Journal of Applied Pharmacy
ISSN: 1920-4159

Huq et al., J App Pharm 2016, 8:3
DOI: 10.21065/1920-4159.1000226

Research Article Open Access

Biological Evaluation of Native and Exotic Plants of Bangladesh

Samin Huq[1], Mohammad Shawkat Ali[2], Ridwan Islam[3]*, Farzana Manzoor[4] and Imon Rahman[3]

[1]*Department of Pharmacy, BRAC University, Bangladesh*
[2]*Department of Clinical pharmacy and Pharmacology, University of Dhaka, Bangladesh*
[3]*Department of Pharmacy, BRAC University, Bangladesh*
[4]*Sanofi Bangladesh Ltd, Bangladesh*

Abstract

The study was designed to determine the phytochemical constituents and to evaluate several biological activities of crude methanol extracts of a native plant, *Murdannia nudiflora*, an exotic plant, *Tradescantia pallida*, and a common spice, *Piper nigrum*, as well as their organic soluble fractions. Preliminary phytochemical screening confirmed the presence of several secondary metabolites.The petroleum ether extract of *P. nigrum* exhibited the highest degree of cytotoxicity (LC_{50}=3.524 µg/ml) whereas that of *M. nudiflora* and *T. pallida* were quite low. Antioxidant studies of the plant extracts yielded prominent results where the free radical scavenging potential of *M. Nudiflora* and *T. Pallida* were found to be 95.76 ± 1.34 mg/100g and 97.15 ± 0.96mg/100g of AAE, respectively, whereas, IC_{50} values of *M. nudiflora* and *T. pallida* were $1.51x10^{-10}$µg/ml and $4.209x10^{-13}$µg/ml, respectively, justifying the presence of high concentration of flavonoids. In addition, the analgesic studies of *M. nudiflora* and *T. Pallida* revealed significant inhibition of writhing upon induction of pain by acetic acid ($p<0.001$, $p<0.05$, respectively). The chloroform extract of *P.nigrum* showed 61.27% inhibition of hypotonic solution induced and 96.61% inhibition of heat induced haemolysis, while the methanolic extract of the plant produced 30.74% of clot lysis confirming a moderate thrombolytic activity. Thus, the present study strives to provide a scientific basis of the traditional uses of the medicinal plants.

Keywords: Phytochemical screening; Cytotoxicity; Free radical scavenging; Analgesic; Membrane stabilizing; Thrombolytic activities

Abbreviations: AAE= Ascorbic acid equivalent; GAE= Gallic acid equivalent; TAE= Tannic acid equivalent; LC_{50}= Median lethal concentrations; OD=Optical density; ME: Methanol fraction; PETSF: Petroleum ether portion; N-Hex: n-hexane portion; CH: Chloroform portion; SK: Streptokinase

Introduction

Phytomedicine refers to the use of any plant part for medicinal purposes [1]. Estimates of 25% of currently used prescribed drugs are chemically altered natural products and another 25% comes directly from the arena of herbal medicine [2]. In addition, evidences drawn from the studies of Arvigo et al (1993) showed an estimate of 119 chemical substances were obtained from 90 different plant species. In 74% cases, an active substance was isolated from a plant which had been used in the traditional system of medicine, revealing the primary importance medicinal plants in the healthcare system [2]. This is due to the fact that, plants contain various secondary metabolites serving as the source of novel therapeutic compound conferring improvement of human health with controlled adverse effects. Various medicinal products like anti-microbial agents, cardiotonic agents, Central nervous system (CNS) stimulants, nutraceuticals, sweeteners, animal feed, etc, are derived from the natural source [3-5], natural product can also have the potentiality of anticancer agent [6].Thus the present study aims at the preliminary phytochemical screening and the evaluation of diverse pharmacological activities of the methanol extract of *Murdannianudiflora, Tradescantiapallida* and *Piper nigrum* as well as their organic soluble partitionates (Figures 1 and 2).

The plants employed for the study had some notable compound that have elucidated significant therapeutic activity such as anthrocyanidins isolated from *Tradescantia pallid* was investigated as potential food pigment as well as various flavonoid compound isolated from the seeds of *Piper nigrum* such as piperine, Limonen, Sabinene in abundance as well as b-cryophyllene, Piperolein B, Dehydropipernonaline, Piperlongumininehas been investigated for its therapeutic role as major antioxidant compounds.

Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers [7,8]. Therefore, a number of

Figure 1: Whole Plant of Doveweed (*Murdannianudiflora*).

***Corresponding author:** Ridwan Islam, Senior Lecturer, Department of Pharmacy, BRAC University, Bangladesh, Tel: 8801720634427; E-mail: ridwan2039@gmail.com

Received: June 06, 2016; **Accepted:** June 23, 2016; **Published**: June 28, 2016

Citation: Huq S, Ali MS, Islam R, Manzoor F, Rahman I (2016) Biological Evaluation of Native and Exotic Plants of Bangladesh. J App Pharm 8: 226. doi: 10.21065/1920-4159.1000226

Figure 2: Whole Plant of Purple Heart (*Tradescantiapallida)*

reports on the isolation and testing of plant derived antioxidants have been described during the past decade [7-10]. Brine shrimp lethality bioassay serves as a preliminary tool to assess the cytotoxicity as well as the anti-tumor activity of bioactive natural products [11].

Pain is a symptom as an outcome of injury and disease conditions whose management is always critical and vital i.e., to provide comfort to the patients, consequently, derived analgesic drugs are becoming significant day by day [12].

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 [13]. As a result, membrane stabilizers that interfere in the release and/or the action of mediators like histamine, serotonin, prostaglandins, leukotrienes etc., could reduce the inflammation significantly [14]. Thrombolytic drugs, such as urokinase and streptokinase, play a crucial role in the management of coagulation disorders e.g., cerebral venous sinus thrombosis (CVST) i.e., a disorder often accompanied by significant morbidity and mortality [15].

In view of the above, the present study strives to highlight the antioxidant potential, cytotoxicity and analgesic activity of *M. nudiflora* and *T. pallida* as well as the membrane stabilizing, thrombolytic and cytotoxic abilities of *P. nigrum*.

Materials and Methods

Plant materials

***Murdannianudiflora*:** *M. nudiflora* (L) Brenan, commonly termed as Doveweed, is an annual herb being one of the only two recognized species of the genus *Murdannia* under the family Commelinaceae [16]. Widely distributed in wet place, the plant is mainly found in low elevated areas as well as in the tropical and subtropical regions, with rarely can be seen among grass [17,18].Traditionally used in the cure of asthma, leprosy, stomach complaints, piles, the plants has been also imparted its use as an astringent. The root paste mixed with goat milk has been prescribed orally for the cure of asthma, however, the whole plant paste in combination with a common salt has been used to cure the affected areas of leprosy [19]. Evidences shows the presence of significant group of phytochemicals such tannins, flavonoids and alkaloids while the ethanolic extract has shown significant level of analgesic effect using hot plate test ($P<0.05$) where morphine sulphate has been used as standard [20].

