Vol. 14(8), pp. 428-437, August, 2020
DOI: 10.5897/JMPR2020.6999
Article Number: 2775D0564285
ISSN 1996-0875

http://www.academicjournals.org/JMPR

ACADEMIC JOURNALS
expand your knowledge

Journal of Medicinal Plants Research

Full Length Research Paper

Comparison of antioxidant role of methanol, acetone and water extracts of *Andrographis paniculata* Nees

Monsur Ahmad[1], Nor Mohammad[2,3], Abdullah-Al-Maswood[1], Md. Abdul Aziz[4], Md. Ashraful Alam[5], Md. Shahadat Hossain[6], Md. Rakibul Islam[6] and Md. Giash Uddin[3,5*]

[1]Department of Pharmacy, Faculty of Science, Noakhali Science and Technology University, Noakhali-3814, Bangladesh.
[2]Department of Chemistry, Faculty of Science, University of Chittagong, Chittagong-4331, Bangladesh.
[3]Pratyasha Health Biomedical Research Center, Dhaka-1230, Bangladesh.
[4]Department of Pharmacy, School of Science and Engineering, Southeast University, Dhaka-1213, Bangladesh.
[5]Department of Pharmacy, Faculty of Biological Sciences, University of Chittagong, Chittagong-4331, Bangladesh.
[6]Department of Pharmacy, Faculty of Health Sciences, Atish Dipankar University of Science and Technology, Dhaka-1230, Bangladesh.

Received 10 June, 2020; Accepted 1 July, 2020

Antioxidant properties of plants can help thwart a plethora of conditions including cancer, bronchitis, asthma, ulcers, coronary heart diseases, scabies, insomnia, senile debility, diabetes, dysentery, etc. In this study, *Andrographis paniculata* (Family: Acanthaceae) was investigated for antioxidant activity. 1, 1-Diphenyl-2-picryl hydrazyl (DPPH), reducing power assay, total antioxidant capacity, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), nitric oxide test, total phenolic and flavonoid from methanol, acetone, and water extracts were investigated employing various established *in-vitro* system. The results divulged that methanol extract had significant activity in quenching of DPPH, reducing power, ABTS, nitric oxide, antioxidant capacity when compared to other extracts. All extracts were correlated with two specific standard antioxidants ascorbic acid and butylated hydroxyanisole. The methanol extract at 500 µg/ml showed the highest scavenging activity of DPPH (91.78 ± 0.02%), ABTS (99.76 ± 0.05%). Total antioxidant capacity was 83.35 ± 0.13 AAE/mg. Reducing the power of methanol extract was significant compared to all other fractions. Nitric oxide scavenging activity was maximum in acetone extract (80.73 ± 0.05%). The quantitative estimation of the extract revealed a considerable amount of flavonoids and conversely, the amount of phenolic compounds was poor. The flavonoid content (592.34 ± 0.13 mg QE/g of dry extract) was maximum in methanol extract but phenolic content of methanol and acetone extract was very similar. This study strongly suggests that methanol extract exhibits more potent antioxidant activity than other extracts of acetone and water.

Key words: *Andrographis paniculata,* antioxidant potential, methanol, acetone, water, DPPH, ABTS

INTRODUCTION

The use of herbal medicines has rapidly increased in the last few decades and is expanding swiftly across the world day by day (Ekor, 2014; Welz et al., 2018; Ali et al., 2019). Around 75-80% of individuals of developing

*Corresponding author. E-mail: giash.uddin@cu.ac.bd.

countries and 25% of individuals of the developed countries are using traditional herbal medicines for their primary source of treatment (Bodeker et al., 2005; Bandaranayake, 2006; Mukherji, 2008; Hussain et al., 2018). With the introduction of many more herbal products in the market to suffice the surging demand for herbal medicines, public health issues surrounding their safety have become a major concern for the community. Albeit, some herbal medicines have been known for their potential biological activity, and are widely used, the vast majority of them yet remain untested, and their uses are poorly monitored as well. This scenario is also prevalent in Bangladesh, where herbal medicines play a pivotal role in the treatment of different diseases. Top pharmaceutical companies are now opening their herbal plants to appease the burgeoning exigency of herbal medicines in the country. Nevertheless, there are no specific parameters up to date based on which we can control the quality of these therapeutic plants. MDA is the end product of lipid peroxidation. Increased level of MDA generates oxidative stress. Antioxidants work against oxidative stress by converting superoxide-free radicals into hydrogen peroxide and oxygen molecules (Das et al., 2020). This study was conducted to evaluate the antioxidant potential of *Andrographis paniculata.* However, In Bangladesh*, A. paniculata* commonly referred to as ' Kalmegh' in Bangla, has markedly been pervasively consumed for ages as one of the most vital medicinal plants.

