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Research Article

ANTIOXIDATIVE AND ANTIMICROBIAL ACTIVITIES OF FOUR IMPORTANT MEDICINAL HERBS OF BANGLADESH

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ABSTRACT

The evaluation of total phenolic contents, antioxidant properties and antimicrobial activities of four medicinal plants such as *Centella asiatica, Holarrhena antidysenterica, Euphorbia hirta, Alstonia scolaris* were performed using 80% methanol as a solvent. They were screening out to investigate their phytochemical properties. Preliminary phytochemical studies revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins as the chemical class present in the extracts. The results suggest the phytochemical properties of the plant for curing various ailments. Preliminary antioxidative activities of the samples were also determined and among them we found that *Centella asiatica* contained highest total poly phenols (77.40 mg GAE/g sample) as well as DPPH radical scavenging activity (88.16%) and reducing power (OD=1.36). This shows that the plant may be potent source of natural antioxidants. The antimicrobial activity can be determined only in *Centella asiatica* and *Holarrhena antidysenterica* methanolic extract. However both of them showed considerable level of activity against standard strains and clinical isolates of some gram positive and gram negative bacteria. The obtained results provide a support for the use of these plants in traditional medicine and for its further investigation.

KEYWORDS: *Alstonia scolaris,* Antimicrobial, Antioxidant, *Centella asiatica, Euphorbia hirta, Holarrhena antidysenterica.*

INTRODUCTION

More than 500 medicinal plants have so far been enlisted in Bangladesh and are used by traditional practitioners of the Indian sub-continent and also in other countries for treatment of various diseases like bronchitis, asthma, anemia, diarrhea, dysentery, typhoid, jaundice, rheumatism, hemorrhages, ulcers, gonorrhea, hypertension, cardiac failure, cancer and many others. Some mentionable ones are *Adhatoda vasica, Allium sativum, Aloe sp, Alstonia sp, Andrographis paniculata, Asparagus racemosus, Atropa belladona, Azadirachta indica, Calotropis procera, Datura sp, Ephedra sp, Holarrhena antidysenterica, Jatropha sp, Momordica sp, Ocimum sp, Rouwalfia serpentina, Terminalia arjuna, Tinospora sp, Vinca sp,* etc. Plants are rich sources of bioactive compounds as they posses various secondary metabolites like alkaloids, flavonoides, phenolic acids, terpinoides, glycosides, tannins, saponins, quinolines, essential oils that have regulatory or defensive role[1]. Many natural substances in plants have antioxidative activity of which phenolics are one of the most notable groups. Phenolic compounds that has antioxidative activity, can play an important role in preventing body cells from i) injuries by Hydrogen peroxide ii) damage of unsaturated fatty acids by Lipid peroxides iii) absorbing and neutralizing free radicals. Anti-oxidative phenols are thought to be beneficial to suppress the oxygen reactive species, which may cause aging or carcinogenesis. Antioxidants from natural sources are most acceptable one as they are natural non-synthetic product with least side effect. Medicinal herbs also represent a rich source from which novel antibacterial and antifungal chemotherapeutic agents may be obtained. So it is important to investigate the antioxidant and antimicrobial properties of plants to screen its beneficial effect.

Centella asiatica is a small herbaceous plant with slender runners, grows commonly in damp places all over the country. *Holarrhena antidysenterica* is a laticiferous deciduous shrub with white flowers, grows in the forests of Dhaka, Mymensingh and Chittagong. *Euphorbia hirta* is a small annual herb with loose globosely heads of small flowers, grows as a low-growing common weed in waste places and roadsides throughout the country. *Alostonia scolaris* is a tall evergreen tree, grows wild and planted in all districts. Many ethnobotanical uses of these plants are already known and commonly used in traditional medicine. These medicinal plants have been used in traditional medicine for hundreds of years with reputation as efficacious remedies although there may not be

sufficient data to substantiate their efficacy[1]. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. In this context, studies on total phenolic content, anti-oxidative and anti-microbial activities of various extracts of the plants were studies.

