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Preliminary phyto-chemical screening and comparative antioxidant potential of *Alstonia scholaris* leaves extracts

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Abstract

Yet to now natural products, or its' derivatives are in service to humankind as the best source of molecules to control the pest and disease problems of human beings. The present work investigated total phenolic content, total flavonoid content, reducing power capacity, total antioxidant capacity, DPPH free radial scavenging activity of four different extracts of the plant *A. scholaris* which belongs to the family Apocynaceae. Total phenolic content (21.92± 0.13 mg GAE/g) and total flavonoid content (16.61± 0.06 mg CatE/g) were found to be highest in Dia-ion resin adsorbed fraction, and the same fraction showed the highest total antioxidant activity with absorbance 1.049±0.014 at 100 µg/mL as well as highest DPPH radical scavenging activity with IC_{50} value 24.90 µg/mL. The Iron reducing power of the different extractives and standard exhibited the following order: Ascorbic acid> DRAF > CLF > EAF > PEF and the total antioxidant activity of different extractives and standard exhibited the following order: Ascorbic acid> DRAF > PEF > EAF > CLF.

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Introduction

Bangladesh is a good repository of natural vegetative and medicinal or toxic plants, and people use them in various ways: to control infections as well as diseases caused by resistant strain of organisms and different types of pest insects. The use of plant extracts and phytochemicals can be of great significance in therapeutic treatments (Dahlia *et al.*, 2017; Ikram & Inamul, 1984) not only in our country but also in developed ones. But it is true that quite a large number of plants have still been untouched or have not been touched fully from which significant results can be obtained to control the pest and disease problems of human beings. *A. scholarisis* is one of such plant under the family Apocynaceae.

It has been documented pharmacologically and clinically in the world, which endowed phytochemicals with marked activity on human pathogenic bacteria (Ray & Majumdar, 1976; Farnsworth, 1988; Rastogi & Mehrotra, 1991; Asolkaret 1992; Perry & Metzer, 1998; Khan *et al.*, 2002). However, *A. scholaris* plants synthesizes toxic substances which act as a defense for themselves against infections, insects and herbivores, and it often affect the organisms that feed on them (Teixeira *et al.*, 2003). The extracts of A. scholaris possesses inhibitory effect on microbs, deterrent or lethal activity on organisms that cause disease or damage such as *S. aureus*, *B. subtilis*, *E. coli*, *S. typhi*, *Candida albiens* (fungal) etc. (Karthishwaran, 2010).

The aqueous extract of the plant *A. scholaris* (Linn) showed mollucicidal and anti-cholinesterase activity against the snail *Lymnaea acuminta* (Singh *et al.*, 2003). Three alkaloids: picrinine, vallesamine and scholaricine isolated from A. *scholaris* (Linn) leaf, by Shang *et al.* (2010) were found active as anti-inflammatory, anti-tussive, and anti-asthmatic and analgesic.

The crude extract of *A. scholaris* Linn, which was also tested positive for the presence of alkaloids, provided 31%-84% protection against castor oil-induced diarrhoea in mice (Shah *et al.*, 2010).

Taking consideration of the medicinal values of these plants many of these medicinal plants are used as food supplement as well as in many herbal industries for their herbal preparation and formulation. To identify effective and specific phytoconstituents researcher from all around the world are investigating plants materials as theirs valuable sources. To achieve such goal screening of phyto-chemicals and their antioxidant significance is needed to discover and develop novel therapeutic agents having improved and effective efficacy. Although, some study reported its biological activities on these species in many countries of the world but depending on geographical location, environmental and also ecological factors its bioactive principle may varied in some extent. So, the present investigations aims to study the antioxidant efficacy of different extracts of medicinal plant *Alstonia scholaris* grown in Bangladesh based on phyto-chemical screening and in vitro antioxidant tests.

Materials and methods

All the reagents and chemicals used for the presence work were purchased from Thomas Baker (Mumbai, India), Bdh (England), Fluka (Switzerland) and E. Merck (Germany) supplied by local vendors. Commercial alcohol (rectified spirit) and absolute alcohol were available from Carew and Company, Darsana, Chuadanga. The solvents used mainly in this work were benzene, acetone, tetrahydrofuran (THF), ethyl acetate, chloroform, n-hexane, petroleum ether, methanol, absolute alcohol, toluene etc. The solvents were dried and distilled when necessary.

Collection of plant materials

The *Alstonia scholaris* plant leaves were collected from the cultivated adjacent areas of BCSIR, Rajshahi. The collected materials were washed thoroughly in water, chopped, air dried for a week at 35-40^{o}C and pulverized in electric grinder. Dried ground leaves of *Alstonia scholaris* were exhaustively extracted with ethanol in Soxhlet apparatus. The resulting juicy extract was filtered through Whatman

paper No.1 and concentrated under reduced pressure at 45°C using the Buchi Rotavapor R-200 to obtain a crude residue (23.5%).The process was repeated several time for maximum yield. Then water triturate part was collected from crude extract. The water triturate fraction was passed through a previously well packed dia-ion resin column which was selectivity collect only the phenolic group containing compounds. Then the materials, which were bound in resin column, collected by passing methanol solvent. Then Petroleum ether, Ethyl acetate and Chloroform solvents were passing through the residue respectively. Finally Petroleum ether, Ethyl acetate and Chloroform triturate were collected.

Preliminary phyto-chemical analysis

Preliminary phytochemical screening of crude ethanol extract and its different fractions was done using various published qualitative chemical tests procedures for several classes of natural products (Sofowara, 1993 & Harborne, 1973).

Estimation of total phenolic

Total phenolic content of different extractives of *A. scholaris* were determined employing the method as described by Singleton *et al.* (1965) which involves Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard. The absorbance of the solution was measured at 760 nm using a UV spectrophotometer (ThermoSpectronic 20, USA) against blank.