A. paniculata (Family: Acanthaceae) is plentifully abundant in Southeast Asia where it engenders from seed on all kinds of soil (Purohit et al., 2006) and has the ubiquitous presence of bitter andrographolide. Since, it has a wide spectrum of pharmacological action (Singha et al., 2003; Mamatha 2011), it is termed “king of bitters”. With the virtue of the combined effect of the arabinogalactan proteins and andrographolides, this plant has also been reported to exhibit antimicrobial action (Roy et al., 2010). *A. paniculata* was propounded by *Charaka Samitha* for the treatment of jaundice (Sharma, 1983) and has been used against sluggish liver in colic dysentery as well as dyspepsia (Handa and Sharma, 1990; Sheeja et al., 2006; Tatiya et al., 2012). In addition, it turns out to have beneficial effects on hepatitis and dysentery (Singha et al., 2007). The new leaf juice obtained from the plant is an established household remedy in treating colic pain, diminished appetite, irregular stools (Madav et al. 1995, Sheeja et al., 2006; Nugroho et al., 2012). The most opulent and sought-after constituent andrographolide isolated by Valdiani et al. (2014) carries certain other activities mainly liver protection (Nagalekshmi et al., 2011), anticancer activity (Menon and Bhat, 2010), anti-diabetic activity (Nugroho et al., 2012) and antimalarial activity (Dua et al., 2004).

Studies in terms of seeking bioactivities such as the antioxidant potential of myriad plants including *A. Paniculata* have always been deemed with utmost priority since the effectiveness can deviate concerning geographic ancestry, age of the plant, part of the plant used as well as the use of the solvents for extraction. Recently, an increase of immense interest for plant-derived natural antioxidants has been substantially on the rise due to their promising possibility to be used as an effective therapeutic drug, nutraceuticals, food, cosmetics because they have multifacetedness when it comes to activity and serves enormous opportunity in turning the tide of imbalance in the body.

According to Hamdard Laboratories (WAQF), in Bangladesh, the annual demand for Kalmegh (*A. paniculata*) is 1,000 tons (Hasan 2003). Bangladesh satiates this needs mostly by importing from foreign countries (e.g., India, China, etc.) and the rest is accrued from wild sources and cultivated in the country, especially in some parts of the Rajshahi division. But, still now, no such research has been done to determine the quality of Kalmegh cultivated in the country particularly. Since the antioxidant properties of any products can be implicated in the retardation of numerous diseases like cancer, coronary heart diseases, bronchitis, asthma, ulcers, scabies, insomnia, senile debility, diabetes, dysentery, etc., for the first time, this study was designed to determine the antioxidant properties of Kalmegh samples available in Bangladesh. Therefore, the main objective of this study is to assess the antioxidant activity of Kalmegh and to perform a comparative study among these samples.

MATERIALS AND METHODS

Plant materials collection

A. paniculata (Kalmegh) samples (stem along with leaves) were garnered from Owshudi Gram, Natore, Bangladesh, during September 2018. Having properly identified by an expert botanist, a voucher specimen (DACB Accession Number: 35939) of the plant sample was then deposited to Bangladesh National Herbarium, Mirpur, Dhaka. After collection, cleansing and chopping of the sample into small pieces, in the next step dried samples were grained into powder with the help of a cyclone grinder machine. The powdered samples were eventually used for different experiments.