MATERIALS AND METHODS

Collection of the samples

Centella asiatica, Holarrhena antidysenterica, Euphorbia hirta, Alstonia scolaris were freshly collected from Jahangirnagar University, Savar, Dhaka campus and these were taxonomically identified by the specialist.

Preparation of extracts from the plant parts

About 400g of powdered materials of each were placed in clean flat bottomed glass containers (4L) and soaked in 1.3 liters of 80% ethanol for the samples separately. The containers were sealed for a period of seven days with occasional shaking and stirring. The whole mixtures were then underwent coarse filtration through a piece of clean, white cotton followed by filtration through Whatmann filter paper. The filtrates were concentrated using a rotary evaporator to obtain the crude extracts.

Phytochemical screening

The crude leaf extract and the fractions were subjected to different qualitative tests to find out the presence of chemical constituents. These were identified by characteristic color changes using standard procedure[2,3,4]. Molisch's and Fehling's reagents were used to investigate the presence of carbohydrates and reducing sugar respectively. Hagger's reagent, Wagner's reagent, Mayer's reagent and Dragendroff's reagents were used to test alkaloids while $FeCl_3$ test and Keller Killiani's test were carried out for glycosides and cardenolides respectively. Borntrager's test was conducted to test the anthraquinone glycosides; flavonoids were tested using Lead acetate, Alkali, $FeCl_3$ and Conc. H_2SO_4; $FeCl_3$, Ammonia and Lead acetate were used to test the phenolic compounds. Concentrated H_2SO_4 was used to detect terpenoids whereas acetic anhydride was used to check the presence of triterpene. The presence of phytosterols or steroids was indicated by the Salkowski's test while the presence of saponins was confirmed by Foam test.

Determination of antibacterial activity

Antibacterial sensitivity was determined by Disc diffusion method[5]. Three gram positive bacteria *Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus* and nine Gram negative bacteria *Erwinia* sp., *Escherichia coli*, *Prtoteus mirabilis, Pseudomonas* sp., *Salmonella* sp., *Salmonella typhi, Serratia* sp., *Shigella flexneri* and *Vibrio cholerae* were used to determine antibacterial activities. The sterile filter paper disc (8 mm diameter) containing the dry extracts (10 mg, 20mg and 30mg), standard antibiotic disc (10 µg streptomycin) and negative control (Blank disc soaked with solvent) were used in the experiment and the result was recorded as the mean values of three replications.

Determination of antioxidant activity

The antioxidant activities of the extracts were measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl(DPPH) free radical by following method[6]. Each extract (0.1 ml) was added to 3 ml of a 0.004% Methanol (MeOH) solution of DPPH separately. Absorbance at 517 nm was determined after 30 min, and the percent inhibition activity was calculated as

$$\text{The DPPH radical scavenging activity}(\%) = \frac{\text{control absorption-corrected absorption}}{\text{control absorption}} \times 100$$

IC_{50} values concentration of sample required to scavenge 50% of free radicals were calculated following by Brand-Williams method.

Determination of total phenol content

The total concentration of phenolic compounds (TPH) in the extracts were determined as described previously by Folin-Ciocalteu method[7] using the Folin-Ciocalteu Reagent (FCR) with gallic acid (GA) as the standard and expressed (mg) as gallic acid equivalents (GAE)/g of extract[6]. The Folin-Ciocalteu reagent (FCR) is used for the colorimetric assay of phenolic and polyphenolic antioxidants. It works by measuring the amount of the test substance needed to inhibit the oxidation of the reagent[7]. The calculation was as

$$\text{Total phenolic content} = \frac{\text{sample absorbance}}{\text{absorbance of 100 µM galic acid}} \times 10 \times 50 \times 0.18814 \text{ mg GAE/g sample}$$

Determination of reducing power

The reducing capacities of various fractions were determined following Oyaizu.

