British Journal of Pharmaceutical Research
4(6): 739-750, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Comparative Phytochemical Screening and *In vitro* Evaluation of Biological Activities between Aqueous and Ethanolic Extract of *Momordica charantia* L. Fruits

Abhijit Das[1], Palash Karmakar[1*], Md. Golam Kibria[1], Pankaj Chandra Debnath[1], Mohammad Safiqul Islam[1] and Mohammad Mafruhi Sattar[2]

[1]Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh.
[2]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Author AD designed and wrote the protocol, conduct the study, performed the statistical analysis and wrote the first draft of the manuscript. Author PK supervised the whole research. Authors MGK and PCD helped for plant collection, processing, literature searches and correction of the draft. Authors MSI and MMS helped to develop the methodology and monitored the overall data analysis. All authors read and approved the final manuscript.

Original Research Article

Received 4th October 2013
Accepted 26th November 2013
Published 1st February 2014

ABSTRACT

Aims: The study was conducted to compare the presence of different phytochemicals and biological activities like cytotoxicity, anthelmintic activity, antioxidant and free radical scavenging activities between aqueous and ethanolic extract of *Momordica charantia* L. Fruits.
Methodology: The cytotoxic assay was undertaken using brine shrimp lethality test (BSLT) while the anthelmintic activity was carried out with the determination of time of paralysis and death of earthworm (*Pheritima posthuma*) at five different concentrations. Antioxidant and free radical scavenging activities were measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method and determining the total phenolic contents.

**Corresponding author: Email: pknstu@gmail.com;*

Results: Study revealed that several phytochemicals were found common for both of the extracts, though proteins and amino acids were only found in ethanolic extract. Both the extracts showed mild cytotoxic activity where the ethanolic extract showed better potency (LC_{50}=24.245 µg/ml) than aqueous extract (LC_{50}=24.515 µg/ml). In case of anthelmintic activity, ethanolic extract was also found significantly potent than aqueous extract at five different concentrations. IC_{50} values for the total antioxidant activity were 304.41±0.903 µg/ml and 479.05±1.393 µg/ml for ethanolic and aqueous extract, respectively. Ethanolic extract contained a significantly higher concentration of total phenols (71.08 ± 0.380 mg of GAE/g of extract) in comparison to aqueous extract (57.33±0.520 mg of GAE/g of extract).
Conclusion: Therefore, in all aspects of the study, ethanolic extract was found more potent than aqueous extract. It can be concluded that *M. charantia* fruits are abundant of various phytochemicals and possess versatile biological activities.

Keywords: Momordica charantia; phytochemicals; brine shrimp lethality; anthelmintic activity; antioxidants.

1. INTRODUCTION

Momordica charantia L. is primarily an anti-diabetic medicinal plant belongs to the family Cucurbitaceae [1,2,3] and is widely cultivated as a vegetable crop in many tropical and subtropical regions of the world such as Bangladesh, India, Asia, South America [4]. It is a slender climbing annual vine with long-stalked leaves and yellow, solitary male and female flowers. It is commonly known as Bitter gourd in English and *Karela* in Bengali and Hindi [5]. *M. charantia* consist of different types of biologically active chemical compounds such as alkaloids, saponins, glycosides, resins, reducing sugars, phenolic compounds, fixed oil and free acids [5,6].

Bioactive compounds at some higher doses are seldom toxic to living body [7]. Brine shrimp lethality bioassay is a rapid, inexpensive and comprehensive method for analyzing the natural product extracts, fractions as well as pure and synthetic compounds for their bioactivity [8]. In this method *in vivo* lethality in a simple zoological organism (Brine shrimp nauplii) is used as a subsidiary monitor for screening and discovering of new bioactive natural products. This bioassay indicates a wide range of pharmacological activities including cytotoxicity as well as antimicrobial, antiviral, anthelmintic, pesticidal and anti-tumor activities etc. of the compounds [7].

Beside this, helminthiasis is a macro-parasitic disease observed in humans and animals which reflects serious social and economic problems all around the world particularly in the countries of Third World. In this disease, a part of the body is infested with parasitic worms like Roundworms (Nematodes), Tapeworms (Cestodes) or Flukes (Trematodes) [9]. World Health Organization (WHO) estimates that about 2 billion of people throughout the world are affected by parasitic worm infection and the reason of it is associated with poor management practices and inadequate control measures [10,11]. Helminthiasis is much more prevalent in the developing countries and assume that about 57% population of the developing countries will be infected by helminthiasis by the year 2025 [12,13].