***Tradescantia pallida*:** *T. pallida* (commonly known as Purple Heart), widely distributed from Canada to northern Argentinais generally commercialized as ornamental houseplant. In traditional systems of medicine, the plant has shown its potential as anti-inflammatory and anti-toxic supplement in addition to its ability of improving blood circulation obtained from the evidence in Taiwanese traditional medicine [21]. In terms of its environmental value, the plant has been found out to be an effective removal of volatile organic pollutant from the air [22].

Extensive studies over its antioxidant potential of the leaf extract revealed a total flavonoid content of 10.6 ± 4mg GAE (Gallic Acid Equivalent)/100mg while an estimate of total tannin content was 13.6 ± 2.1mg TAE (Tannic Acid Equivalent)/100mg [23]. The studies further showed the free radical scavenging capacity of the leaf extract to be 103.1 ± 36.9mg AAE (Ascorbic Acid Equivalent)/100 g [23]. Isolation of chemicals from the plant extract has led to discovery of two anthocyanidins, a notable flavonoid group namely, cyaniding-g 3, 73'-triglucoside with 3 molecules of ferulic acid and an extra terminal glucose while the other lacking the terminal glucose in studies done by Shi et al, 1995 for its potential use as food pigment [24]. In terms of its antimicrobial potential, the leaf extract has shown various degree of zone of inhibition of 5-10 mm against various gram-positive and gram-negative micro-organisms while a zone of inhibition of more than 10 mm has been showed against *Staphylococcus epidermis* [23]. In terms of environmental values, the plant has shown its ability to survive in presence of chromium concentration raging 5-20 mg of L(01)Cr(VI) in hydrophobic environment while the plant has shown indication of accumulation of heavy metals like Chromium as per studies conducted by Sinha et al, 2014 [25].

Piper nigrum: The seed of *Piper nigrum* Linn a common spice known as white pepper, is considered as the king of spice. It is native to India and tropical countries such has Indonesia (Java in particular) as well as Malaysia, Madagascar and different parts of South East Asia [26] which showed rich values in terms of therapeutic properties available throughout the year [27]. Traditional system of medicine has given potential leads of pepper in epilepsy and snake bite. While Syrian books of medicine found in 5th Century AD has shown pepper as medicine for illnesses like constipation, dysentery, earache, gangrene, heart disease, herniation, hoarness, digestive problems, insect bites, insomnia, pain at the joints, liver, lung disorders, tooth decay and toothache while the root has shown its potential in the form of ghees, powder, enemas and balks for the remedy of abdominal tumor [28].

Presence of high degree of polyphenol with addition to its potential in free radical scavenging has led to the emphasis of its antioxidant value as well as its significant role in the prevention of various oxidative stress and its associated disorders which however has not been confirmed with evidence on in vivo studies [29].

The spice has also showed to affect the growth rate of *Streprococcus mutans* which is a strong indicative of its antimicrobial properties [30].

Collection of the plant materials

Doveweed was collected from Savar, Dhaka whilePurple Heart was collected from the National arboriculture Department, Ministry of Public Works, and Government of the People's Republic of Bangladesh. A White Pepper seed was collected from a local market. After collection, the plant materials were identified and confirmed by the National Herbarium, Ministry of Environment and Forests, Government of the People's Republic of Bangladesh, Mirpur, Dhaka (DACB Accession No: 40297, 40299) (Figures 3 and 4).

Maceration

The study materials were subjected to the process of shed drying for a period of a month with minimal exposure to direct sunlight and the materials had undergone grinding after cutting down into small pieces.

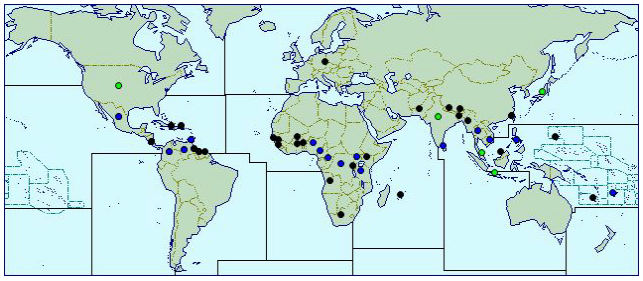

Figure 3: Distrubution Map of Doveweed [cabi.org].

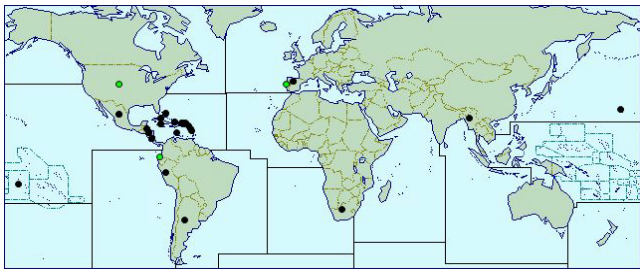

Figure 4: Distrubution map of Purple Heart [cabi.org].

The plant materials were first dissolved into 2 lit of methanol while the White Pepper seeds were dissolved individually under 500ml of methanol, chloroform and n-hexane (Active Fine Chemicals, Bangladesh). The contents were kept for a weeks, occasionally stirred and shaken. The dissolved materials were filtrated firstly through cotton and afterwards through Whatman No.1 Filter Paper (Extra Large Size) which was concentrated to obtain crude extracts of different solvent of the study materials involving rotary evaporator (Heidolph Rota-Vap 1500) at an elevated temperature range of 35-50°c involving a rotation speed of 80-100 rpm.

Phytochemical identification

Phytochemical Screening was carried out to for the qualitative identification of major groups of phytochemicals found in the plant extract. Around 30mg of the crude plant extract was dissolved in 30ml of distilled water with a few drop of DMSO (Fischer Chemicals Limited) , added to facilitate dissolution of the crude extract. Phytochemical identification tests of carbohydrates, tannins, saponins, flavonoids, alkaloids, protein was carried out as per the standard procedures [31] (Figures 5 and 6).

Evaluation of cytotoxic potential

Preliminary cytotoxic potential of the study materials were evaluated using Brine Shrimp Lethality Bioassay [32]. Using simulated conditions for 48 hours, Brine shrimp was hatched in artificial sea water. The mother concentration of the crude extract were prepared by dissolving in DMSO (Di-methyl sulphoxide) from which serial dilution has taken place by adding equal amount of dissolved sample and DMSO. The dissolved samples of various concentrations were added to pre-marked vial of simulated sea-water having 10 nauplii in each of them. Vincristine sulphate was used for the evaluation of positive control of cytotoxicity while negative control of the study was conducted using 5ml of simulated sea water containing 10 nauplii where DMSO was added. The vials were left for 24 hours and the surviving nauplii were counted to evaluate cytotoxicity of the samples by visual observations.

The mortality data were analyzed using probit analysis and linear regression method which were expressed in terms of median lethal concentration (LC_{50}) value obtained by utilizing regression equation (Figure 7-13).