RESULTS

The weight percentage yields of the crude extracts of the samples were shown in the Table 2. The sample yields were 10 to 18 %(w/w). These crude extracts (20 mg) were dissolved in ethanol (1ml) for experiments.

Table 2: Weight of crude extract and percentage yield of crude extract of the samples

Plant samples	Percentage yield of extracts
Centella asiatica	16.2
Holarrhena antidysenterica	15.5
Euphorbia hirta	10.5
Alstonia scolaris	18.4

Phytochemical Screening of the samples

Phytochemical studies

Preliminary phytochemical investigation of ethanolic extract of the plant materials was carried out for qualitative determination of the groups of organic

compounds present in them, by using standard procedure to identify the constituents as described by Trease and Evans and Harborne. The extracts revealed the presence of alkaloids, tannins, cardiac glycosides, reducing sugars, saponins, flavonoids and steroids. Terpenoids and phytostrol were present in the samples.

Table 3: Result of the samples in phytochemical screening tests

Sl. no.	Name of the test	Results			
		Centella asiatica	***Holarrhena antidysenterica***	***Euphorbia hirta***	***Alstonia scolaris***
01	Alkaloid test	++	+++	+++	++
02	Tanin test	+++	+++	+++	+++
03	Saponin test	+	+++	+++	++
04	Flavonoid test	++	+++	++	++
05	Anthraqinon glycoside test	+	+	-	-
06	Cardiatic glycoside test	+	+	+	+
07	Phenol test	++	++	++	++
08	Terpenoid test	+	-	+	+
09	Phytosterol test	+	-	+	+
10	Steroid test	++	+++	+	++
11	Amino acid test	+	+	+	+

"+++" = highly present, "++" = moderately present, "+" = slightly present and "-"= absent

Total phenolic content of the extracts

It has been recognized that the phenolic compounds are class of antioxidant agents which act as free radical terminators[8]. The Folin-Ciocaltue reagent method is actually not an antioxidant test but instead an assay for the quantity of oxidizable substance, that is, phenolic compounds[9]. Table 4 shows the content of total phenolic compounds ranged from 37.09 to 77.40 mg GAE/g extract. *Centella asiatica* with 77.40 mg GAE/g extract of total phenolic content had the highest amount of this substance among the plants in this research. The compounds such as phenolic substances, which contain hydroxyls, are responsible for the radical scavenging effect in the plants[10,11]. According to our study, the high contents of these phytochemicals in *Centella asiatica* can explain its high radical scavenging activity. Extract of *H. antidysenterica* contained comparatively lower amount (37.1).

Table 4: Total phenolic content of the samples (100 times dilution)

Sample	Absorbance at 700 nm			Average	Total phenolic content (mgGAE/g)
	R-1	R-2	R-3		
Centella asiatica	0.593	0.593	0.611	0.599	77.40
Holarrhena antidysenterica	0.283	0.281	0.297	0.287	37.1
Euphorbia hirta	0.546	0.513	0.519	0.526	68.0
Alostenia scolaris	0.379	0.370	0.378	0.376	48.54
GA100µM	0.721	0.730	0.735	0.728	
GA200µM	1.468	1.446	1.443	1.45	

Antioxidant assay

DPPH radical-scavenging activity

DPPH is a useful reagent for investigating the free radical-scavenging activities of compounds. In the DPPH test, the extracts were able to reduce the stable radical DPPH to the yellow-colored diphenyl hydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction[12]. As seen in Table 6, the methanolic extract of all plants exhibited considerable DPPH radical scavenging activity. At 100 µg/ml concentration, extract of *C. asiatica* showed the highest DPPH radical scavenging activity (88.16%) followed by bark of *A. Scolaris* (72.11%), *E. hirta* (57.00%), and *H. antidysenterica* (50.62%). Since *C. asiatica* exhibited strongest activity it could be used as antioxidative substrate.