It is evident that the development of different types of cell disorders and chronic diseases are associated with the oxidative stress which initiates with the increased formation of free radicals [14] Oxidative stress is one type of chain reaction which damages cell components

like proteins, lipids and nucleic acids leading to cell death [15]. Antioxidants from both natural and synthetic origin are capable of inhibiting or delaying the oxidation of an oxidizable substrate in a chain reaction [16]. Evidences reveal that increased uptake of fruits and vegetables can diminish the risk of oxidation as well as help the body to decrease oxidative injury [5,15]. Thus, interest in finding natural antioxidants without any adverse effect has gained importance.

Our traditional system of medicine and folklore are using the whole medicinal plant or a part for the treatment of all types of diseases successfully since the time immemorial. It includes antibacterial, anthelmintic, anti-inflammatory antioxidant, antitumor, cytotoxic agents [17]. This is because the traditional medicines act as an easily available and effective source of medicines to people with broad spectrum actions like high percentage of cure with single therapeutic dose, cost effective and free from toxicity [18,19]. To create a scientific evidence for the natural herbs *M. charantia* fruit is selected for the cytotoxic, anthelmintic and antioxidant activity. The present study was carried out to assess the phytochemical screening as well as the evaluation the cytotoxic, anthelmintic and antioxidant activities between aqueous and ethanolic extract of *M. charantia* which may be helpful for the development of new novel drugs.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

The fruits of *M. charantia* were collected from the local market of Noakhali assuring that the fruits were free from pesticide. After collection the taxonomic identification was carried out with the help of taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (Accession No. DACB: 37656).

2.2 Collection and Authentication of Worm

The earthworms, *Pheritima posthuma* (annelida) weighing about 0.8-3.04 g in weight as well as about 3-5 cm in length and 0.1-0.2 cm in width were collected from the moist soil of Noakhali Science and Technology University, Sonapur, Noakhali, Bangladesh. The earthworms were identified by the department of Fisheries and Marine Science of Noakhali Science and Technology University.

2.3 Preparation of Aqueous and Ethanolic Extract

After the collection of fruits in fresh condition they were allowed to wash and then sun dried. The fruits were then dried in an oven at a reduced temperature (less than 50ºC) to make them suitable for grinding. Finally the dried fruits were converted into coarse powder by crushing them by high capacity grinding machine.

For aqueous extraction 500 g of air dried fruit powder was immersed in 3000 ml of distilled water in a beaker and kept for maceration for 10 days with occasional shaking. At the end of 10^{th} day it was filtered using filter cloth and whatman® filter paper and allowed to evaporate. Thus a brown colored semisolid mass of the extract was obtained [20].

To prepare ethanolic extract 500 mg of dried powdered sample was soaked in 2500 ml of 99.8% ethanol (Merck KGaA, Darmstadt, Germany). After 15 days the solution was filtered

using filter cloth and Whatman® filter paper No.1. The resulting filtrates were then evaporated in water bath maintained at 45ºC to dryness and thus a blackish-green semisolid mass of the extract was obtained [20].

2.4 Phytochemical Screening

Small quantity of freshly prepared ethanolic and aqueous extracts of *M. charantia* fruits were subjected to preliminary quantitative phytochemical investigation for the detection of phytochemicals such as carbohydrates, phytosterols, alkaloids, glycosides, proteins, flavonoids, tannins, saponins, phenols, terpenes, fats & fixed oils etc. using the following standard methods [21-23].

2.5 *In vitro* cytotoxic assay

The cytotoxicity was conducted using brine shrimp lethality test [24]. The brine shrimp eggs were placed in 1 liter of sea water, aerated and hatched for 48 hours at 37ºC to become nauplii. After 48 hours ten brine shrimp nauplii were placed in a small container filled with seawater. *M. charantia* fruit extracts serially diluted with DMSO (Dimethyl Sulfoxide) were then added to the container. The lethality of brine shrimp was observed after 24 hours of treatment was given. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality was plotted and the values of LC_{50} were calculated using Microsoft Excel 2007®. Vincristine Sulphate was used as positive control.