Evaluation of antioxidant potential

Antioxidant potential of the plant extracts were evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Method in order to determine the free radical scavenging ability and to have a quantitative measurement of the overall antioxidant potential of the sample [33]. Ascorbic Acid was used as positive control while methanol contributed as negative control of the study.

The mother solution was prepared by dissolving the plant material in methanol and subsequent serial dilution has taken place by dissolving the sample in equal amount of methanol. 1.8 ml of methanol

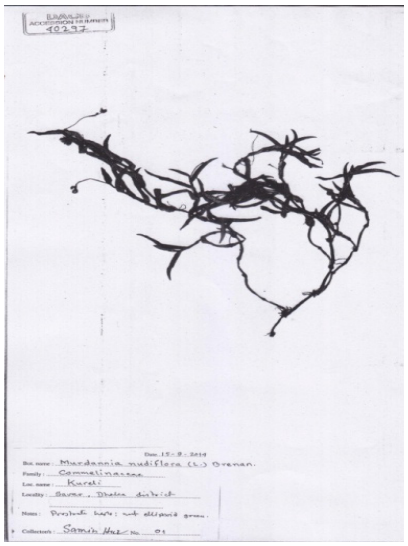

Figure 5: Herbarium Sheet of *Murdannianudiflora*(DACB Accession No.: 40297).

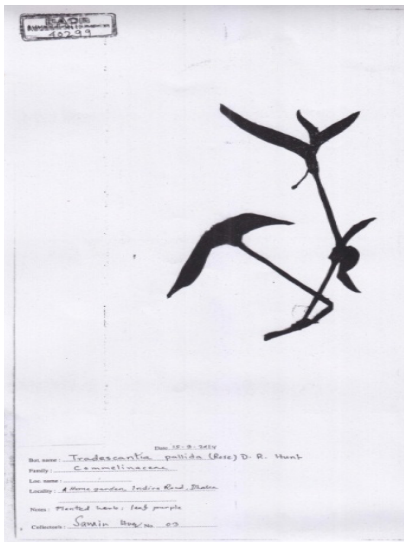

Figure 6: Herbarium Sheet of *Tradescantiapallida* (DACB Accession No.:40299).

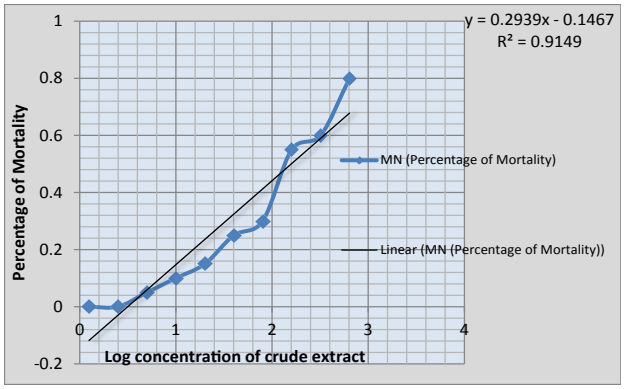

Figure 7: Cytotoxic Assay of *Murdannianudiflora* Crude Methanolic Extract.

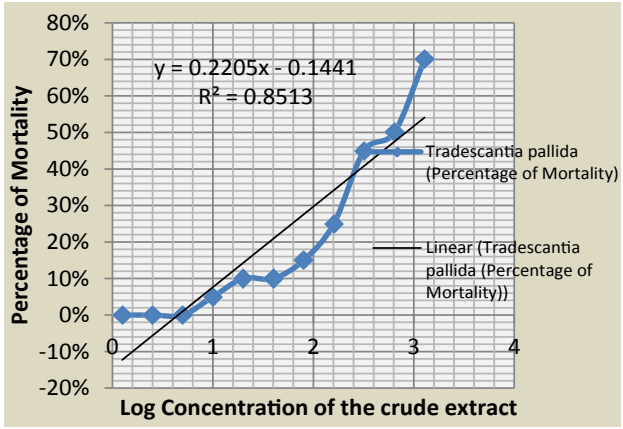

Figure 8: Cytotoxic Assay of *Tradescantiapallida* Crude Methanolic Extract.

was added to each of the pre-marked tubes of both samples at various concentration and positive control to obtain a final solution of 2 ml and was mixed thoroughly. 2 ml of DPPH was added to the pre-marked tubes and shaken well and was incubated in dark conditions for 30 m. Absorbance of the sample and control solutions was measured at 517nm by UV Spectrophotometer.

DPPH Radical Scavenging Activity was determined using the equation (% inhibition)=[{(A_0-A_s)/A_0}x 100], where A_0=Absorbance of the blank, A_s=Absorbance of the sample/standard

The equivalent value was determined using the equation: % of $FRS_{(sample)}$/% of $FRS_{(AA)}$x100 and IC_{50} values were determined using linear regression and probit analysis in Microsoft Excel 2007.

In-vivo analgesic activity

Analgesic potential was tested using acetic acid induced writhing method. Study was conducted in two experimental test groups (Dose: 100 mg/kg and 200 mg/kg of body weight) for the crude plant extract while control group received oral saline (0.9% NaCl). Dichlofenac Na (25 mg/kg of body weight) was taken as standard for the study.

Test materials were orally administered using long needle with ball shaped end. Acetic acid (0.7%) was administered intra-peritonially to each of the testing animals after 30 minutes of oral administration of the sample. The counting of writhing was started after an interval of 5 m continued till 15 m allowing a time space of 10 m for each groups. Similar sets of conditions were applied for the control and standard groups as well (Figure 14).

Comparison studies were conducted using percentage of inhibition calculated using the following equation: % of inhibition of writhing = (1-Mean writhing $_{(sample)}$/Mean writhing $_{(control)}$) x100.

Significance analysis of the study was conducted using t-test analysis ($p<0.05$) in IBM SPSS 17.0.

Evaluation of the membrane stabilizing activity

Whole blood from human under standard condition was collected and clotting was prevented using EDTA (Ethylene diamine tetra acetic acid). The blood sample was washed thrice with isotonic solution (154 mM NaCl) in 10 mM sodium phosphate buffer of pH 7.4 via centrifuge action for 10 m at 3000g to collect the stock erythrocyte suspension.

To conduct hypotonic solution-induced haemolysis, test sample consisted of stock erythrocyte suspension (0.50ml) with 5ml of hypotonic solution (50 mM NaCl) in 10mM sodium phosphate buffer

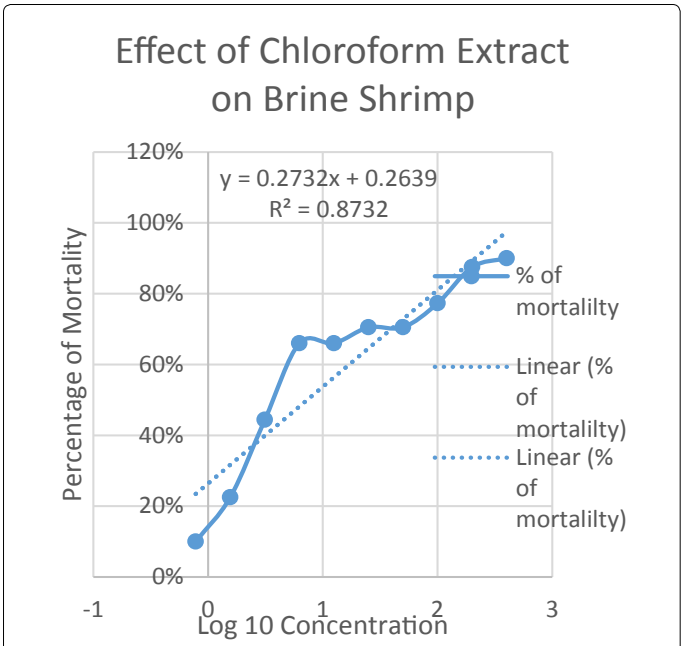

Figure 9: Cytotoxic Assay of Chloroform Extract of *Piper nigrum*.

