-tumour properties [2]. Furthermore, The crude methanolic extracts of *C. odorata* showed a moderate anthelmintic potency in dose dependent manner. Chemicals like phenols, tannins and alkaloids etc. are responsible for anthelmintic property. By acting on the central nervous system of worms, alkaloids may cause paralysis of it. Besides, the main function of anthelmintic drug is to cause a flaccid paralysis of the worm [27].

CONCLUSION

In the context of the above discussion, it can be revealed that all the extracts of *C. odorata* showed significant antioxidant activity. These extracts also possess moderate membrane stabilizing and anthelmintic and mild cytotoxic activity. However, further investigations, based on these preliminary researches are required to explore the bioactive molecules which are responsible for the extracts' activities as well as their mechanisms of action.

Table 01: Preliminary phytochemical screening of methanolic extract of *C. odorata*

Sl. No.	Phytochemicals	Test	Observation
i.	Alkaloids	a) Wagner's test	+
		b) Hager's test	++
ii.	Carbohydrates	a) Molisch's test	-
		b) Benedict's test	-
		c) Fehling's test	-
iii.	Glycosides	a) Legal's test	-
iv.	Saponins	a) Froth Test	-
		b) Foam test	-
v.	Phytosterols	a) Libermann-Burchard's test	+
		b) Salkowski's test	-
vi.	Phenol	a) Ferric Chloride Test	+
vii.	Tannins	a) Ferric Chloride Test	+
viii.	Flavonoids	a) Alkaline reagent test	-
		b) Lead acetate test	-
ix.	Proteins and amino acids	a) Xanthoproteic Test	-
		b) Ninhydrin Test	-
x.	Terpenes	a) Copper acetate Test	+
xi.	Fats and Fixed oils	a) Soap test	+

Key: (+) = Present, (++) = significantly present and (−) = Absent

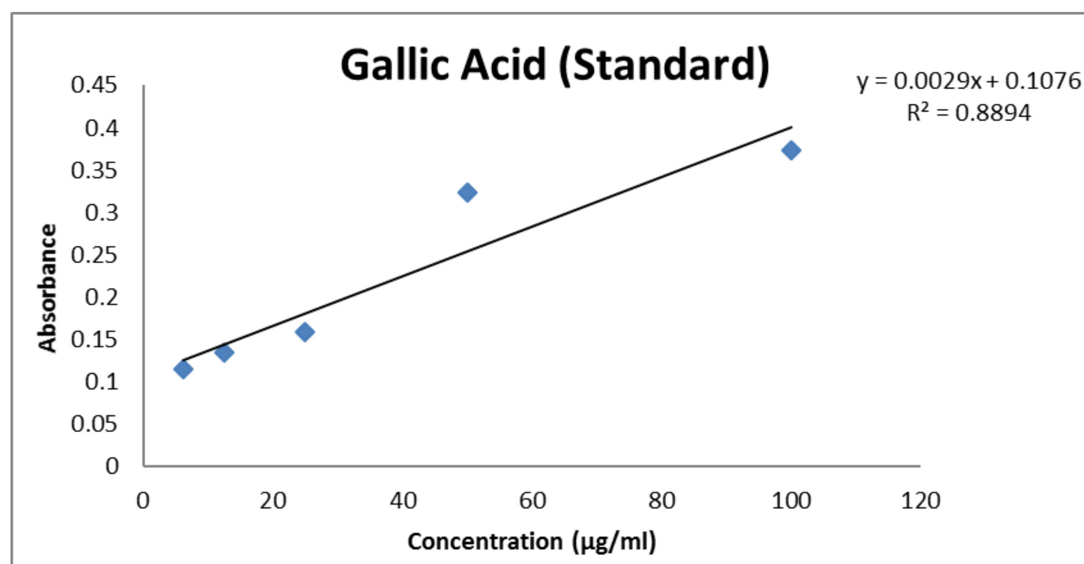

Figure 01: Total phenolic content of gallic acid (Standard)

Table 02: Determination of total phenolic content of crude methanolic extracts of *C. odorata* (CME), PESF, EASF and CSF.