2.6 *In vitro* anthelmintic Assay

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al. with minor modifications [25]. Adult earthworms were used to study the anthelmintic activity because they are anatomically and physiologically resemble with the intestinal roundworm parasites of human being [26,27]. They are widely used as effective tools for anthelmintic study because of their easy availability [28-30]. All the worms were washed with normal saline water to remove all fecal matters. Albendazole was used as the standard drug for the study. Extracts were weighed and dissolved in 10 mL of distilled water to obtain the concentrations of 10,20,30,40 and 50 mg/ml. Earthworms were divided into twelve groups (each containing five worms) in petri dish. Both the extracts (aqueous and ethanolic) were applied to the petri dishes and the time of paralysis and death was determined. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death time of worms was counted after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50ºC) followed by fading away of their body color.

2.7 *In vitro* antioxidant assay

2.7.1 DPPH free radical scavenging activity

The ability of ethanolic and aqueous extracts of *M. charantia* to scavenge 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals was estimated as previously described by Ramalingam et al. [31]. *M. charantia* extracts (2 ml) with ten different concentrations (ranging from 500 to 0.977 µg/ml) were mixed with 3 ml of a 0.1 M ethanolic solution of DPPH. The absorbance was measured by a spectrophotometer at 517 nm at 30 min intervals against a blank (pure ethanol). The percentage of radical scavenging activity was,

$$\text{Radical scavenging (\%)} = [(A_0 - A_1 / A_0) \times 100]$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample extracts. Lower absorbance values show higher free radical scavenging activity. Butylated hydroxytoluene (BHT) was used as a reference standard in different concentrations (ranging from 500 to 0.977 µg/ml). The 50% inhibitory concentration value (IC_{50}) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals [32].

2.7.2 Determination of total phenolic contents

Total phenolic contents were determined according to the method described by Demiray et al. using gallic acid as standard [33]. The extract samples (0.5 ml of different dilutions) were mixed with Folin-ciocalteu reagent (2.5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (2 ml, 7.5% w/v) was then added. The mixture was incubated for 20 minutes at room temperature. The absorbance of the sample was measured at 760 nm by UV-spectrophotometer after 20 minutes. The total phenolic content of the samples were measured using the standard curve prepared from gallic acid solution with different concentrations (6.25, 12.5, 25, 50 and 100 µg/ml). The phenolic contents of the sample were expressed as mg of GAE (gallic acid equivalent)/gm of the extracts.

2.8 Statistical Analysis

Data were processed and analyzed using both MS Excel version 2007® and SPSS (version 16.0, IBM Corporation, NY, USA). The lethal concentrations of plant extracts were determined using linear regression analysis (MS Excel version 2007®) and finally the LC_{50} was derived from the best-fit line obtained**.** The data of anthelmintic studies were reported as mean ± standard error mean while the data of antioxidant studies were expressed as mean ± standard deviation. For determining the statistical significance and standard error mean, analysis of variance (ANOVA) at 5% level significance was employed. $P<0.05$ was considered significant [34].

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Preliminary phytochemical screening of both aqueous and ethanolic extracts of *M. charantia* fruits revealed the presence of alkaloids, carbohydrates, glycosides, phytosterols, phenols, flavonoids, saponins, terpenes, fats & fixed oils. Both of the extract lacked tannin, although proteins & amino acids were present only in ethanolic extract (Table 1). Considering the study of both extracts (i.e. aqueous and ethanolic) of *M. charantia* fruits, it is much evident that ethanolic extract contains higher concentration of active phytochemical agents such as alkaloids, glycosides, phenols and terpenes which are all found abundantly in *M. charantia* than aqueous extract [35]. This information is supported by the fact that ethanol is a better extractant than water [20]. This might be why the biological activities of the ethanolic extract were more potent than that of the aqueous extract though equal concentrations were used in all cases.