Table 5: DPPH radical scavenging activity of the samples

Sample	Absorbance at 517 nm			Average	Activity (%)
	R-1	R-2	R-3		
Centella asiatica	0.075	0.078	0.075	0.076	88.16
Holarrhena antidysenterica	0.320	0.317	0.314	0.317	50.62
Euphorbia hirta	0.259	0.286	0.284	0.276	57.00
Alostonia scolaris	0.201	0.199	0.138	0.179	72.11
Control	0.640	0.647	0.640	0.642	

Reducing power

The reducing capability of a compound may serve as a significant indicator of its potential antioxidant activity. Table 7 shows the reductive activity of the extracts at a concentration of 400 µg/mL in phosphate buffer. The most reducing activity comes from the *Alostonia scolaris* (O.D. 1.781) followed by *Centella asiatica* (O.D. 1.36). *Holarrhena antidysenterica* and *Euphorbia hirta* also has some activity.

Table 6: Reducing power of the samples

Sample	OD at 700 nm			Average absorbance
	R-1	R-2	R-3	
Centella asiatica	1.369	1.341	1.357	1.356
Holarrhena antidysenterica	0.704	0.692	0.723	0.706
Euphorbia hirta	0.295	0.405	0.312	0.337
Alostonia scolaris	1.792	1.723	1.781	1.76
Ascorbic acid 100µg/ml	1.886	1.1.790	1.616	1.764
Ascorbic acid 200µg/ml	2.773	2.798	2.786	2.786

Antimicrobial activity of the samples

Methanolic extracts (80%) of the *Centella asiatica* and *Holarrhena antidysenterica* were tested for their antimicrobial activity by using disc diffusion method at 250mg/ml concentration, were shown in the table 7. The extract of *Centella asiatica* displayed the height level of activity against *Bacillus cereus* and *Salmonella typhi.* The inhibition zone was 17 mm and 13 mm. Activity was also detected against *Bacillus subtilis, Staphylococcus aureus, E. coli* and *Salmonella typhi.* Gram negative bacteria*, Shigella flexneri* were showing no activity.

Table 7: Antibacterial activity (inhibition zone) of the bark of *Holarrhena antidysenterica* using disc diffusion method

Bacterial strains	Type	Inhibition zone (mm) (120µl/ml)			Average inhibition zone (mm)	Standard
		R-1	R-2	R-3		
Bacillus subtilis	+	14.00	15.25	13.25	14.16	22.3
Bacillus cereus	+	17.50	16.50	17.25	17.17	25.3
Salmonella paratyhpi	-	14.5	15.33	15.00	14.93	23.2
Vibrio colerae	-	12.00	10.00	10.33	10.77	22.0
Escherichia coli	-	14.50	14.75	12.10	13.78	22.4
Proteus mirabilis		12.50	11.00	13.75	12.42	18.3
Pseudomonus aerus	-	13.75	15.25	14.00	14.33	18.3
Shigella flexneri	-	8	8	8	8	15.7

Table 8: Antibacterial activity (inhibition zone) of *Centella asiatica* using disc diffusion method.

Bacterial strains	Type	Inhibition zone (mm) (120µl/ml)			Average inhibition zone	Standard
		R-1	R-2	R-3		
Bacillus subtilis	+	13.0	12.0	14.25	13.08	22.3
Bacillus cereus	+	11.5	12.5	10.0	11.33	25.3
Salmonella paratyhpi	-	11.0	11.33	12.0	11.44	23.2
Vibrio colerae	-	15.0	12.0	12.25	13.08	22.0
Escherichia coli	-	14.5	14.75	12.1	13.78	22.4
Proteus mirabilis		12.5	11.0	13.75	12.42	18.3
Pseudomonus aerus	-	13.75	15.25	14.0	14.33	18.3
Shigella flexneri	-	8.0	8.0	8.0	8.0	15.7