Chemicals, reagents and equipment

1, 1-Diphenyl-2-picryl hydrazyl (DPPH), aluminum chloride ($AlCl_3$), disodium hydrogen phosphate (Na_2HPO_4), $FeCl_2.4H_2O$, $FeCl_3$, Ferrozine, Folin–Ciocalteu reagent, L-Ascorbic acid, Sodium carbonate (Na_2CO_3), KH_2PO_4, $K_3Fe(CN)_6$, Sodium nitroprusside, Griess reagent (2% H_3PO_4, 0.1% naphthalene diamine dihydrochloride, 1% sulphanilamide), phosphate buffer and Trichloroacetic acid were taken from Sigma Chemical Co. (USA). The methanol for the mobile phase was HPLC grade (Merck, Germany). Deionized water was obtained from a water purification system. All other chemicals and reagents were of analytical quality. Soxhlet apparatus (Fisher Scientific, USA), Rotary Vacuum Evaporator (Labfirst Scientific and Industrial, China), UV VIS spectrophotometer SPECORD 205 (Analytik Jena, Germany) were used in this study.

Preparation and extraction of extracts

The powdered samples of *A. paniculata* were then subjected to extract preparation. For extract preparation, powdered samples were extracted with solvents using a Soxhlet apparatus. The extraction solvents used for the extraction were methanol, acetone, and water. Then the extracted solvents were evaporated and concentrated using a rotary evaporator. Finally, it was desiccated in a vacuum drier and stored for further analysis (WHO, 2011).

Antioxidant assay

DPPH radical scavenging activity

The stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was assessed concurring with the modified method explicated (Chang et al., 2001; Gupta et al., 2004; Uddin et al., 2020). Concisely, 2 ml volume of 0.1 mM DPPH solution having purple color and another 2 ml of plant sample of varying concentrations (5, 10, 20, 40, 60, 80, 100, 250 and 500 µg/m) were first brought together and stirred vigorously for 15 s. To get a uniform mixture followed by keeping in the dark at room temperature for a time period of half an hour. After the specified interval, a UV/Visible spectrophotometer was used to read absorbance against a condign blank at 517 nm (Model 205, Germany). The antioxidant viability was estimated from the ability of the extract to eradicate the purple color that appeared in DPPH solution and the percentage of DPPH radical-scavenging activity was computed as:

$$\text{DPPH radical-scavenging activity (\%I)} = \frac{A_0 - A}{A_0} \times 100$$

Where A_0 is the absorbance of the control solution; A is the absorbance of the DPPH solution containing plant extract. IC_{50} values for the plant extract and standard were obtained by analysis of the respective percentage scavenging of DPPH radical.

Determination of reducing power assay

It was performed with faint modifications, based on the method expounded by Oyaizu (1986). First of all, a mixture of 0.75 ml of plant extract at varying concentrations with 1% potassium hexacyanoferrate (0.75 ml) and 0.2 M potassium dihydrogen phosphate (0.75 ml, pH 6.6) was conducted with subsequent incubation in a water bath at 50°C for 20 min. The mixture was then subjected to centrifugation for 10 min at 800 rpm after the addition of trichloroacetic acid (10%). After that, the obtained upper layer was blended with 0.1% ferric chloride (w/v, 0.1 ml) and distilled water (1.5 ml) for ten minutes. The optical density (OD) against a blank was ascertained at 700 nm. Increased OD value of the mixture of reaction indicates increased reducing power.

Total antioxidant capacity determination

The total antioxidant capacity was ascertained following the phosphomolybdenum assay method (Prieto et al., 1999) which operates via the reduction of Mo (VI) to Mo (V) by extracting and subsequently forming a green phosphate-Mo (V) complex in an acidic state. The extract was permitted to amalgamate with 3.0 ml of reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, 4 mM ammonium molybdate), and afterward, the reaction mixture was incubated at 95 ^{0}C for 90 min. Upon cooling at room temperature, a The antioxidant activity was elicited as the number of ascorbic acids UV-Visible spectrophotometer was used to measure the solution's absorbance against an opposite blank at a wavelength of 695 nm. tantamount in gram.