Table 1. Preliminary phytochemical screening of aqueous and ethanolic extracts of *M. charantia* L. Fruits

Sl. No.	Phytochemicals	Aqueous extract	Ethanolic extract
1.	Alkaloids	+	+
2.	Carbohydrates	+	+
3.	Glycosides	+	+
4.	Saponins	+	+
5.	Phytosterols	+	+
6.	Phenols	+	+
7.	Tannins	-	-
8.	Flavonoids	+	+
9.	Proteins and amino acids	-	+
10.	Terpenes	+	+
11.	Fats & fixed oils	+	+

(+) = Presence of phytochemicals and (-) = Absence of phytochemicals

3.2 Cytotoxic activity

The lethal concentration (LC_{50}) of the test samples after 24 hours was determined by a plot of percentage of the shrimps died against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis. The lethality of the extracts to brine shrimps was determined and the results are given in the Table 2. Vincristine Sulphate (VS) was used as positive control and the LC_{50} value was found as 0.839 µg/ml. The LC_{50} values of aqueous and ethanolic extracts were found to be 24.515 µg/ml and 24.245 µg/ml, respectively (Table 2). Ethanolic extract of *M. charantia* fruits showed better cytotoxic activity than aqueous extract though the difference of cytotoxicity between two extract was negligible. Ethanol has been known more effective to dissolve active compounds in cells than water. Hence, it was easier to extract the intracellular ingredients from plant materials penetrating the cellular membrane [36]. It was reported by Tiwari et al. that several active compounds such as anthocyanin, saponins, tannins, flavones, and polyphenols etc. can be easily obtained if organic solvents (ethanol, methanol, n-hexane) are used as solvent in the extraction technique [37]. These compounds are known to be scavenger of free radical, quencher of reactive species, hydrogen donor, activator of antioxidant enzymes, inducer of detoxification, differentiation promoter of normal cell, tumor production and proliferation cell inhibitor, and inducer of apoptosis [24]. Besides, some of these bioactive compounds are shown to have inhibitory action on carcinogenesis such as triterpenoids, saponin showed its cytotoxicity on different types of cell lines [38]. It was also proved that flavonoid effectively suppressed the proliferation of a human colon carcinoma cell line (COLO 201) through apoptosis induction while phenolic compounds showed anticancer activity on cancer colon cell by arresting the cell cycle [39,40].

Table 2. Cytotoxic potential of aqueous and ethanolic extracts of *M. charantia* L. fruits along with Vincristine Sulphate

Sample	LC_{50} (µg/ml)	Regression Equation	R^2
Vincristine Sulphate	0.839	y = 34.02x + 52.58	0.952
Aqueous Extract	24.515	y = 35.23x + 1.05	0.982
Ethanolic Extract	24.245	y = 36.44x - 0.456	0.968

3.3 Anthelmintic Activity

The extracts of *M. charantia* produced significant anthelmintic activity in a dose dependent manner and the activity of both aqueous and ethanolic extracts was comparable with that of standard drugs [Table 3]. From the study, it was observed that both the extracts of *M. charantia* showed not only paralysis but also death of earthworms. With the higher doses concentration of 40 & 50 mg/ml the effects of aqueous (paralysis time of 37.2±0.97, 24.0±0.45 minutes and death time of 71.8±1.68, 46.4±0.60 minutes, respectively) and ethanolic extracts (paralysis time of 17.6±0.68, 11.6±0.40 minutes and death time of 41.6±1.29, 32.6±0.40 minutes, respectively) were comparable with the standard drug albendazole (paralysis and death time of 56.2±0.20 and 77.4±0.24 minutes, respectively) at the concentration of 10 mg/ml. The results showed that both the extracts possess wormicidal activity and may be used as an anthelmintic. It was also clear that, the time for paralysis and death decreases with the increasing of concentrations in a manner where the paralysis and death time for aqueous extract was twice than that of the ethanolic extract in each case (Table 3). Parasitic helminthes affect human being and animals causing a chronic and debilitating disease which ultimately leads to death. Our traditional medicines hold a great promise as a great source of easily available effective anthelmintic agents to the people especially in developing countries. Many plants have reported to possess anthelmintic activity *in vitro* and *in vivo* [27]. In this study, several phytochemicals were detected in both of the extracts. The possible reason for anthelmintic activity of both the extracts of the plant might be the presence of alkaloid, polyphenol, flavonoid and terpene compounds [41]. These compounds may act on the Central Nervous System (CNS) of the parasites causing paralysis and death of worms, interfere with the energy generation in the helminthes by uncoupling the oxidative phosphorylation or they bind to free proteins in the gastrointestinal tract of the host animal or to glycoprotein on the cuticle of the parasite and causes death [42].