Estimation of total flavanoids

Total flavonoid content of different extractives of *A. scholaris* leaves were determined by aluminium chloride colorimetric method using catechin as reference compound (Zhishen *et al.*, 1999). A volume of 125µL of extract is added to 75 µL of a 5% $NaNO_2$ solution. The mixture was allowed to stand for 6 min, then 150 µL of aluminium trichloride (10%) was added and incubated for 5 min, followed by the addition of 750 µL of NaOH (1M). The final volume of the solution was adjusted to 2500 µL with distilled water. After 15 min of incubation the mixture turned to pink and the absorbance was measured at 510 nm. The total flavonoids content was expressed as mg cat.E/g DM.

Determination of reducing power capacity

The reducing power of methanol extract and different fractions of *A. scholaris* leaves were evaluated by the method of Oyaizu, (1986) using potassium ferricyanide [K3Fe (CN) 6] (1%) solution. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The iron reducing power of different extracts of *A. scholaris* leaves were estimated by measuring the formulation of Perl's Prussium blue at 700 nm.

Determination of total antioxidant capacity

Total antioxidant activity of different extracts of *A. scholaris* leaves were determined by the method of Prieto *et al.*, (1999) with some modifications.

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol and carotinoids. This method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound. 3 ml of reaction mixture containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 1 % ammonium molybdate was added into the experimental samples and incubated at 95°C for 90 minutes to complete the reaction and measured the absorbance of the solution at 695 nm.

Determination of free radical scavenging activity

DPPH free radical scavenging activity of the ethanolic extract and its different fractions were carried following the method described by Braca *et al.*, (2001). Briefly, 0.1 ml of extract with various concentrations was added to 3 ml of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percentage inhibition activity was calculated from $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/ standard. IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration (µg/ml) versus % inhibition.

Results

Preliminary phyto-chemical screening of crude ethanol extract and its different extractives of *Alstonia scholaris* leaves were carried out and listed in Table 1. Preliminary phyto-chemical screening revealed the presence of potential phyto-constituent like tannins, glycosides, steroids and alkaloids in the extractives with varying quantity. All the tested parameters were observed in Dia-ion resin fraction and comparatively higher than other extractives.

Table 1. Preliminary phyto-chemical screening of crude ethanol extract and its different fractions of *Alstonia scholaris*

Phytochemical constituents	Crude ethanol extract	Petroleum ether fraction	Chloroform fraction	Ethyl acetate fraction	Dia-ion resin adsorbed fraction
Saponins	–	–	–	–	–
Tannins	+	–	–	–	+ + +
Glycosides	+	+	–	–	+ +
Steroids	+ +	+ +	+	+	+ +
Alkaloids	+ + +	–	+ +	+	+ + +

Here, + = Present in the mild amount, + + = Present in the moderate amount,
+ + + = Present in the large amount, – = Not present.

Table 2. Cooperative study of total phenolic and flavanoid content of different fractions of ethanolic extract of *Alstonia scholaris*.

Name of fractions	Total phelolic GAE/g of dried sample Mean ±STD	Total flavanoid Cat.E/g of dried sample Mean ±STD
Chloroform fraction (CLF)	3.44± 0.07	9.96± 0.16
Petroleum ether fraction (PEF)	3.39± 0.26	10.91± 0.17
Ethyl acetate fraction (EAF)	5.67± 0.20	9.31± 0.37
Dia-ion resin adsorbed fraction (DRAF)	21.92± 0.13	16.61± 0.06

Total phenolic content of different fractions of *Alstonia scholaris* were shown in Table 2 and Fig. 2. Among the fraction, the highest phenolic content was found in Dia-ion resin adsorbed fraction (21.92± 0.13 mg GAE/g of dried extract), followed by ethyl acetate fraction (5.67± 0.20 mg GAE/g of dried extract), Chloroform fraction (3.44± 0.07 mg GAE/g of dried extract), and Petroleum ether fraction (3.39± 0.26 mg GAE/g of dried extract).

Total flavonoid content of different fractions of *A. scholaris* has been shown in Table 2 and Fig. 2. Among the fractions, the highest total flavonoid content was found in Dia-ion resin adsorbed fraction (16.61± 0.06 mg CatE/g of dried extract), followed by Petroleum ether fraction (10.91± 0.17 mg CatE/g of dried extract), Chloroform fraction (9.96± 0.16 mg CatE/g of dried extract), and Ethyl acetate fraction (9.31± 0.37 mg CatE/g of dried extract).

The iron reducing capacity of the four different fractions of *A. scholaris* extract such as petroleum ether fraction, chloroform fraction ethyl acetate and Dia-ion resin adsorbed fraction was investigated and shown in Fig.4. Among the four different extractives Dia-ion resin adsorbed fraction showed the highest iron reducing capacity with absorbance of 2.468±0.006 at 80μg/mL concentration, followed by Chloroform fraction with absorbance 1.487±0.019 at 80 μg/mL, while Ethyl acetate fraction showed iron reducing capacity with absorbance of 0.998±0.023 at 80 μg/mL and petroleum ether fraction showed the iron reducing capacity with absorbance 0.836±0.005 at 80 μg/mL.