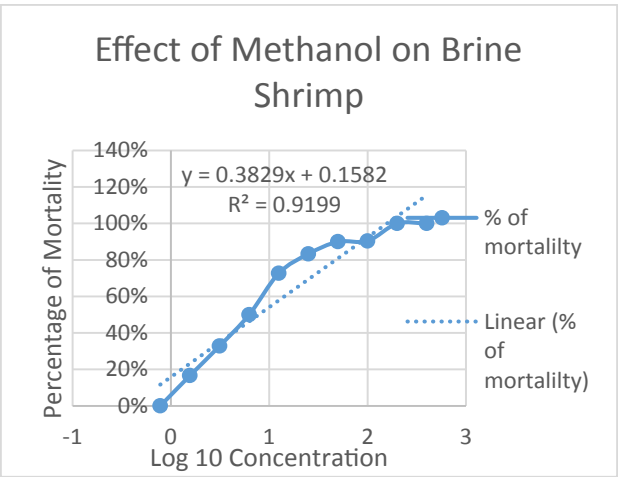

Figure 10: Cyotoxic Assay of Methanolic Extract of *Piper nigrum*.

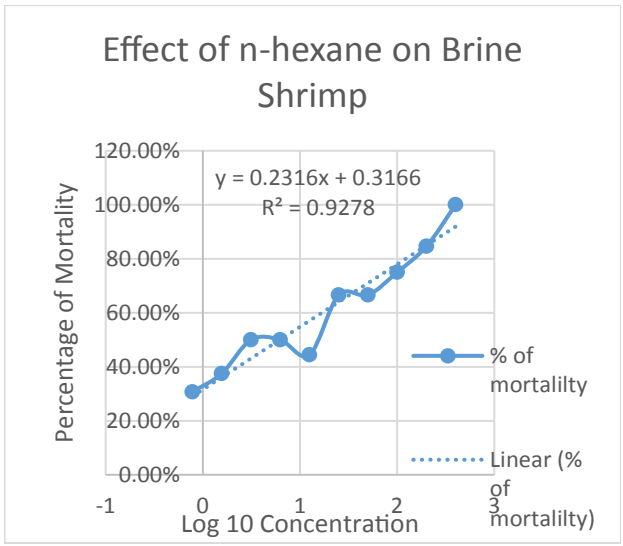

Figure11: Cytotoxic Assay of n-hexane Extract of *Piper nigrum*.

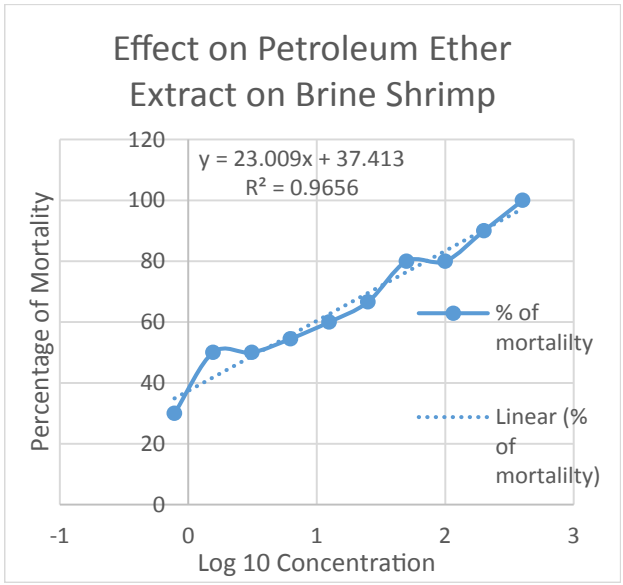

Figure12: Cytotoxic Assay of Petroleum Ether Extract of *Piper nigrum*.

containing the different crude extract (1.0 mg/ml). Acetyl Salicylic acid (0.10 mg/ml) was used as reference standard which has undergone similar set of experimental preparation. Incubation of the mixtures was done at room temperature for 10minutes, followed by centrifugation at 3000 g for 10 m and measurement of absorbance of the supernatant at 540nm. Percentage of inhibition was calculated using the following equation: % inhibition of haemolysis=100x{(OD_1-OD_2)/OD_1}, where OD_1= Optical density of hypotonic-buffered saline solution (control) and OD_2=Optical density of test sample in hypotonic solution (Figure 15).

On the other hand, to evaluate membrane stabilizing potential using heat induced haemolysis, aliquot of 5ml containing 1 mg/ml of different extract of the sample dissolved in isotonic solution was put into two duplicate sets of centrifuge tube [31]. Same amount of vehicle was added to another tube as control. 30µl of erythrocyte suspension was added to each tube and gentle inversion was applied to mix properly. Two different sets of incubation was applied, one pair of the tubes was under 54°C for 20 minutes while the other pair was maintained at 0-5°C in an ice bath. Centrifugation at 1300 g of the reaction mixture was allowed for 3 m and absorbance of the supernatant was measured at 540 nm.

Percentage of inhibition or acceleration was calculated using the following equation: % inhibition of haemolysis=100x[1-(OD_2-OD_1)/OD_3-OD_1]

Where, OD_1= Test sample unheated, OD_2=Heated test sample and OD_3=Control sample heated.

Evaluation of thrombolytic activity

Dry Crude Extract (100 mg) was suspended in 10ml of distilled to be kept overnight after which the soluble supernatant was decanted

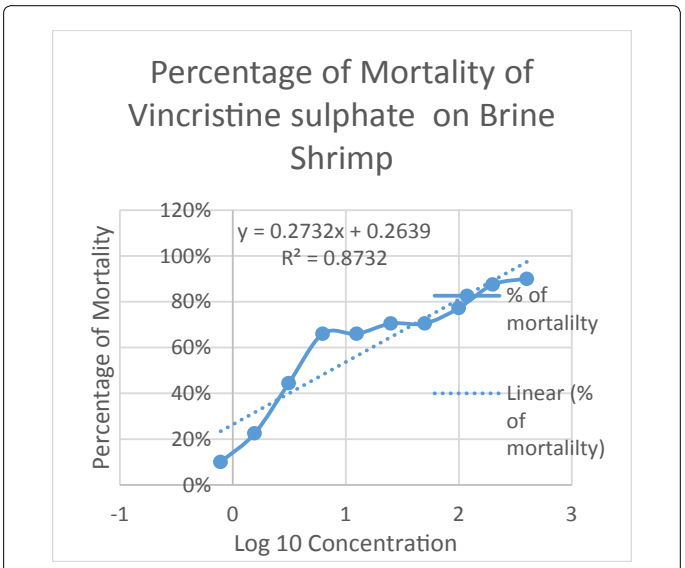

Figure13: Cytotoxic Assay of Vincristine Sulphate (Positive Control).