Table 3. Evaluation of anthelmintic activity between aqueous and ethanolic extracts of *M. charantia* L. fruits

Test Substance	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Control (Distilled water)	-	-	-
Standard (Albendazole)	10	56.2±0.20	77.4±0.24
	10	84.0±0.84[a]	125.8±1.16[a]
	20	62.6±0.40[c]	104.6±0.40[a]
Aqueous extract	30	46.8±0.86[b]	88.2±1.61[b]
	40	37.2±0.97[a]	71.8±1.68[c]
	50	24.0±0.45[a]	46.4±0.60[a]
	10	34.4±0.75[a]	66.4±1.03[b]
	20	29.0±0.32[a]	54.8±0.37[a]
Ethanolic extract	30	25.6±0.40[a]	47.6±0.51[a]
	40	17.6±0.68[a]	41.6±1.29[a]
	50	11.6±0.40[a]	32.6±0.40[a]

Each values is represented as mean ± standard deviation (n = 5). Data are found to be significant by testing through one way ANOVA at 5 % level of significance (P=0.05). [a]P <0.001, [b]P=0.01, [c]P=0.05 compared to reference drug.

3.4 Antioxidant activity

3.4.1 DPPH free radical scavenging activity

The 50% inhibitory concentration (IC_{50}) of ethanolic extract (IC_{50}=304.41±0.903 µg/ml) was significantly (P=0.05) lower than aqueous extract (IC_{50}=479.05±1.393 µg/ml) (Table 4). These *M. charantia* extracts had a lower scavenging activity than BHT (IC_{50}=19.656±0.252 µg/ml) which was used as standard. Fig. 1 shows that ethanolic extract contained significantly (P < 0.05) more antioxidant and free radical scavenging activity than aqueous extract. These data revealed that the percentage of free radical inhibition increased with the increasing of concentration of both the extracts. However, the percentage of free radical inhibition was higher in ethanolic extract when compared with aqueous extract. Free radicals are chemical species containing one or more unpaired electrons which contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of different tissues, injury of central nervous system, gastritis, cancer and AIDS [27]. The DPPH assay is one of the most common and relatively quick methods used for testing radical scavenging activity of various plant extracts [14]. The results of this study indicated that the IC_{50} in ethanolic extract was significantly lower than the IC_{50} in aqueous extract suggesting that the ethanolic extract had better scavenging activity than the aqueous extract. In this study, we also found a dose dependent relationship in the DPPH assay i.e., the activity increased with the increasing of concentration for both the extracts. Again, the antioxidant activity of *M. charantia* fruits may be due to the dissolution nature of phytochemicals in different solvents. It was reported by Ansari et al. that extract of heated methanol and water of *M. charantia* revealed higher free radical antioxidant activities than a cold extract [43]. Another study stated that ethanolic extract of *Momordica dioica* Roxb leaves were found to possess high free radical antioxidant activity than aqueous extract [32]. Therefore, these results suggest that the difference in free radical scavenging of these two extracts may be due to differential solubility of the *M. charantia* compounds in the solvents.

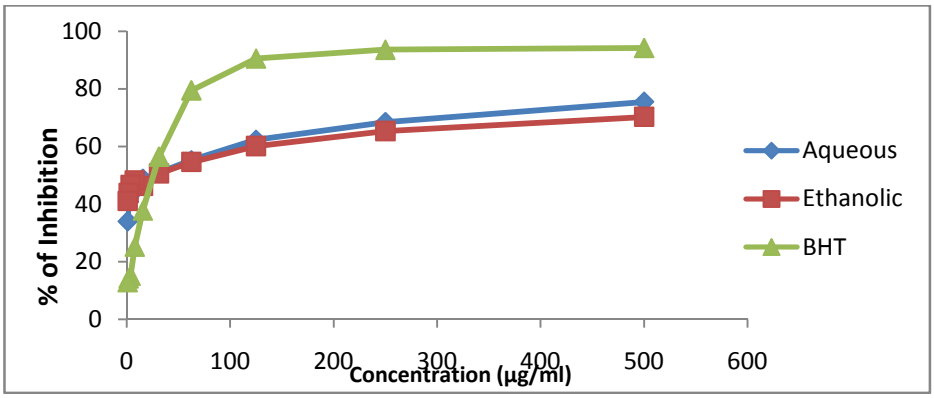

Fig 1. Comparative DPPH radical scavenging activity of aqueous and ethanolic extracts of *M. chrantia* fruits along with BHT