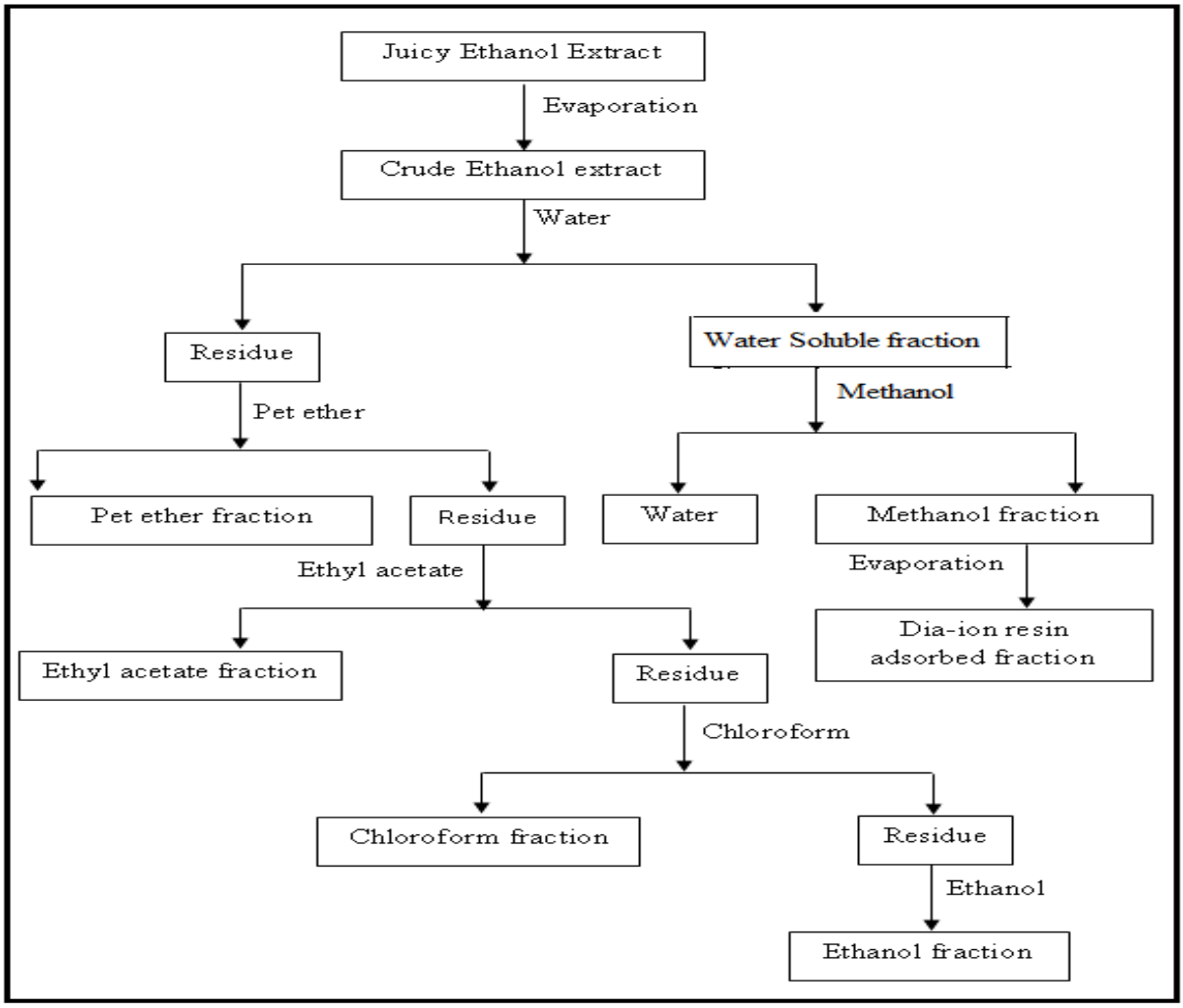

Fig. 1. Extract preparation flow chart.

Antony *et al.*, (2011) reported 84.62 µmoles/mg at conc. 2.0 mg *Alstonia scholaris* extracts. The reducing power of the different extractives and standard exhibited the following order: Ascorbic acid> DRAF > CLF > EAF > PEF.

Total antioxidant activity of different fractions of ethanolic extract of *A. scholaris* such as Dia-ion resin adsorbed fraction, chloroform fraction, Ethyl acetate fraction and petroleum ether fraction were investigated (Fig.5). Among the fractions, Dia-ion resin adsorbed fraction showed the highest total antioxidant activity with absorbance 1.049±0.014 at 100 µg/mL. Whereas, the Petroleum ether and Ethyl acetate fraction showed the absorbance 0.974±0.033 at 100 µg/mL and 0.947±0.027 at 100 µg/mL respectively. Chloroform fraction showed the lowest total antioxidant activity with absorbance 0.609±0.014 at 100µg/mL concentration. The total antioxidant activity of different extractives and standard exhibited the following order: Ascorbic acid> DRAF > PEF > EAF > CLF.

Among the fractions of the extract, the highest DPPH radical scavenging activity was found comparatively higher in Dia-ion resin adsorbed fraction having IC_{50} value 24.90 µg/mL ((Fig.6) than other extractives. On the other hand, chloroform fraction showed DPPH radical scavenging activity with IC_{50} value 73.30 µg/mL, followed by ethyl acetate fraction with IC_{50} value 40.90 µg/mL and petroleum ether fraction showed DPPH radical scavenging activity with IC_{50} value 113.63 µg/mL (Fig.7). Antony *et al.*, (2011) reported 80.69% scavenging activity in EAF of *Alstonia scholaris* extracts at 50 mg conc.