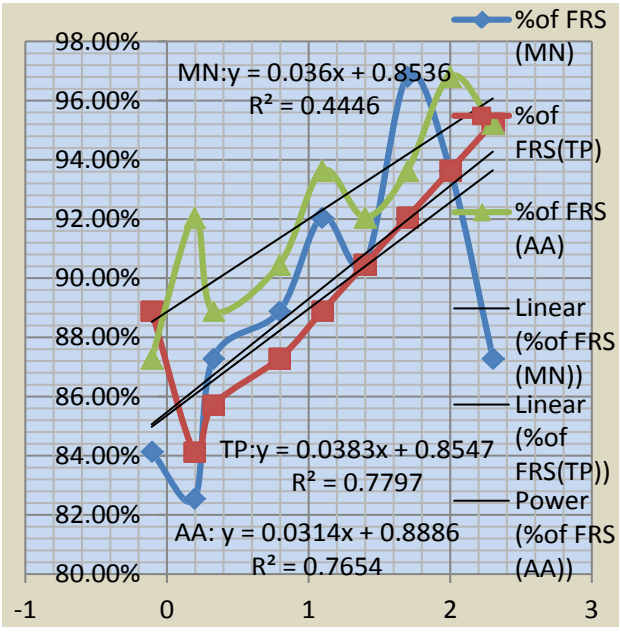

Figure 14: Evaluation of DPPH Free Scavenging Power of plant extract (MN=*MurdanniaNudiflora*, TP= *Tradescantiapallida,* AA=Ascorbic Acid).

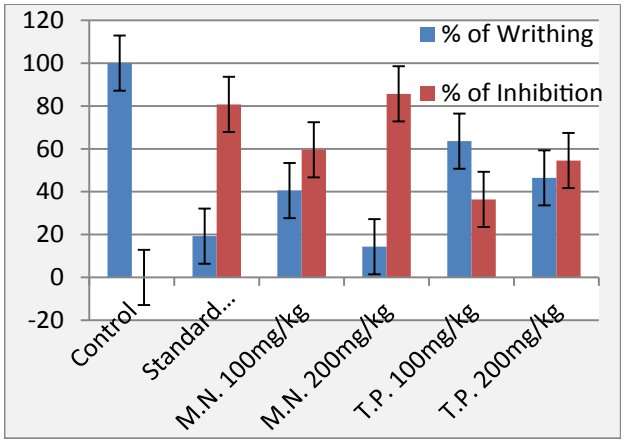

Figure15: Percentage of inhibition of writhing by different groups exposed to acetic acid induced writhing.

and filtered while commercially available streptokinase of 15,00,000 International Unit (IU) was collected and mixed properly with 5ml distilled water which was kept as a stock from which 100µl was used for *in-vitro* thrombolysis.

Whole blood was drawn from human healthy volunteer, without having a history of taking oral contraceptive or anticoagulant therapy from which 1ml of blood was transferred to previously weighed micro centrifuge tube allowed to form clots.

Aliquots of venous blood drawn from healthy volunteers (5 ml) were distribute five different pre weighed sterile micro centrifuge tube to be incubated at 37°C for 45 minutes followed by removal of serum without disturbing the clot completely and further weighing the tubes to determine clot weight.

100µl of aqueous solutions of different crude extracts along was added separately. 100µl of streptokinase and 100µl of distilled water were taken as positive control and negative control, respectively, and were added to the control tubes separately. The tubes were then incubated at 37°C for 90 minutes and visual observation of clot lysis has been conducted. Following incubation, the removal of released fluid has taken place and its subsequent weight was taken to observe the weight difference upon clot disruption. Percentage of clot lysis can be determined using the following equation:

% of clot lysis=(weight of released clot/ clot weight)x100.

Results and Discussions

Phytochemical screening

Phytochemical Screening of the plant materials confirmed the presence of alkaloids notable for its action against inflammation and pain, tannins significant for imparting anti-microbial and anti-tumor activity, compounds like saponins playing important role for its anti-hepatotoxic, anti-ulcerogenic, anti-carcinogenic activity as well as polyphenols like flavonoids for imparting significant antioxidant action that would combat against diseases related to oxidative stress as well as imparting its anti-bacterial action. Carbohydrate test showed positive results for *M. nudiflora* in the event of Fehling's test which is an indication of presence of reducing sugar (Table 1). However, no protein molecules have been identified.

Evaluation of cytotoxicity

Poor degree of cytotoxicity has been showed by *M. nudiflora* and *T. pallida*in comparison to the positive control employed for the study. Taking LC_{50} value into consideration, *M. nudiflora*has a LC_{50} value of 158.638µg/ml , while *T. pallida* has LC_{50}value of 833.85µg/ml in comparison to the standard drug having LC_{50} value of 0.5926µg/ml. Identification of cytotoxic glycosphingolipid 2,β-O-D-glucopyranosyl-2(2`-hydroxy-Z-6-enecosamide) sphingsonie from ethanolic extract of a herb of the similar genus named *Murdannia loriform* draws to the inference on the diversity of the notable secondary metabolite of the plant species belonging to same genus. This leads to another degree of potentiality that the plant extracts would be safe if ingested at high level indicative of its value as food supplement.

On the other hand, the seeds of *Piper nigum* have showed relatively high degree of cytotoxicity under the influence of various solvent. It was observed that the petroleum ether extract had the highest degree of cytotoxicity (LC_{50} value=3.524µg/ml), closest to that of the standard drug used, while n-hexane extract had LC_{50} value of 7.8101µg/ml, which is also indicative of high degree of cytotoxicity potential (Table 2). Similar trend of LC_{50} values was found in studies conducted in the same procedure using the similar sets of solvent extract of dried leaves of *Moringa oleifera* where LC_{50} values of petroleum ether extract was found out to lower than the LC_{50} values of n-hexane extract [34]. The difference in biological activity between the pet-ether and the n-hexane soluble fractions may be attributed to the different sets of chemical compounds isolated from the partitionates. While steroidal compounds like stigmasterol were isolated from petroleum ether partitionate [35], n-hexane fraction yielded polyphenols and flavonoids [36].

Evaluation of antioxidant activity

After spectroscopic analysis, it was found out that the plant extract possesseda good degree of free radical scavenging capacity. *M. nudiflora* showed FRS capacity of 95.76 ± 1.34mg/100g of Ascorbic Acid while that of *T. pallida* was somewhat similar having FRS value of 97.15 ± 0.96mg/100g of Ascorbic Acid (Table 3).