2.8.2 Determination of total phenolic content

Table 4 also shows the total phenolic contents of aqueous and ethanolic extracts of *M. charantia* fruits. Total phenol compounds were reported as gallic acid equivalent by the reference to a standard curve (y = 0.002x+0.107; R^2 = 0.889). The results showed that the

total phenol contents of ethanolic extract (71.08±0.380 mg of GAE/ gm of extract) were significantly (*P*=0.05) higher than the contents of aqueous extract (57.33±0.520 mg of GAE/ gm of extract). The present study also estimated the phenolic contents of ethanolic and aqueous extracts of *M. charantia* fruits. It was reported that *M. charantia* is a powerful source of both phenolic compounds as well as other phenolic acids such as gallic acid, gentisic acid, catechin and epicathecin. Studies have also showed that, the different levels of antioxidant activities in plants may be due to not only differences in their phenolic contents but also in their phenolic acid components [44]. Because of hydroxyl groups in the phenolic compounds they may directly contribute to the antioxidant activity and have a critical role in scavenging free radicals [14]. Again, recent studies have shown that fruit and vegetable phenols and polyphenols such as flavonoids prevent free radical damage and lipid peroxidation [45]. The high content of total phenolic components in the ethanolic extract may have led to the better results found in the total antioxidant activity and free radical scavenging ability when compared with the aqueous extract.

Table 4. Evaluation of antioxidant activity between aqueous and ethanol extracts of *M. charantia* L. Fruits

Sample	IC_{50} (µg/ml)	Total phenol content (mg of GAE /g of extract)
BHT	19.656±0.252	--
Aqueous Extract	479.05±1.393[a]	57.33±0.520[a]
Ethanolic Extract	304.41±0.903[a]	71.08±0.380[a]

Data represents mean ± standard deviation (n=3) of duplicate analysis. Data are found to be significant by testing through one way ANOVA at 5 % level of significance (P=0.05). [a]P <0.001, [b]P=0.01, [c]P=0.05 compared to reference drug.

4. CONCLUSION

M. charantia fruits are traditionally used as vegetable by the local people of Bangladesh and several countries in the world. The study detects that *M. charantia* fruits are abundant of wide range of phytochemical constituents and possess varying degree of biological activities. The biological activities of ethanolic extract have shown more potent activity than aqueous extract, although the possible mechanism of the activities of *M. charantia* fruits can't be explained on the basis of the study results. As the current study confirmed that fruits of *M. charantia* showed several biological activities with phytochemical constituents so taking into consideration of all the findings it can be mentioned that *M. charantia* fruits can contribute major role in drug research. The plant may be further explored for its phytochemical profile to recognize the active constituents accountable for its versatile activities.

ACKNOWLEDGMENT

The authors are thankful to Dr. Mohammad Salim Hossain, Chairman, Department of Pharmacy, Noakhali Science and Technology University for his cordial support and providing the laboratory facilities to perform the research work.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