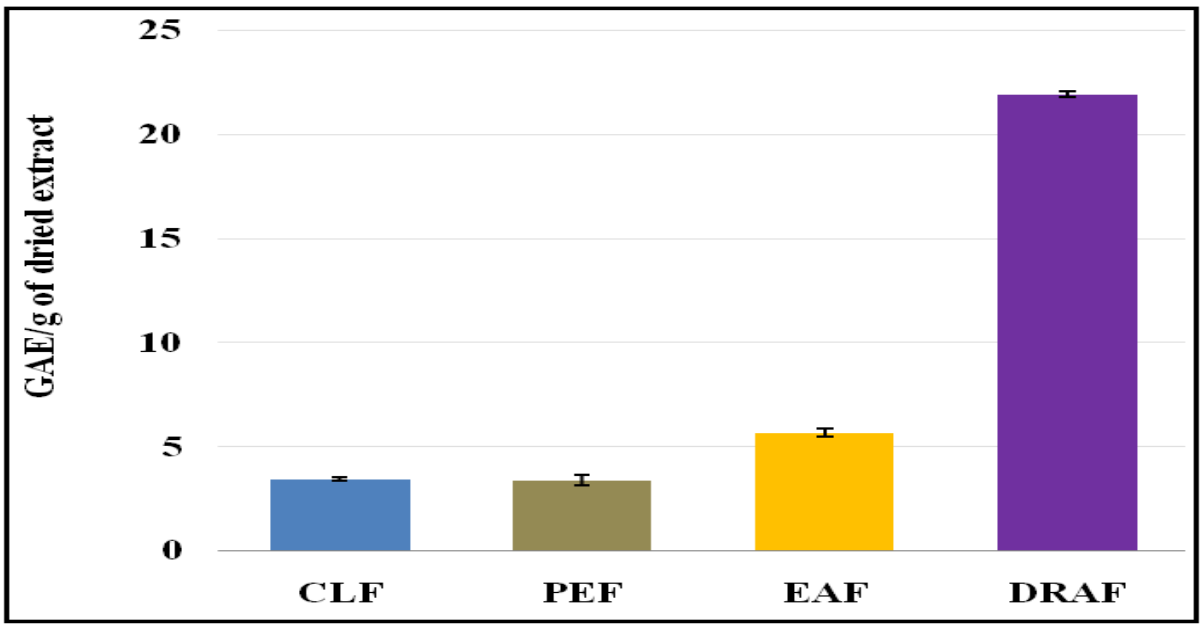

Fig. 2. Total phenolic content (mg GAE/g of dried extract) of different extractives of *A. scholaris* leaves.

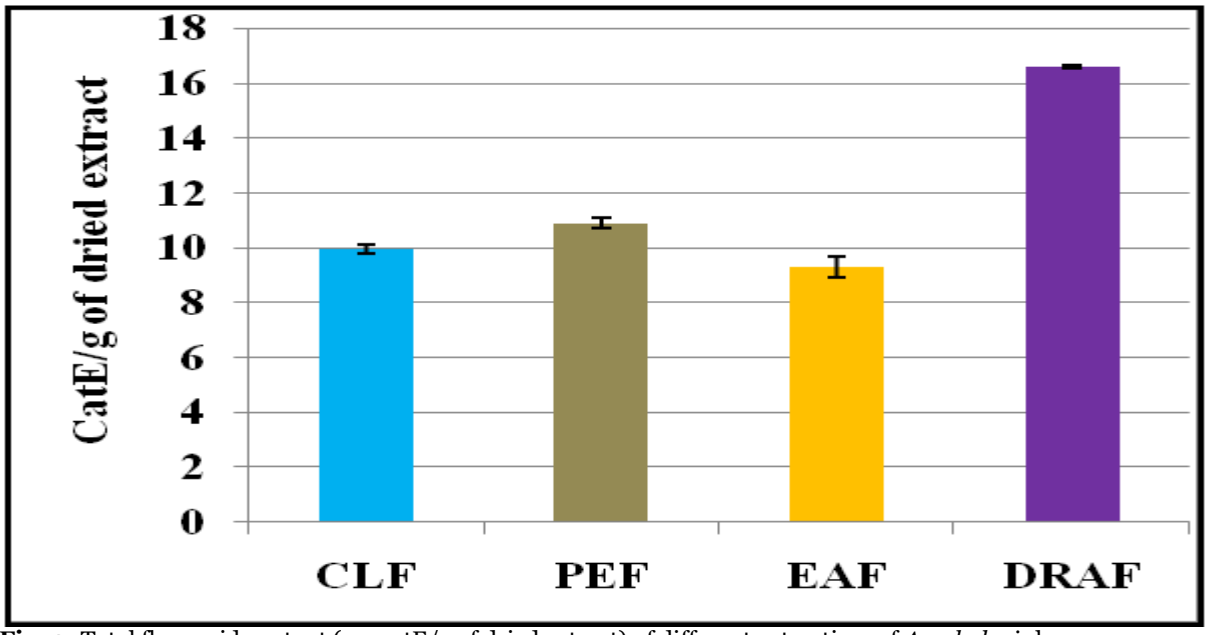

Fig. 3. Total flavonoid content (mg catE/g of dried extract) of different extractives of *A. scholaris* leaves

Discussion

For the preparation of relatively safe and non-toxic drugs for cure, isolation and characterization of pharmacologically active compounds from medicinal plants continue till today and hence so many compounds like alkaloids, polyketides, terpenoids, flavonoids etc. have been reported by many researcher (Butler, 2004). *Alstonia scholaris* leaves extractives were screened for preliminary phyto-chemical and the results thus obtained showed that the crude extracts contained alkaloids, steroids, tannins and glycosides but absence of saponins were noticed (Table1). Cherian and Augusti, (1995) stated that tannins and alkaloids exhibit hypoglycemic activities and used for treating intestinal disorders like diarrhea and dysentery as stated by Akinpelu and Onakoya, (2006).