Comparing the IC_{50} value of plant extracts in comparison to standard used showed good degree of antioxidant potential by *M. nudiflora* having an IC_{50} value of $1.51x10^{-}10$µg/ml, while *T.pallida* possessed an IC_{50} value of $5.48x10^{-10}$µg/ml.Ascorbic Acid showed IC_{50} value of $4.209x10^{-}13$µg/ml (Table 4).

The results showed by the plant extracts in terms of free radical scavenging capacity as well as IC_{50} values gives us a good indicative of the rich degree antioxidant potential the plant extracts possess in addition to the presence of high flavonoid content.

Analgesic potential evaluation

Analgesic potential of the crude plant extracts of *M. nudiflora* and *T. pallida* was evaluated using acetic acid induced writhing method and was found out to be statistically significant ($p<0.001$ and $p<0.05$, respectively) in comparison to control group.

M. nudiflora exhibited maximum inhibition of writhing at a dose of 200 mg/kg of body weight, having an inhibition of writhing of 85.67% which is higher than the standard utilized, while 100 mg/kg of body weight dose showed inhibition of writhing of 59.47%.

On the other hand, *T. pallida i*nhibited writhing by 54.55% at a 200 mg/kg of body weight, while the dose of 100mg/kg of body weight showed 36.41% inhibition of writhing. The inhibitions of writhing at a dose of 200

Si. No.	Test	Reagents Involved	*Tradescantiapallida*	*Murdannianudiflora*
1.	Carbohydrate			
	Molisch Test	α-napthol	-	-
	Fehling's Test	Fehling's reagent	+	-
2.	Tannins			
	10% Potassium Dichromate	$K_2Cr_2O_7$	++	++
	5% Ferric Chloride	$FeCl_3$	++	+++
	1% Lead Acetate		+++	++
3.	Alkaloids			
	Mayer's Test	HgI and KI	++	+++
	Dragendroff's Test	Bismuth Nitrate, Tartaric Acid and Potassium Iodide	+++	++
	Hager's Test	Picric Acid	++	++
4.	Saponins	Distilled Water	++++	++++
5.	Proteins	Biuret's solution	-	-
6.	Flavonoids	Zinc Ribbon and Conc. HCl	++	++
'++++' = Present in high amount, '++' = Present in moderate amount, '+' = Present in Trace Amount, '-'=Absent				

Table 1: Phytochemical Evaluation of *Murdannia nudiflora*.

Si. No.	Name of the plant	Concentration for LC_{50}	Regression Equation	R^2
1.	*Murdannianudiflora*	158.638 µg/ml	y=0.2939x-0.1467	0.9149
2.	*Tradescantiapallida*	833.85 µg/ml	y=0.2205x-0.1441	0.8513
3.	*Piper nigum (Chloroform Extract*	7.3148 µg/ml	y=0.2732+2639	0.8732
4.	*Piper nigum (methanolic extract)*	7.8101 µg/ml	y=0.3829+.1582	0.9199
5.	*Piper nigum (n-hexane extract)*	6.1927 µg/ml	y=0.2316+.3166	0.9278
6.	*Piper nigum (petroleum ether extract)*	3.524 µg/ml	y=0.2301+.3741	0.9278
7.	Vincristine sulphate	0.5926 µg/ml	y=0.3054x + 0.5694	0.8042

Table 2: LC_{50} values of the plant extracts and the standard.

Name of the Sample	Free Radical Scavenging Capacity (mg/100g of Ascorbic Acid)
Murdannianudiflora	95.76 ± 1.34
Tradescentapallida	97.15 ± 0.96

Table 3: DPPH Free Radical Scavenging of Plant Extract Equivalent to Ascrobic Acid per 100g.

Sl. No.	Name of the Sample	IC_{50}	Regression equation	R^2
1.	*Murdannia nudiflora*	$1.51x10^{-10}$µg/ml	y=0.036x+0.8536	0.4446
2.	*Tradescantia pallida*	$5.48x10^{-10}$µg/ml	y=0.0383x+0.8547	0.7797
3.	Ascorbic acid	$4.209x10^{-13}$µg/ml	y=0.0314x+0.8886	0.7654

Table 4: IC_{50} values of the plant extract and standard.

mg/kg of body weight of *T. pallida* and for both the selected doses of *M. nudiflora* were found out to be statistically significant (Table 5).

Presence of high degree of alkaloids obtained in phytochemical studies of the plant extract in relation to its potential of inhibition of writhing gives us potential lead of obtaining sources of new generation of analgesics. Since the etiology of pain generation is highly dependent upon the generation of prostaglandin and leukotriene derivatives, the results obtained from the study gives us potential directives in the discovery of analgesic products from natural sources highly acceptable taking the risks and adverse effects involved in administration of synthetic NSAIDs into consideration.

Membrane stabilizing capacity evaluation

In terms of hypotonic solution induced haemolysis, chloroform extract of white pepper exhibited an inhibition of 61.27%, while acetyl salicylic acid (standard) possessed an inhibitory capacity of 71.9%, which is an indicative of its high degree ofanti-inflammatory property (Table 6).

In terms of heat-induced haemolysis, all extracts of different solvent showed high degree of membrane stabilizing activity in comparison to the standards used(acetyl salicylic acid, % of inhibition=57.42%). The highest degree of inhibition of heat-induced haemolysis of the seeds of *P. nigrum* was revealed by the chloroform extract (96.61%), while methanol extract (86.76%), petroleum ether extract (79.34%) and n-hexane extract (94.01) also possessed high degree of membrane stabilizing potential (Table 7) (Figure 16).

Thrombolytic Activity

Evaluation of thrombolytic activity of the seeds of *P. nigrum* upon dissolving in different solvents was assessed in comparison to thrombolytic drugs like Streptokinase (30,000 IU) with respect to its percentage of lysis (76.06%). It was found out that all the portions exhibited low degree of thrombolytic activity in comparison to the standard. The maximum thrombolytic activity was showed by methanol extract (30.74%) while the lowest was showed by n-Hexane portion (10.60%) (Table 8) (Figures 17 and 18).

Conclusion

In the world of advanced medical discovery where the compatibility of synthetic product is an issue in the illustration of therapeutic benefit with the added concern of toxicity and side effects, scientific justification of the natural product in therapeutic activity would serve as a basis for the newer avenues of complementary and alternative medicine to disease management as natural product minimizes the issues concerning compatibility of medication with human body. Therefore, this study aimed at identifying new and novel potential bio-active compounds based on the presence of notable phytochemicals and comparative evaluation on major bio-activity of various extract, thus providing the basis of entering into the world of therapeutically active

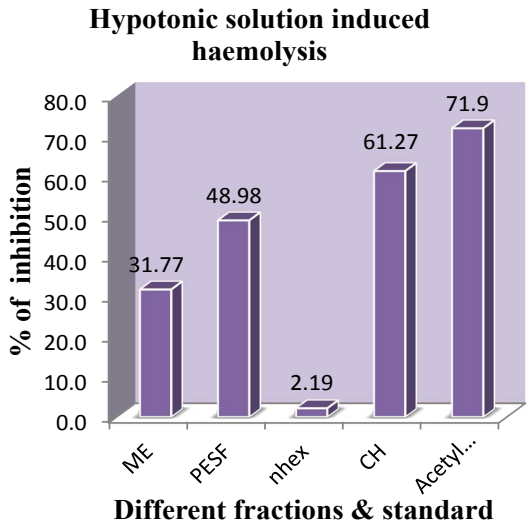

Figure16: Hypotonic Solution Induced Haemolysis of Different Extracts of *Piper nigrum*.