REFERENCES

1. Beloin N, Gbeassor M, Akpagana K, Hudson J, de Soussa K, Koumaglo K, Arnason JT. Ethnomedicinal uses of *Momordica charantia* (Cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. J Ethnopharmacol. 96;2005:49-55.
2. Ullah M, Showkat M, Ahmed NU, Islam S, Abser N. Evaluation of *Momordica charantia* L. fruit extracts for analgesic and anti-inflammatory activities using in vivo assay. Res J Med plant. 2012;6(3):236-44.
3. Bakare RI, Magbaagbeola OA, Akinwande AI, Okunowo OW. Nutritional and chemical evaluation of *Momordica charantia*. J Med Plants Res. 2010;4(21):2189-93.
4. Patil SA, Patil SB. Toxicological studies of *Momordica charantia* Linn seed extracts in male mice. Int J Morphol. 29(4);2011:1212-18.
5. Leelaprakash G, Rose JC, Gowtham BM, Javvaji PK, Prasad S. In vitro antimicrobial and antioxidant activity of *Momordica charantia* leaves. Pharmacophore. 2011;2(4):244-52.
6. Kannan VR, Mathan M, Rajesh, P, Sumathi CS, Balasubramanian V, Ramesh N, Solomon EK. Phytochemical Screening and Anti-diabetic Efficacy in Fruit Extracts of *Momordica charantia* L. by using Alloxan Induced Wistar Albino Diabetic Rats. J Pharm Res Clin Pract. 2011;1(3):88-93.
7. Elhardallou SB. Citotoxicity and biological activity of selected Sudanese medicinal plants. Res J Medi Plant. 2011;5(3):201-29.
8. Nahar UJ, Lina SMM. Evaluation of antioxidant activity and cytotoxic potential of *Cryptocoryne ciliate.* Int Curr Pharm J. 2013;2(2):38-41.
9. Rafi KP, Karthikeyan M, Kannan M, Rajasekar S. Anthelmintic activity of *Nerium olender* flower extract in Indian adult earthworm. J Nat Prod Plant Res. 2011;1(4):40-6.
10. Gaikwad SA, Kale AA, Jadhav BG, Deshpande NR, Salvekar JP. Anthelmintic activity of *Cassia auriculata* L. extracts-In vitro study. J Nat Prod Plant Resour. 2011;1(2):62-6.
11. King CH. Parasites and poverty: The case of schistosomiasis. Acta Tropica. 2010;113(2):95-104.
12. Elliott DE, Summers RW, Weinstock JV. Helminths as governors of immune mediated inflammation. Int J Parasitol. 2007;37(5):457-64.
13. Clewes CAN, Shaw C. Parasite. Brit Med Bull. 2000;56:193-208.
14. Elmastas M, Isildak O, Turkekul I, Temur N. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. J Food Compos Anal. 2007;20:337-45.
15. Rezaeizadeh A, Zakaria ZBAB, Abdollahi M, Meng GY, Mustapha NM, Hamid MB, Ibrahim TABT. Antioxidant and antihyperglycaemic effects of an aqueous extract from *Momordica charantia* fruit in a type II diabetic rat model. J Med Plants Res. 2011;5(14):2990-3001.
16. Jayaseelan RS, Vijayan FP, Madheswaran M, Suresh V, Padikkala J. *In vitro* antioxidant and phytochemical evaluation of Desmodium triangulare (Retz.) Merr root. Int Res J Pharm. 2012;3(4):347-49.
17. Rastogi T, Bhutda V, Moon K, Aswar PB, Khadabad SS. Comparative Studies on Anthelmintic Activity of *Moringa Oleifera* and *Vitex Negundo*. Asian J Rese Chem. 2009;2(2):181-82.
18. Yadav P, Singh R. A Review on Anthelmintic drugs and their future scope. Int J Pharm Pharm Sci. 2011;3(3):17-21.
19. Mali RG, Mahajan S, Patil KS. Anthelmintic activity of root bark of *Capparis spinosa*. Ind J Nat Prod. 2005;21:50-51.
20. Amole OO, Ilori OO. Antimicrobial activity of the aqueous and Ethanolic extracts of the stem bark of A*lstonia boonei*. Int J Phytopharmacol. 2010;1(2):119-23.