These phyto-chemicals have also been identified by several researchers in the leaves of *A. scholaris* (Misra *et al.*, 2011; Antony *et al.*, 2011). Yamauchi *et al.*, (1990) have reported Lagumamine (19-

hydroxytubotaiwine), angustilobine B acid, losbanine (6,7-seco-6-norangustilobine B), tubotaiwine, its oxide, 6,7-secoangustilobine B, 17-o-Acetyl echitamine and echitamine alkaloids in the leaves and bark of *Alstonia sholaris*. However, chloroform faction of 85% ethanolic extract of *A. scholaris* showed the presence of echitamidine-N-oxide-19-O-β-Dglucopyranoside, an indole alkaloid by Reddy, (2016). Alkaloids exhibit variety of chemical structures, therefore responsible for the pharmacological properties of the medicinal plants (Kaushik *et al.*, 2011). As a consequence the presence of phenolics and flavanoids in the leaves of *Alstonia scholaris* enables them to be used as an potential antioxidant which are known to neutralize cell attacking free radicals produce in body of human (Stauth, 2007).

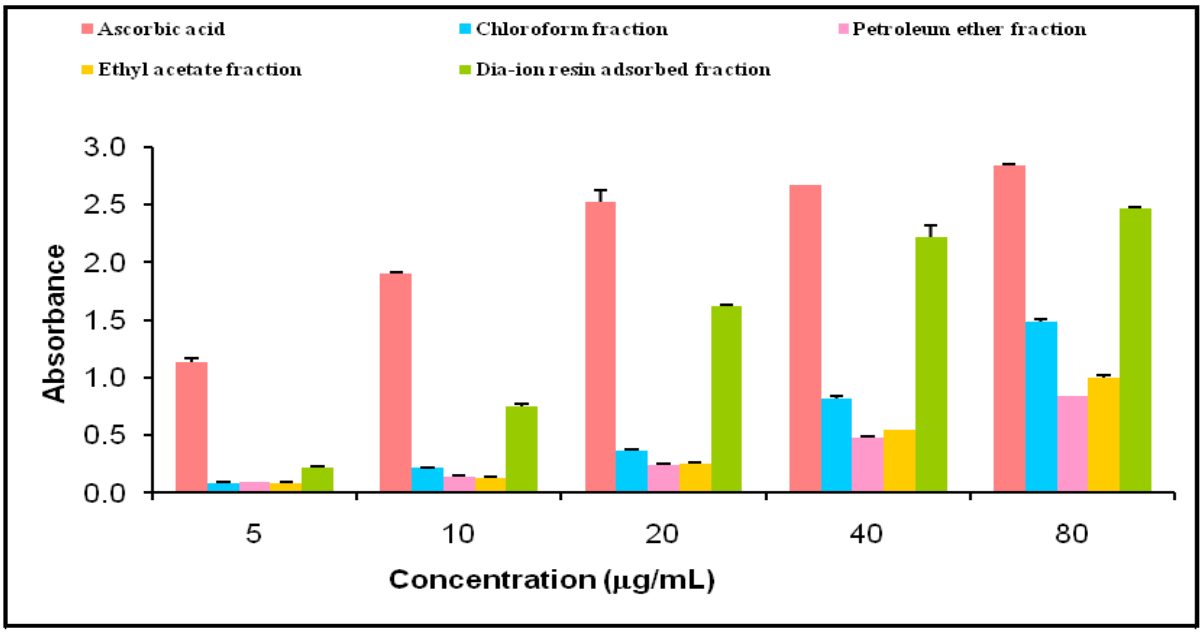

Fig. 4. Reducing power capacity of different fractions of ethanol extract of *Alstonia scholaris* with Ascorbic acid (Standard).

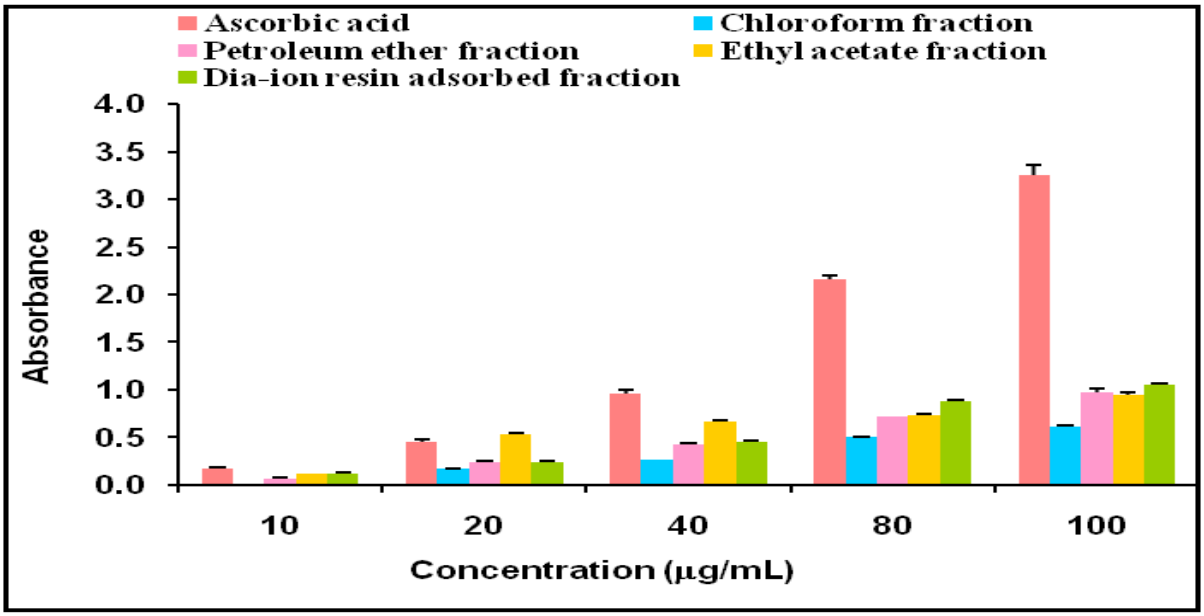

Fig. 5. Total antioxidant activity of different fractions of ethanol extract of *A. scholaris* with Ascorbic acid (Standard).