ABTS radical scavenging activity test

The antioxidant capacity was appraised by Fan et al. (2009), with petty alterations. In succinct, ABTS radical cation was synthesized by reacting with 2.45 mM potassium persulfate solution of 7 mM ABTS and leaving the reaction blend to stay at room temperature in the dark for a period of 16 h. At the time of use, the ABTS solution was then diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Application of 1 ml of each sample with varying concentrations (5 to 250 µg/ml) to 1 ml of ABTS solution was conducted, afterward. At the subsequent stage, the reaction admixture was held to stand for another 6 min at room temperature, and momentarily the absorbance was reported at 734 nm. The ABTS scavenging effect was calculated based on the following formula:

$$\text{ABTS scavenging effect} = \left(\frac{A_o - A_s}{A_o}\right) \times 100$$

Where A_o = Absorbance of control and A_s = Absorbance of sample

Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically (Marcocci et al., 1994; Balakrishnan et al., 2009). In this experiment, a reaction mixture of 5 ml, comprising SNP or sodium nitroprusside (5 mM) dissolved in phosphate buffer (pH 7.3), with the presence or absence of various concentration of plant samples was subjected to incubation for 180 min at 25°C in the existence of a light source (visible and polychromatic). Thus the generation of nitric oxide radical ($NO^{\cdot}$) took place that subsequently combined with oxygen to form nitrite ion which was estimated at every 30 min by amalgamating incubation mixture (1 ml) with Griess reagent of the same volume. Absorbance measured for the chromophore produced due to the reaction was then noted at 546 nm. Estimation of nitrite ion formed with or without adding extracts was performed based on the standard sodium nitrate curve obtained by using a known concentration of the solution. The nitric oxide (NO) radical scavenging activity was expressed as the inhibition percentage (I%) and calculated as:

$$I\ (\%) = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction, and A_{sample} is the absorbance of the test compound. The IC_{50} value was calculated from the plot of inhibition (%) against extract concentration. Ascorbic acid was used as a standard for this study.

Determination of total phenolic content

The total phenolic content of the extracts was gauged in compliance with the modified Folin-Ciocaltu method (Wolfe et al., 2003). The absorbance was noticed at 765 nm with a spectrophotometer (Specord 205, Germany). The standard curve was arranged using 0, 0.25, 0.50, 0.75 and 100 mg/ml solutions of gallic acid in methanol: water (50:50, v/v). Total phenolic content was finally stated as mg of gallic acid equivalent per gram using the equation obtained from a standard gallic acid calibration curve y =6.9104x - 0.0937, R^2 = 0.9972.

Determination of total flavonoids

The aluminum chloride colorimetric method was used for flavonoids

Table 1. DPPH radical scavenging activity of different solvent extracts of *A. paniculata.*

Extract	IC_{50} (µg/ml)
Methanol extract	15.48 ± 0.71[e]
Acetone extract	22.67 ± 1.20[d]
Water extract	13.42 ± 0.71[c]
Ascorbic acid (ASA)	03.27 ± 0.02[a]
BHA	04.48 ± 0.59[b]

Values are expressed as mean ± SD (n=3) and same column with a different letter are significantly different (P < 0.05).

determination (Chang et al., 2002). In this prescribed assay, a quantity of 0.6 ml of fractions and 2% aluminium chloride (6 ml) were blended with each other. Following mixing, incubation of the solution at room temperature was carried out for 1 h and the absorbance of the reaction mixture was then reported at 415 nm wavelength. The calibration curve was attained in the same way using Quercetin solutions instead of fractions at concentrations 0, 0.25, 0.50, 0.75, 100 mg per ml in methanol. Total flavonoids content was expressed as mg of Quercetin equivalent per gram using the equation acquired from a standard Quercetin calibration curve y = 4.7385x + 0.0355; R^2 = 0.9993.

Statistical analysis

All data presented in the study as mean ± SD and were statistically analyzed by SPSS software package (version 20) and MS Excel for windows version 2010.

RESULTS AND DISCUSSION

In this study, crude extracts of *A. paniculata* have been investigated for *m*ethanol, acetone, and water extracts. All extracts were tested for antioxidant potential evaluation. Antioxidant potential was assessed by the DPPH scavenging activity, reducing power assay, total antioxidant capacity, ABTS radical scavenging activity, nitric oxide (NO) scavenging activity, amount of total phenolic and flavonoid content determination.