21. Roopashree TS, Dang R, Rani SRH, Narendra C. Antibacterial activity of anti-psoriatic herbs: *Cassia tora, Momordica charantia and Calendula officinalis*. Int J App Res Nat Prod. 2008;1(3):20-8.
22. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. 17th ed. Pune: Nirali Prakashan; 2007.
23. Dewan SMR, Das A. Investigation of *in vitro* thrombolytic potential and phytochemical nature of *Crinum latifolium* L. leaves growing in coastal region of Bangladesh. Int J Biol Pharm Res. 4(1);2013:1-7.
24. Firdaus M, Prihanto AA, Nurdiani R. Antioxidant and cytotoxic activity of *Acanthus ilicifolius* flower. Asian Pac J Trop Biomed. 2013;3(1):17-21.
25. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. In vitro anthelmintic properties of *Buchholzia coriaceae* and *Gynandropsis gynandries* extract. Pharm Biol. 2001;39:217-20.
26. Chatterjee KD. Parasitology: Protozoology and Helminthology. 6th ed. Calcutta: Sree Saraswaty Press Ltd; 1967.
27. Kumar SVP, Kekuda TRP, Vinayaka KS, Sudharshan SJ, Mallikarjun N, Swathi D. Studies on antibacterial, anthelmintic and antioxidant activities of a macrolichen *Parmotrema pseudotinctorum* (des. Abb.) Hale (Parmeliaceae) from Bhadra wildlife sanctuary, Karnataka. Int J PharmTech Res. 2010;2(2):1207-14.
28. Sangeetha J, Soundarya K, Santhoshm K, Sindhura C. Evaluation of In-vitro Anthelmentic Property of *Passiflora edulis* Linn. Res J Pharm Biol Chem Sci. 2010;1(3):715-18.
29. Dash GK, Suresh P, Kar DM, Ganpaty S, Panda SB. Evaluation of *Evolvulus alsinoides* Linn for anthelmintic and antimicrobial activities. J Nat Rem. 2002;2:182-85.
30. Szewezuk VD, Mongellim ER, Pomilio AB. Antiparasitic activity of *Melia azadirach* growing in Argentina. Molecular Med Chem. 2003;1:54-7.
31. Ramalingam R, Nath AR, Madhavi BB, Nagulu M, Balasubramaniam A. Free radical scavenging and antiepileptic activity of *Leucas lanata*. J Pharm Res. 2013;6(3):368-72.
32. Jain A, Soni M, Deb L, Jain A, Rout SP, Gupta VB, Krishna KL. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica* Roxb. leaves. J Ethnopharmacol. 2008;115(1):61-6.
33. Demiray S, Pintado ME, Castro PML. Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: *Tilia argentea*, *Crataegi folium* leaves and *Polygonum bistorta* roots. World Acad Sci Eng Tech. 2009;54:312-37.
34. Nakagawa S, Cuthill IC. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol Rev. 2007;82(4):591-605.
35. Grover JK, Yadav SP. Pharmacological actions and potential uses of *Momordica charantia*. A Rev J Ethnopharmacol. 2004;93(1):123-32.
36. Alo MN, Anyim C, Igwe JC, Elom M, Uchenna DS. Antibacterial activity of water, ethanol and methanol extracts of *Ocimum gratissimum*, *Vernonia amygdalina* and *Aframomum melegueta.* Adv App Sci Res. 2012;3(2):844-48.
37. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction. Int Pharm Sci. 2011;1:98-106.
38. Man S, Gao W, Zhang Y, Huang L, Liu C. Chemical study and medical application of saponins as anti-cancer agents. Fitoterapia. 2010;81:703-14.
39. Imai M, Kikuchi H, Denda T, Ohyama K, Hirobe C, Toyoda H. Cytotoxic effects of flavonoids against a human colon cancer derived cell line, COLO 201: A potential natural anti-cancer substance. Cancer Lett. 2009;276:74-80.
40. Gonzalez-Sarrias A, Li L, Seeram NP. Anticancer effects of maple syrup phenolics and extracts on proliferation, apoptosis, and cell cycle arrest of human colon cells. J Funct Foods. 2012;4(1):185-96.

41. Bate-Smith EC. The phenolic constituent of plants and their taxonomic significance, dicotylendons. J Linn Soc Bot. 1962;58:95-103.
42. Salhan M, Kumar B, Tiwari P, Sharma P, Sandhar HK, Gautam M. Comparative anthelmintic activity of aqueous and ethanolic leaf extracts Of *Clitoria ternatea.* Int J Drug Dev Res.2011;3(1):62-9.
43. Ansari NM, Houlihan L, Hussain B, Pieroni A. Antioxidant activity of five vegetables traditionally consumed by South-Asian migrants in Bradford, Yorkshire, UK. Phytother Res. 2005;19(10):907-11.
44. Horax R, Hettiarachchy N, Islam S. Total Phenolic contents and phenolic acid constituents in 4 varieties of bitter melons (*Momordica charantia*) and antioxidant activities of their extracts. J Food Sci. 2005;70(4):C275-80.
45. Bernardi APM, López-Alarcón C, Aspée A, et al. Antioxidant Activity in Southern Brazil Hypericum species. J Chil Chem Soc. 2008;53(4):1658-62.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=407&id=14&aid=3506