The estimated total phenolic was in the range of 3.39± 0.26-21.92± 0.13 GAE/g of dried extract, total flavanoid in the range of 9.31± 0.37-16.61± 0.06 CatE/g of dried extract in which more or less similar amount of total phenolic was noticed in CLF and PEF fractions, while total flavanoid between CLP and EAF fractions respectively.

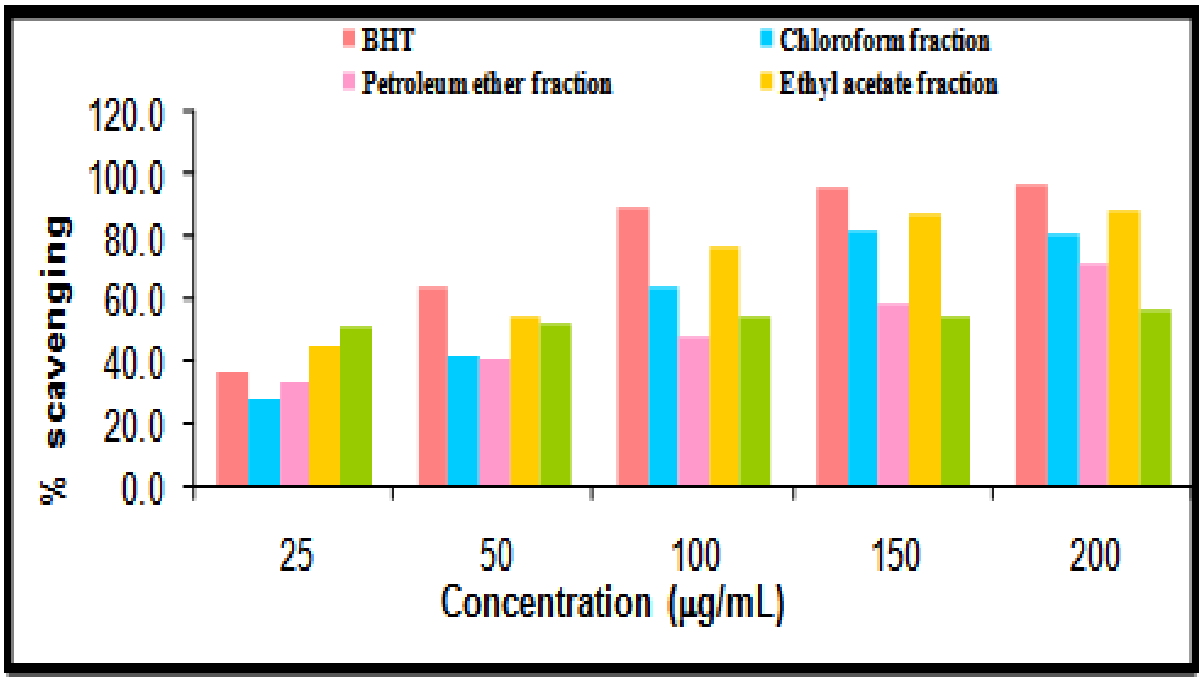

Fig. 6. DPPH free radical scavenging activity of different fractions of ethanolic extract of *A. scholaris* with BHT (standard).

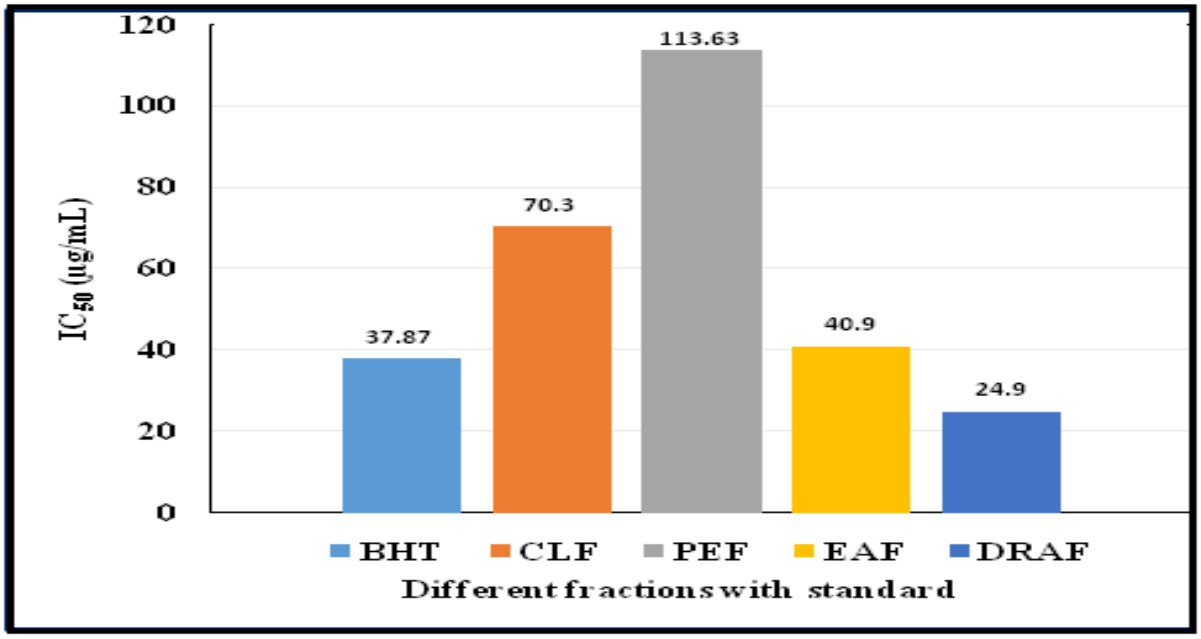

Fig. 7. IC_{50} (µg/ml) of different extractives of *A. scholaris* for free radical scavenging activity by DPPH radical.