Antioxidant assay

DPPH scavenging activity of different solvent extracts

A constant free radical DPPH is decolorized in the existence of antioxidants (Hasanuzzaman et al., 2013) and has an unpaired electron which exhibits absorbance of 515 to 517 nm providing a clear dark purple color. Decolorization of DPPH occurs as it accepts electron generated by antioxidant compound, and is quantitatively measured from the changes in absorbance (Subhan et al., 2008). All extractives showed substantial DPPH free radical scavenging activity suggested by IC_{50} values (Table 1 and Figure 1). The IC_{50} value implies the magnitude of scavenging potential. Scavenging activity of hot methanol, acetone, and water extract of *A. paniculata* was found to ascend with the augmentation in concentration. Though all the samples showed somewhat less radical scavenging activity at a lower concentration, the very significant antioxidant potential was demonstrated compared to standard compound when the concentration was greater with the highest activity at 500 µg/ml. Among the extracts of *A. paniculata,* maximum inhibition of DPPH free radicals at 500 µg/ml was observed (91.78, 92.67 and 86.57%) for methanol, acetone, and water; whereas the standards ascorbic acid and butylated hydroxyanisole both at 500 µg/ml displayed 97.42 and 94.62% inhibition, respectively. The experiment also revealed that the DPPH radical quenching ability of these extracts is concentration-dependent, and scavenging capability is enhanced with gradual increase in concentration.

Reducing power assay of various solvent extracts

Antioxidant potential can also be evaluated by quantifying the reducing power of a substance where a rise in the absorption of a substance is an indicator of heightened reducing power (Li et al., 2017). Increasing antioxidant activity would reduce the cycle of lipid peroxidation and thus protect cell damage (Uddin et al., 2017; Adnan et al., 2019; Amin et al., 2020). With the continued elevation in concentration, reducing the power of methanol, acetone, and water extract of *A. paniculata* was found to climb upward. Figure 2 depicts the comparative reducing power of various extracts and fractions of *A. paniculata* and standard compounds (ASA and BHA). The methanol extract showed noticeably pronounced reducing power than acetone and water extract when compared to selected standards.

Total antioxidant capacity for different solvent extract

Total antioxidant capacity in various solvent extracts and fractions of the *A. paniculata* was also explicated. Total antioxidant capacity was denoted as ascorbic acid equivalents by reference to a standard curve (y = 0.0026x