DISCUSSIONS

Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects.[13-17] Glycosides, flavonoids, tannins and alkaloids have hypoglycemic activities[18,19]. Some study has reported that saponins possess hypocholesterolemic and antidiabetic properties[20]. The terpenoids have also been shown to decrease blood sugar level in animal studies[21]. Steroids and triterpenoids showed the analgesic properties[22]. The steroids and saponins are responsible for central nervous system activities[23]. In the present study, we have found that most of the biologically active phytochemicals were present in the ethanol extracts of plant samples. The medicinal properties of the plant extracts may be due to the presence of above mentioned

phytochemicals. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. Among the samples, we found that *Centella asiatica* consist of highest total polyphenols as well as antioxidative activities. The data shows that the grading of the top polyphenol containing samples is *Centella asiatica* > *Euphorbia hirta* >*Alostenia scolaria*> *Holarrhena antidysenterica*. The antioxidative activity, such as DPPH radical scavenging activity and reducing power of the samples were almost proportional to the total polyphenol in the samples. Therefore, *Centella asiatica* consist of high polyphenol as well as showed a promising antioxidative activity. Reactive oxygenated species (ROS) *in vivo* include super oxide radical, hydrogen peroxide and hypochlorous acid[24]. The DPPH antioxidant assay is based on the ability of DPPH to decolorize in the presence of antioxidants. In case of antioxidant screening (Table 5), the ethanolic extract of *C. asiatica* showed the highest antioxidant activity 88.16%. At the same time, the ethanolic extract of *Alostenia scolaria* also exhibited moderate antioxidant activity (72.11%), where standard ascorbic acid showed free radical scavenging 90%. Ethanolic extracts of the *Euphorbia hirta* and *Holarrhena antidysenterica* also showed more than 50% DPPH scavenging activity. Though the antioxidant activity of a plant extracts depends on the type and the polarity of the extracting solvent, the extracting technique, the purity of the active principle, the antioxidant test, the substrate used and the structural requirement (a number of phenolic and hydroxyl groups on ring structures)[25]. The results obtained in present study indicate that methanolic extracts of *Centella asiatica* and *Alostenia scolaria* inhibits free radical scavenging activity. The overall antioxidant activity of these extracts might be attributed to its flavonoids, phenolic and other phytochemical constituents. These could be a source of natural antioxidant that could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.

Centella asiatica, is a common creeping herb that is claimed to possess various physiological effects. Reports from different places have revealed that *Centella asiatica* has been used for wound healing, memory improvement, treating mental fatigue, bronchitis, asthma, dysentery, leucorrhoea, kidney trouble, urethritis[26], antiallergic and anticancer purposes, curing leucorrhea and toxic fever. It is also commonly used as porridge for feeding pre-school children in Sri Lanka in combating nutritional deficiencies[27].

Centella asiatica and *Holarrhena antidysenterica* appear to be a rich and interesting source for supplementary ethnomedicinal and phytochemical studies. The antimicrobial activity could justify its traditional use in diarrhoea and dysentery. The present findings support the applicability of *Centella asiatica* in traditional system for its claimed uses and can be recommended by the scientific community as an accessible alternative to synthetic antibiotics. The high degree of antimicrobial activity seems to support the folk therapy for infectious and traditional therapeutic claims of this plant. The antimicrobial studies revealed that both the plants tested here posse's considerable level of antimicrobial properties.

CONCLUSION

In order to confirm the anti oxidative effect of these plants further in vitro and in vivo studies will be needed. Even though, this is only a preliminary study of the occurrence of certain properties of *Centella asiatica* and *Holarrhena antidysenterica* an in-depth study will provide a good concrete base of all the phytochemicals functions mention above. Further studies are in progress in our laboratory to isolate the active components.

The findings of the present study suggested that these two plants could be a potential natural source of antioxidants and could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.

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