Extracts	Average Absorbance at 760 nm	Total Phenolic Content (mg of GAE / gm) of Extracts
CME	0.391±0.0015	71.08±0.38
PESF	0.315±0.0040	52.08±1.01
EASF	0.452±0.0015	86.33±0.38
CSF	0.324±0.0036	54.25±0.90

Values are expressed as mean±SD (n=3), GAE = Gallic Acid Equevalents

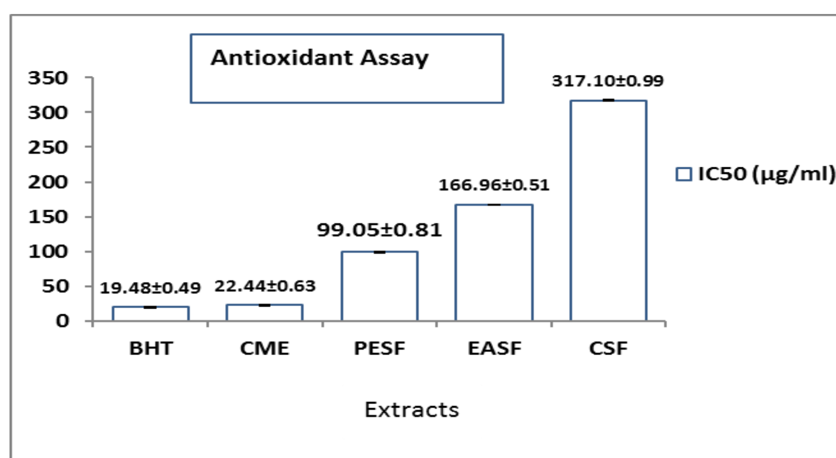

Figure 02: IC_{50} values of standard and four extracts

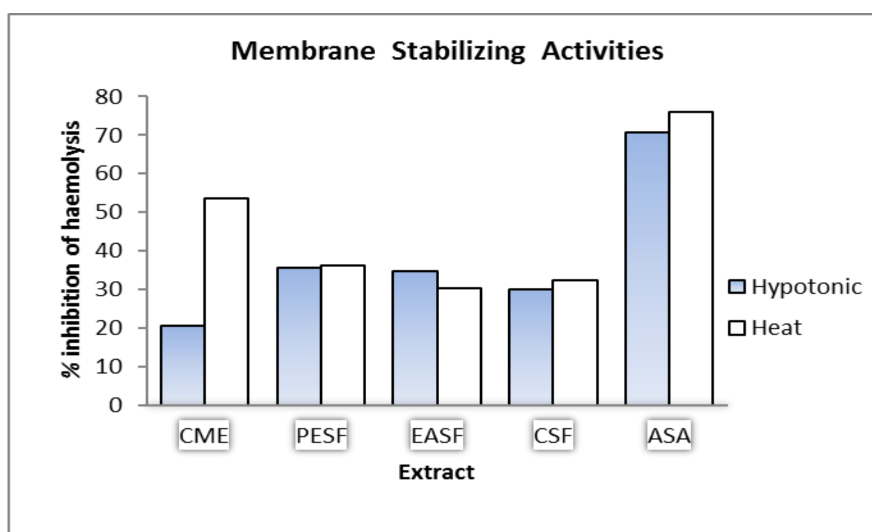

Figure 03: % inhibition of haemolysis of different extractives of *C. odorata* on heat induced and hypotonic solution-induced condition.