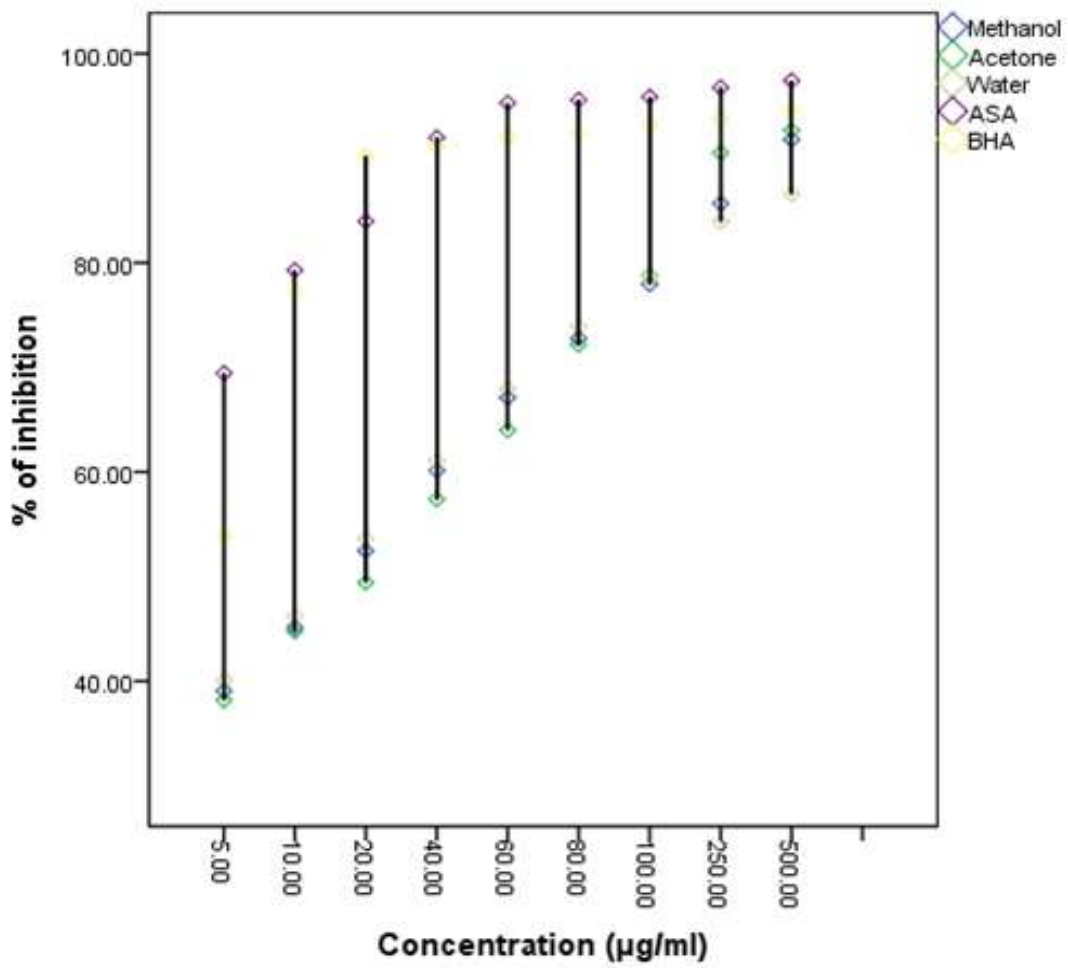

Figure 1. Comparative analysis of different extract and standard sample for DPPH scavenging activity.

+ 0.0156; R^2 = 0.9998) for different extracts of *A. paniculata* (Table 2). Methanol and acetone extracts contained a similar amount of total antioxidant capacity 83.35 ± 0.13% and 82 ± 0.02% (Ascorbic acid equivalent per gram of dry extract) respectively but water extract displayed relatively lower total antioxidant capacity (51.4 ± 0.01%).

ABTS scavenging activity of different solvent extracts

The ABTS scavenging assay is another well-grounded approach taken up to establish antioxidant activity. A blue or green chromophore of $ABTS^{\cdot+}$ is generated as a result of oxidation between potassium persulfate and ABTS. The reduction of $ABTS^{\cdot+}$ formed during the interaction of these two chemicals is assessed with the existence of antioxidants having hydrogen donating property at 734 nm by using a spectrophotometer. The ABTS scavenging is not only an indicator of the antioxidant potential hydrogen donating antioxidants but also chain-breaking antioxidants (Li et al., 2017). The ABTS radical activity of methanol, acetone, and water extracts of *A. paniculata* was seen to rise with the rise in concentration (Figure 3). The IC_{50} value was also computed (Table 3). The scavenging power of methanol and acetone extract provided excellent result but water extract showed weak results when the comparison was made with standard ascorbic acid.

Nitric oxide (NO) scavenging activity of different solvent extracts

NO extricated from sodium nitroprusside gives rise to NO^+ which in turn can modulate the cellular function through the alteration of cellular structure. NO can be even more toxic by reacting with the superoxide and constituting potent oxidant peroxynitrite anion that eventually can decay later to yield both $^{\cdot}OH$ and NO_2 (Awah and Verla, 2010). Nitric oxide scavengers work by