As shown in Figure 8. significant positive correlations (R^2 = 0.863-0.916), were observed between total phenolic and phenolic content and $1/IC_{50}$ values for DPPH assay, indicating the significant contribution of phenolics and flavanoids to these antioxidant assays. As the flavanoids compounds exhibit higher R^2 values

(0.916) for DPPH antioxidant assays than total phenol (R^2 =0.863). Therefore, it is indicated that the antioxidant capacity of different fractions of ethanolic extracts was dominated by flavanoids than phenolic compounds.

The current study results shows that Dia-ion resin adsorbed fraction had the highest amount total phenolic, total flavonoid content and reducing power, total antioxidant and DPPH radical scavenging capacity in comparison with CLF, PEF and EAF fractions. Dia-ion resin is a good adsorbent for separating polyphenolic compounds (Soto *et al.*, 2012; Ogawa *et al.*, 2008) also obtain highly purified polyphenols fractions from the seed shells of *A. turbinate* using Diaion HP-20. Plant polyphenols are potent antioxidants widely distributed and accumulated in large amount in various plants consumed by human beings. Plants polyphenols scavenge DPPH free radicals (Yoshida *et al.*, 1989).

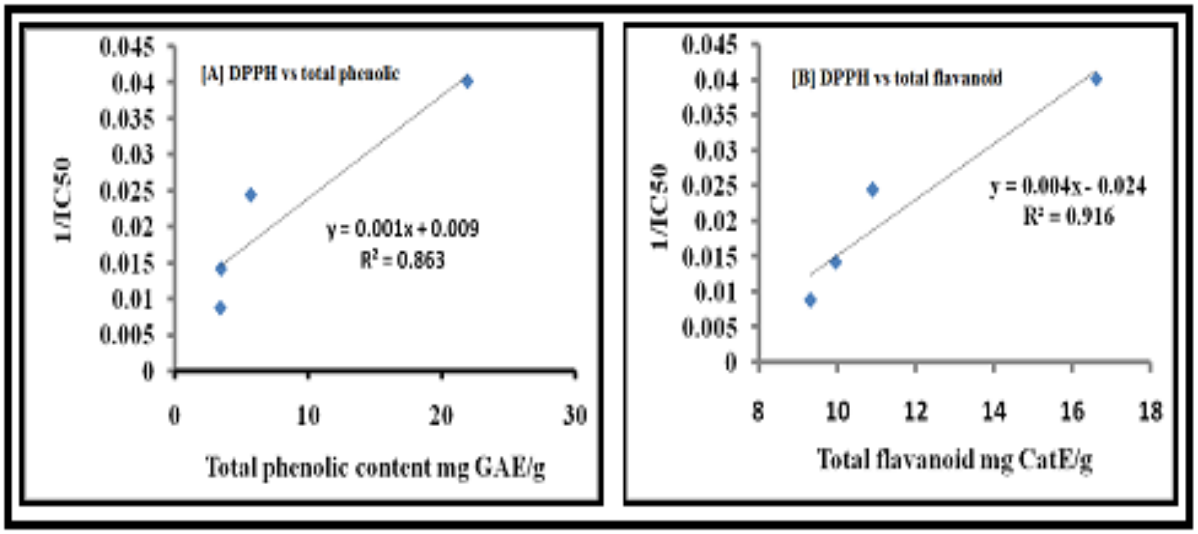

Fig. 8. Correlation graphs for DPPH 1 /IC_{50}values and (A) total phenolic contents, (B) total flavanoid contents in the four ethanoilc extracts of *A. scholaris*.

Total polyphenols were reported significantly negatively correlated to IC_{50} values of DPPH radicals scavenging by Zhao *et al.*, (2011). In numerous in vitro and in vivo experiments, plant flavonoids have shown antioxidant activity (Middleton, 1996) as health-promoting or disease-preventing dietary antioxidant compounds. In this study, similar result was observed for the Diaion adsorbed fraction, which was rich in phenolic contents.

Conclusion

The four different extractives of *Alstonia scholaris* showed the presence of phyto-constituents from moderate to high level in Dia-ion resin absorbed fraction, whereas minute to high in other extracts. The results of the present study also revealed that the different fractions of ethanolic extracts of *Alstonia scholaris* leaves exhibits considerable total antioxidant, iron reducing and free radical scavenging capacity. This result can be strong scientific evidence to use this plant as a useful source of both biological and pharmacological references. Yet, further studies are necessary to clarify a mechanistic way how the plant contributes in these properties.

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References

Akinpelu DA, Onakoya TM. 2006. Antimicrobial activities of medicinal plants used in folklore remedies in south-western. African journal of

Biotechnology **5(11).**

Antony M, Menon DB, Joel J, Lipin D, Arun K, Thankamani V. 2011. Phytochemical analysis and antioxidant activity of *Alstonia scholaris*. Pharmacognosy Journal **3(26),** 13-8.

Asolkaret LV, Kakkar KK, Chakra OJ. 1992. Second supplement to glossary of Indian medicinal plants with active principles, part 1(A-K).XIVII+414, Pub. And Inf. Div. (CSIR), New Delhi.

Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. 2001. Antioxidant principles from *Bauhinia terapotensis*. Journal of Natural Product **64**, 892–895.

Butler MS. 2004. The role of natural product chemistry in drug discovery. Journal of Natural Product, **67**, 2141–2153.

Cherian S, Augusti KT. 1995. Insulin sparing action of leucopelargonin derivative isolated from *Ficus bengalensis* Linn. Indian Journal of Experimental Biology **33, 6**08-11.