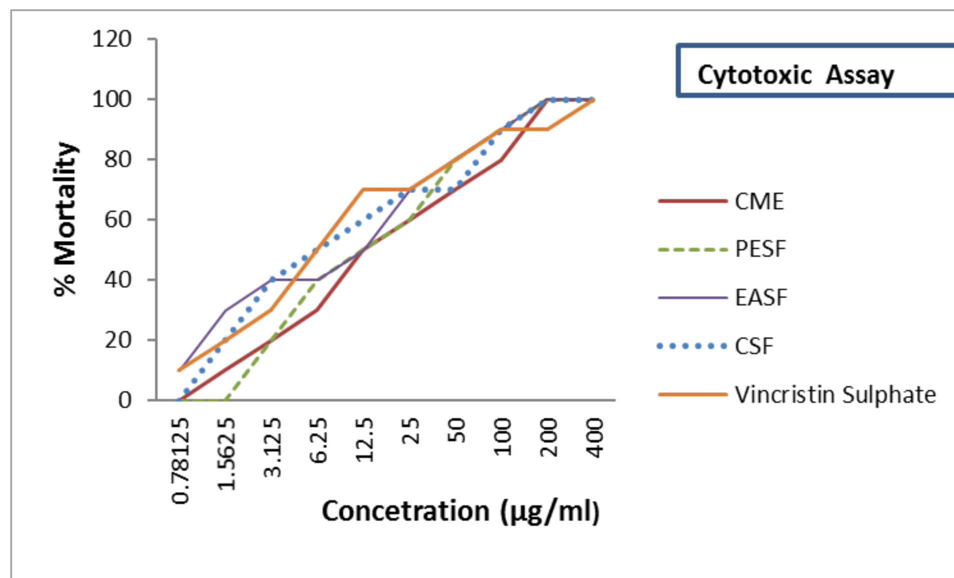

Figure 04: Effect of CME, PESF, EASF and CSF of *C. odorata* on brine shrimp nauplii.

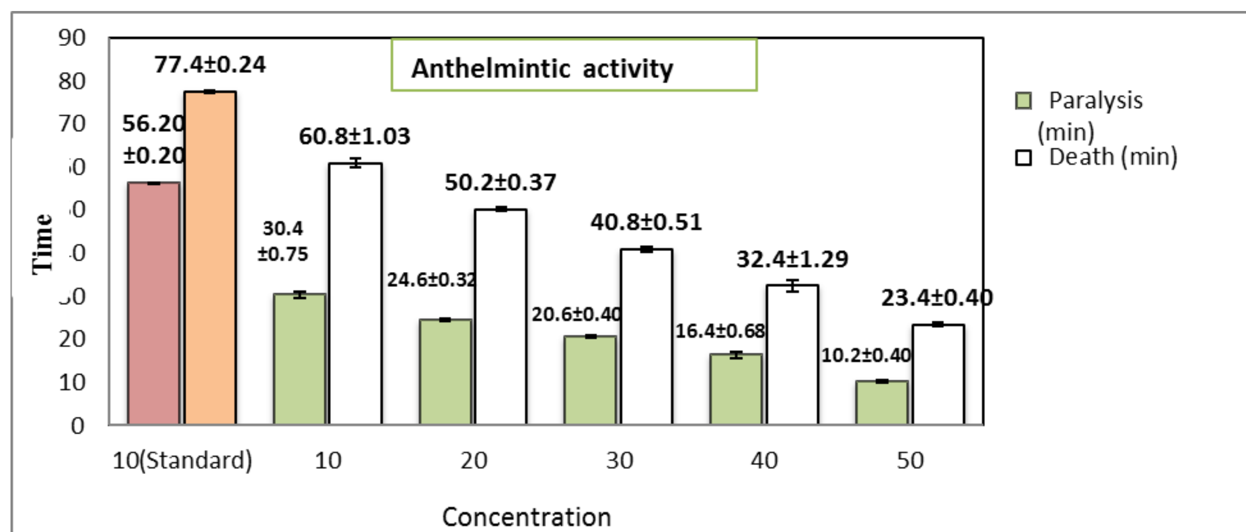

Figure 05: Paralysis and death time of *Pheritima posthuma* for crude methanolic extract of *Chromolaena odorata*