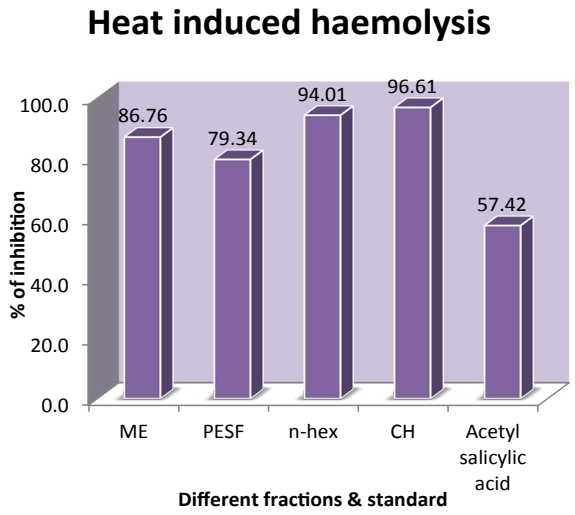

Figure17: Heat Induced Haemolysis of Different Extracts of *Piper nigrum*.

Animal Group	Dose	Mean with STD Error	Standard Deviation	% of Inhibition of writhing
Control		50.583 ± 4.192	10.268	--
Standard	25 mg/kg	9.75 ± 0.772	1.891	80.74
M.N.*	100 mg/kg	20.5 ± 1.779	4.359	59.47
M.N.**	200 mg/kg	7.25 ± 1.419	3.474	85.67
T.P.	100 mg/kg	32.17 ± 2.83	6.94	36.41
T.P. *	200 mg/kg	23.5 ± 0.913	2.236	54.55

Dicholefac sodium (25 mg/kg) was used as standard. Test groups were treated with 100mg/kg and 200mg/kg of MN and TP extracts. Data expressed as Mean ± SEM, n=6. Statistical analysis was done by t-test analysis. *p<0.05, **p<0.001. MN=*M. nudiflora*, TP=*T. pallida*

Table 5: Evaluation of analgesic potential using acetic acid induced writhing method.

natural compounds having the potentiality of providing safe alternative to the adverse effects of synthetic products in disease management. This comparison of various bio-activities of different plants and spices of Bangladesh would allow us in making a comparative justification

Sample code	OD_2	OD_1	% inhibition of haemolysis
ME	0.872	1.278	31.77
PESF	0.652	1.278	48.98
Nhex	1.25	1.278	2.19
CH	0.495	1.278	61.27
Acetyl salicylic acid			71.9

OD_1=Optical density of hypotonic-buffered saline solution (control)
OD_2=Optical density of test sample in hypotonic solutions
ME: Methanol fraction, PETSF: Petroleum ether portion, N-Hex: n-hexane portion, CH: Chloroform portion, SK: Streptokinase

Table 6: Hypotonic induced haemolysis inhibition by different extracts of seeds of *Piper nigrum*.

Sample code	OD_1	OD_2	OD_3	% inhibition of haemolysis
ME	0.162	0.873	0.28	86.76
PESF	0.132	0.81	0.28	79.34
n-hex	0.163	0.805	0.28	94.01
CH	0.032	0.943	0.28	96.61
Acetyl salicylic acid				57.42

Where OD_1= Test Sample Unheated and OD_2=Heated Test Sample, OD_3=Control Sample Heated
ME: Methanol fraction, PETSF: Petroleum ether portion, N-Hex: n-hexane portion, CH: Chloroform portion, SK: Streptokinase.

Table 7: Heat induced haemolysis inhibition by different extracts of seeds of *Piper nigrum*.

Sample code	Clot (before lysis)	Clot (after lysis)	% of lysis
ME	637	195.8	30.74
PETSF	721.2	165	22.88
N-hexane	711.3	75.4	10.60
Chlor	564.6	117.8	20.86
BLANK	608.8	60.3	9.90
SK	705.8	536.8	76.06

ME: Methanol fraction, PETSF: Petroleum ether portion, N-Hexane: n-hexane portion, Chlor: Chloroform portion, SK: Streptokinase

Table 8: Thrombolytic activity of *P. nigrum* seed extracts.

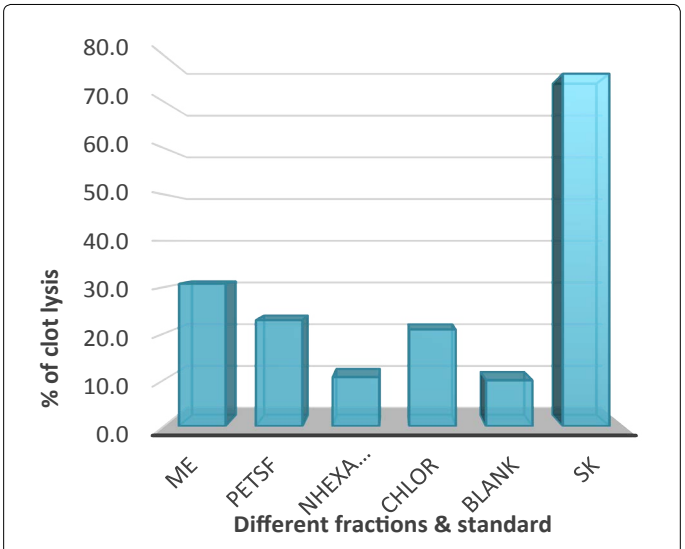

Figure 18: Thrombolytic Activity of Different Extracts of *Piper nigrum*.

on potential areas of further studies in order to validate the scientific evidence concretely. The study has led us to the scientific understanding of the rich antioxidant potential of *M. nudiflora* and *T. pallida*, high degree of cytotoxicity in various extracts of *P. nigrum* seeds as well as statistically significant inhibition of writhing shown by both the plants have opened the avenue of scientific research on the discovery of noble compound that contributes to the therapeutic effect mentioned in the study. In addition, presence of phytochemicals would allow the study on the plants on its anti-microbial activity, anti-carcinogenic activity, and hepatoprotective activities and so on. The results obtained from the bioactivity evaluation would also lead us to the evaluation of various fractions of crude extracts to obtain pure therapeutically significant compounds that impart significant bioactivities which contribute to the betterment of global healthcare.