Dahlia IA, Islam MB, Zaman S, Islam MA, Jalil MA, Ahmed NU, Rahim MA, Mondol MM, Muzahid AA, Sarkar MH. 2017. In-vitro DPPH Free Radical Scavenging Activity of the Plant *Murraya koenigii* Linn (Curry Leaf) in Rajshahi, Bangladesh. Journal of Complementary and Alternative Medical Research **15, 1**-9.

Farnsworth NR. 1988. Sereening plants for new medicines. Wilson EO(Ed.). Biodiversity, pp.83-97, National Academy Press, Washington.

Harborne JB. 1973. Phytochemical methods. Chapman and hall Ltd., London, UK. 49-188.

Ikram M, Inamul H. 1984. Screening of medicinal plants for antimicrobial activities, Fitoterapia **55,** 62-64.

Karthishwaran K, Mirunalini S, Dhamodharan G, Arulmozhi V. 2010. Phytochemical investigation of methanolic extract of the leaves of *Pergularia daemia*. Journal of biological sciences **10(3),** 242-246.

Kaushik P, Kaushik D, Sharma N, Rana AC. 2011. *Alstonia scholaris*: It's Phytochemistry and pharmacology. Chronicles of Young Scientists **2**, 71-78.

Khan AV, Parveen G, Alam MM, Singh VK. 2002. Ethnomedicinal uses of Neem in rural areas of Uttar Pradesh, India. Ethnomed and Pharmacog. II Rec. Prog. Med. Plants, p7.

Middleton E, Jr. 1996. Biological properties of plant flavonoids: an overview. Int Pharmacognosy, **34,** 344-348.

Misra CS, Pratyush K, Sagadevan LD, James J, Veettil AK, Thankamani V. 2011. A comparative study on phytochemical screening and antibacterial activity of roots of *Alstonia scholaris* with the roots, leaves and stem bark. International Journal of Research in Phytochemistry and Pharmacology, **1(2),** 77-82.

Ogawa S, Kimura H, Niimi A, Katsube T, Jisaka M, Yokota K. 2008. Fractionation and structural characterization of polyphenolic antioxidants from seed shells of Japanese horse chestnut (*Aesculus turbinata* BLUME). Journal of Agricultural and Food Chemistry **56(24),** 12046-51.

Oyaizu M. 1986. Studies on product of browning reaction prepared from glucose amine. Japanese Journal of Nutrition **44,** 307-315.

Perry LM, Metzer J. 1998. Medicinal plants of east and south Asia, attributed properties and uses. Massachisetts and London: MIT press, Cambridge.

Prieto P, Pineda M, Aguilar M. 1999. Spectrophotometric quantitation of antioxidant

capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. Analytical Biochemistry **269**, 337-341.

Rastogi R, Mehrotra BN. 1991. Compendium of Indian Medicinal Plants, Vol. III 1: 497, CSIR Publication, India.

Ray PG, Majumdar SK. 1976. Antimicrobial activity of some Indian Plants. Economic Botany, **30(4),** 317-329.

Reddy DS. 2016. Phytochemical Analysis of Active Constituents of *Alstonia scholaris* and their Cytotoxicity in vitro. International Journal of Pharmaceutical Sciences and Research **7(8),** 3262.

Shah AJ, Gowani SA, Zuberi AJ, Ghayur MN, Gill AH. 2010. Antidiarrhoeal and Spasmolytic Activities of the methanolic Crude Extract of *Alstonia scholaris* L. are mediated through Calcium Channel Blockade. Phytotherapy Research **24,** 28-32.

Shang JH, Cai XH, Feng T, Zhao YL, Wang JK, Zhang LY, Yan M, Luo XD. 2010. Pharmacological evaluation of *Alstonia scholaris*: Anti-inflammatory and analgesic effects. Journal of Ethnopharmacology **29(2)**, 174-81.

Singh SK, Singh A. 2003. Molluscicidial and Anti-cholinerstase Activity of *Alstonia scholaris* plant against Freshwater Snail *Lymnaea acuminate*. Pakistan Journal of Biological Science **6(16),** 1442-1446.

Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture **16(3),** 144-58.

Sofowara A. 1993. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria 289-300.

Soto ML, Conde E, González-López N, Conde MJ, Moure A, Sineiro J, Falqué E, Domínguez H, Núñez MJ, Parajó JC. 2012. Recovery and Concentration of Antioxidants from Winery Wastes. Molecules **17(3),** 3008-3024.

Stauth D. 2007. Studies Force New View on Biology of Flavanoids. Oregon State University, USA.

Teixeira RO, Camparoto ML, Mantovani MS. 2003. Assessment of two medicinal plants Psidium guajava L. and *Achilleamille folium* L. In vitro and in vivo assays. Genetics and Molecular Biology **26(4),** 551-555.

Yamauchi T, Abe F, Padolina WG, Dayrit FM. 1990. Alkaloids from leaves and bark of *Alstonia scholaris* in the Philippines. Phytochemistry, **29(10),** 3321-3325.

Yoshida T, Mori K, Hatano T, Okomura T, Uehara I, Komagoe K, Fujita Y, Okuda T. 1989. Studies on inhibition mechanism of autoxidation by tannins and flavonoids. Radical-scavenging effects of tannins and related polyphenols on 1,1-diphenyl-2-picrlhydrazyl radical. Chemical and Pharmaceutical Bulletin **37(7)**, 1919-1921.

Zhao S, Liu JY, Chen SY, Shi LL, Liu YJ, Ma C. 2011. Antioxidant Potential of Polyphenols and Tannins from Burs of *Castaneamollissima* Blume. Molecules **16,** 8590-8600.

Zhishen J, Mengcheng T, Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry **64,** 555-559.