REFERENCES

1. Amin MN, Dewan SMR, Noor W, Shahid-Ud-Daula AFM. Eur J Exp Biol, 2013; 3(1): 449-454.
2. Amin MN, Banik S, Ibrahim M, Moghal MMR, Majumder MS, Siddika R, Alam MK, Jitu KMRM, Anonna SN. Int J Pharmacognosy and Phytochem Res, 2015; 7(1): 8-15.
3. Dewan SMR, Amin MN, Adnan T, Uddin SMN, Shahid-Ud-Daula AFM, Sarwar G, Hossain MS. J Pharm Res, 2013; 6(6): 599-603.
4. Islam T, Das A, Shill KB, Karmakar P, Islam S, Sattar MM. J Med Plants Res, 2015; 9(5): 151-158.
5. Gaikwad SA, Kale AA, Jadhav BG, Deshpande NR, Salvekar JP. J Nat Prod Plant Resour, 2011; 1(2): 62-66.
6. Rashed-Al-Qayum, Khan MD, Moghal MMR, Amin MN, Hossain MS, Hossain MD. Der Pharmacia Sinica, 2013; 4(3):183-187.
7. Majumder MS, Amin MN, Moghal MMR, Banik S, Kar A, Hossain MM. J Sci Res, 2014; 6(2):339-345.
8. Raju GS, Moghal MMR, Dewan SMR, Amin MN, Billah MM. Avicenna J Phytomed, 2013; 3(4): 313-320.

9. Majumder MS, Hossain DMS, Amin MN, Moghal MMR, Banik S, Hossain MM. World journal of pharmacy and pharmaceutical sciences, 2014; 3(5): 58-72.
10. Pandith H, Zhang X, Liggett J, Min K, Gritsanapan W, Baek SJ. ISRN Dermatology, 2013, Article ID 168269, 8 pages.
11. Akinmoladun AC, Ibukun EO, Dan-Ologe IA. Sci Res Essays, 2007; 2(6):191-194.
12. Bhargava D, Mondal CK, Shivapuri JN, Mondal S, Kar S. J Inst Med, 2013; 35(1):53-56.
13. Van Wagenen BC, Larsen R, Cardellina JH, Ran dazzo D, Lidert ZC, Swithenbank C. J Org Chem, 1993; 58: 335-337.
14. Ahmed J, Sultana N, Dewan SMR, Amin MN, Uddin SMN. International Journal of Pharmamedix India, 2013; 1(2): 222-232.
15. Choi HY, Jun EJ, LIM BO, Chung IM, Kyung SH and Park DK. Int J Pharm, 2000; 14: 250-253.
16. Majumder MS, Amin MN, Moghal MMR, Banik S, Kar A, Hossain MM. J Sci Res, 2014; 6(2): 339-345
17. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. Pharm Biol, 2001; 39(3): 217-220.
18. Uddin SMN, Amin MN, Shahid-Ud-Daula AFM, Hossain H, Haque MM, Rahman MS, Kader MA. J Med Plant Res, 2014; 8(37): 1127-1133.
19. Duh PD. J. Agric. Food Chem, 1994; 42:629-632.
20. Kulisic T, Radonic A, Katalinic V, Milos M. Food Chem, 2004; 85(4): 633-640.
21. Tanaka M, Kuie CW, Nagashima Y, Taguchi T. Nippon Suisan Gakkaishi, 1988; 54: 1409-1414.
22. Madaan R, Bansal G, Kumar S, Sharma A. Indian J Pharm Sci, 2011; 73: 666-669.
23. Henriquez C, Almonacid S, Chiffelle I, Valenzuela T, Araya M, Cabezas L, Simpson R, Speisky H. Chilean J Agric Res, 2010; 70: 523-536.
24. Kumar SVP, Kekuda TRP, Vinayaka KS, Sudharshan SJ, Mallikarjun N, Swathi D. Int J Pharm Tech Res, 2010; 2:1207-1214.
25. Elmastas M, Isidak O, Turkekul I, Temura N. J Food Compos Anal, 2007; 20:337-345.
26. Umukoro S, Ashorobi RB. Afr J Biochem Res, 2006; 9: 119 -124.
27. Aziz A, Raju GS, Das A, Ahmed J, Moghal MMR. Adv Pharm Bull, 2014; 4(1):15-19.