References

1. Barrett B, Kiefer D, Rabago D (1999) Assessing the risks and benefits of herbal medicine: an overview of scientific evidence. Altern Ther Health Med 5: 40-49.
2. Arvigo R, Ballick M (1993) Rainforest Remedies.Lotun Press, Twin Lakes.
3. Kong JM, Goh NK, Chia LS, Chia TF (2003) Recent advances in traditional plant drugs and orchids. Acta Pharmacol Sin 24: 7-21.
4. GortziO, Lalas S, Chinou I, Tsaknis J (2008)Reevalution of bioactivity and antioxidant activity of Myrtuscommunis extract before and after encapsulation in liposomes. Eur Food Res Technol226: 583-590.
5. Pavithra PS, Sreevidya N, Verma RS (2009) Antibacterial and antioxidant activity of methanol extract of Evolvulus nummularius. Indian J Pharmacol 41: 233-236.
6. Guo XK, Sun HP, Shen S, Sun Y, Xie FL, et al. (2013) Synthesis and evaluation of gambogic acid derivatives as antitumor agents. Part III. Chem Biodivers 10: 73-85.
7. Buyukokuroglu ME, Gulcln I, Oktay M, Kufrevioglu OI (2001) In vitro antioxidant properties of dantrolene sodium. Pharmacol Res 44: 491-494.
8. Shahidi F, Wanasundara PK (1992) Phenolic antioxidants. Crit Rev Food Sci Nutr 32: 67-103.
9. Pietta A, Sionetti P, Mauri P (1998) Antioxidant activity of selected medicinal plants. J Agric Food Chem 46: 4487-4490.
10. Velioglu YS, Mazza G, Gao YL, OomahBD (1998) Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agric Food Chem. 46: 4113-4117.
11. Rahman MM, Mian MY, Faysal AA, Rahman SMA, Islam R, et al. (2015) Evaluation of membrane stabilizing, cytotoxic and anti-diarrheal activities of leaves of CalotropisGiganteaR.BR.World J Pharm Sci 3(12): 2364-2367.
12. Islam R, Sikder MAA, Khan RA, Ahmed M, Rashid MA (2014). Membrane stabilizing, thrombolytic, analgesic, anti-diarrheal and CNS activities of Polyalthialongifolia. South Asian J ExpBiol 4: 104-109.
13. Omale J, Okafor PN (2008) Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of Cissusmultistriata.AfrJournBiotechnol 7: 3129-3133.
14. Shinde J, Taldone T, Barletta M, Kunaparaju N, Hu B, et al. (2008) Alpha-glucosidase inhibitory activity of Syzygiumcumini (Linn.) Skeels seed kernel in vitro and in Goto-Kakizaki (GK) rats.Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, 8000 Utopia Parkway, Jamaica, NY 11439, United States.
15. Watson RD, Chin BS, Lip GY (2002) Antithrombotic therapy in acute coronary syndromes. BMJ 325: 1348-1351.
16. Zhu Ye LHS (1952) Plant Description: Murdannianudiflora. Kew Bull 7: 189.
17. Rojas LA, Mora A, Rodriguez H (2002) Effect of minimum and conventional tillage in rice (Oryzasativa L) in the Northern Huetar Region in Costa Rica. AgronomiaMesoamericana 13: 111-116.
18. USDA-NRCS (2002)ThePlants Database Version 3.5. National Plant Data Center. Baton Rouge, USA.
19. Panda A, Mishra MK (2011)Ethnomedicinal Survey of some wetland plants of South Orissa and their conservation.Ind J TradKnowl 10: 296-303.
20. Patwari BA, Ahmed AB, Dast T, Sengupta R (2014) Phytochemical screening and analgesic effects of ethanolic extracts of plant Murdannianudiflora(L) Brenan (Commelinaceae) in albino mice using hot plate method.Int J Pharm Sci 6: 512-515.
21. Yang DS, Pennisi SV, Son KC, Kays SJ (2009) Screening of Indoor Plant for Volatile Organic Polluant Removal Efficiency.HortSci 44: 1377-1381.
22. Li TSC (2006) Taiwanese Native Medicinal Plant: Phytopharmacology and Therapeutic Values. CRC Press Boca Raton Florida USA 2006:1-328.
23. Tan JB, Yap WJ, Tan SY, Lim YY, Lee SM (2014) Antioxidant Content, Antioxidant Activity, and Antibacterial Activity of Five Plants from the Commelinaceae Family. Antioxidants (Basel) 3: 758-769.
24. Shi Zu, Lin MI, Francis FJ (1992)Anthocyanins of Tradescantiapallida: Potential Food Colorant. Journ of Food Sci, Wiley, 57: 761-765.
25. Sinha V, Pakshirajan K, Chaturvedi R (2014) Chromium(VI) accumulation and tolerance by Tradescantia pallida: biochemical and antioxidant study. Appl Biochem Biotechnol 173: 2297-2306.
26. Shanmugpriya K, Saravana PS, Mohammad P, William B(2012) Antioxidant potential of pepper leaves and its antimicrobial potential against some pathogenic microbes.IndJourn of Nat Prod and Res 3: 570-577.
27. http://www.botanical-online.com/.
28. Kadam PV, Yadav FA, Patel FA, Karjikar MJ (2013)Pharmacognostic, phytochemistry and physicochemical studies of Piper nigrum Linn fruit (Pipereaceae).Int Res J Pharm 4: 189-193.
29. Gabriel A, Vinson J, Oben J, Ngogang JY (2006) Comparative analysis of the in vitro antioxidant activity of white and black pepper.Nut Res 26: 659-663.
30. Yona OS, Nenny P, Delvi F, Sumarno RP (2013) White Pepper Extrac (Piper nigrumL) as Antibacterial Agent for Streptococcus mutansin-vitro. IOSR Journ of Dent and Med Sci 4: p25.
31. Ghani MA (2003) Meidicinal Plants of Bangladesh with Chemical Constituents and Uses, Asiatic Society of Bangladesh, Dhaka.
32. Yildrim A, Mavi A, Kara AA (2003) Antioxidant and antimicrobial activities of PolygonumcognatumMeissn extracts.JournSci Food Agri 83: 64-69.
33. Ahmed M, Shikha HA, Sadhu SK, Rahman MT, Datta BK (2001) Analgesic, diuretic, and anti-inflammatory principle from Scoparia dulcis. Pharmazie 56: 657-660.
34. Mohammad S, Hossain MI, Bahar MAN, Akhter S, Haque MA, et al (2012). Preliminary Phytochemical Screening, In-Vitro Antioxidant and Cytotoxic Activity of Five Different Extract of MoringaOleifera Leaf. Journ App Pharm Sci 2: 65-68.
35. Kamboj A, Saluja (2011). Isolation of stigmaterol and ß-sitosterol from petroleum ether extract of aerial parts of Ageratum conyzoides (Asteraceae). IntJourn Pharm and Pharm Sci 3: 94-96.
36. Kumar S, Malhotra R, Kumar D (2010) Euphorbia hirta: Its chemistry, traditional and medicinal uses, and pharmacological activities. Pharmacogn Rev 4: 58-61.