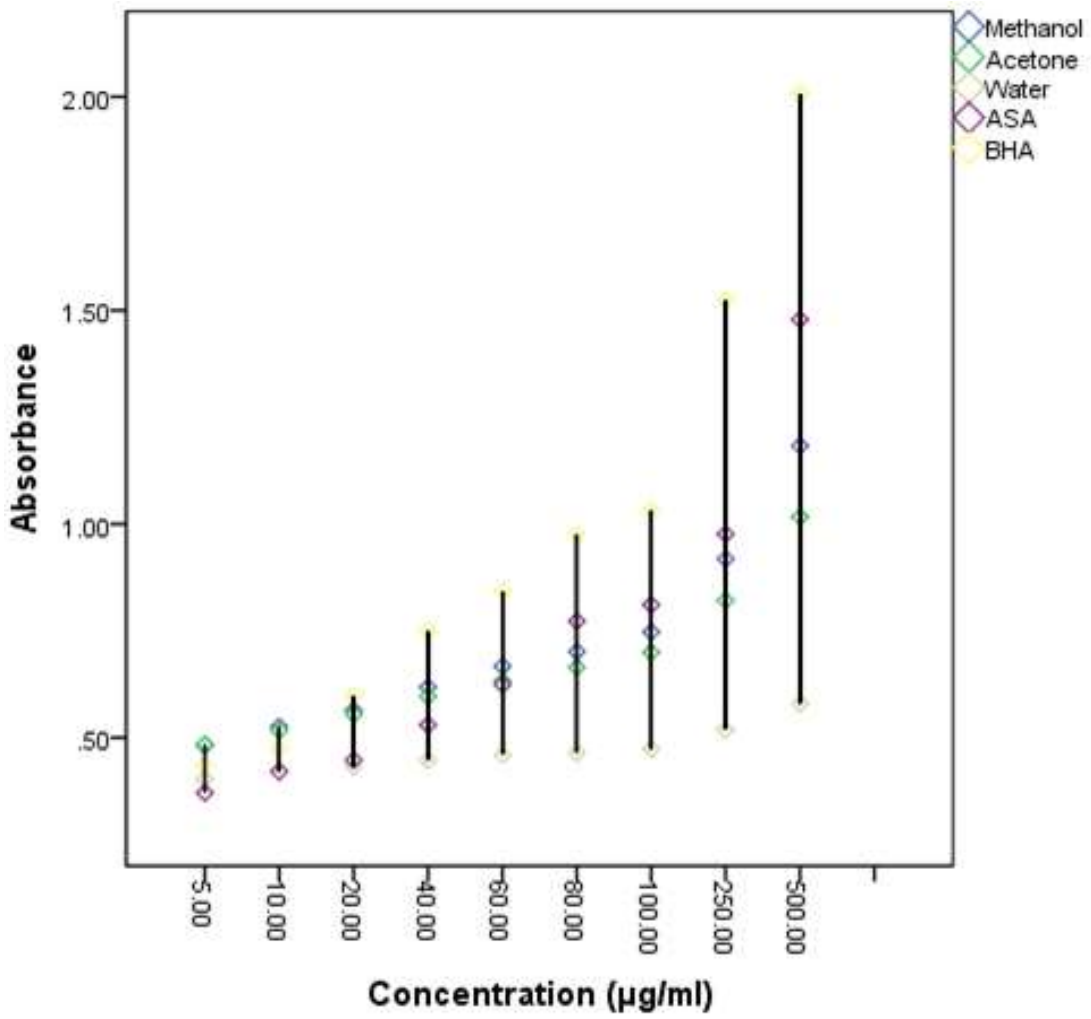

Figure 2. Comparative reducing power assay of different solvent extracts with ascorbic acid and butylated hydroxy anisole.

Table 2. Total antioxidant capacity in different solvent extracts and fractions of *A. paniculata.*

Extract	Absorbance for 500 ppm at 695 nm	Total antioxidant capacity (AAE/gm)
Methanol extract	0.1817 ± 0.0003	83.35 ± 0.13[a]
Acetone extract	0.1790 ± 0.0002	82.00 ± 0.02[a]
Water extract	0.1178 ± 0.0005	51.40 ± 0.01[b]

Values are expressed as mean ± SD (n=3) and same column with a different letter are significantly different (P < 0.05).

competing with oxygen and thereby resulting in the synthesis of reduced NO_2. The NO scavenging activity was seen to enhance with the increase in the concentration of methanol, acetone, and water extract which showed good activity in comparison to standard ascorbic acid. Figure 4 and Table 4 delineate the comparative analysis of different extracts and IC_{50} among the extracts of *A. paniculata.*

Total phenolic and flavonoid content of different solvent extracts

Polyphenols are the significant plant compounds that confer antioxidant potential. Typical phenolics are known as phenolic acids and flavonoids (Demiray et al., 2009). The antioxidant potential of the plant varies due to phenolics. Studies have been able to shed light upon the

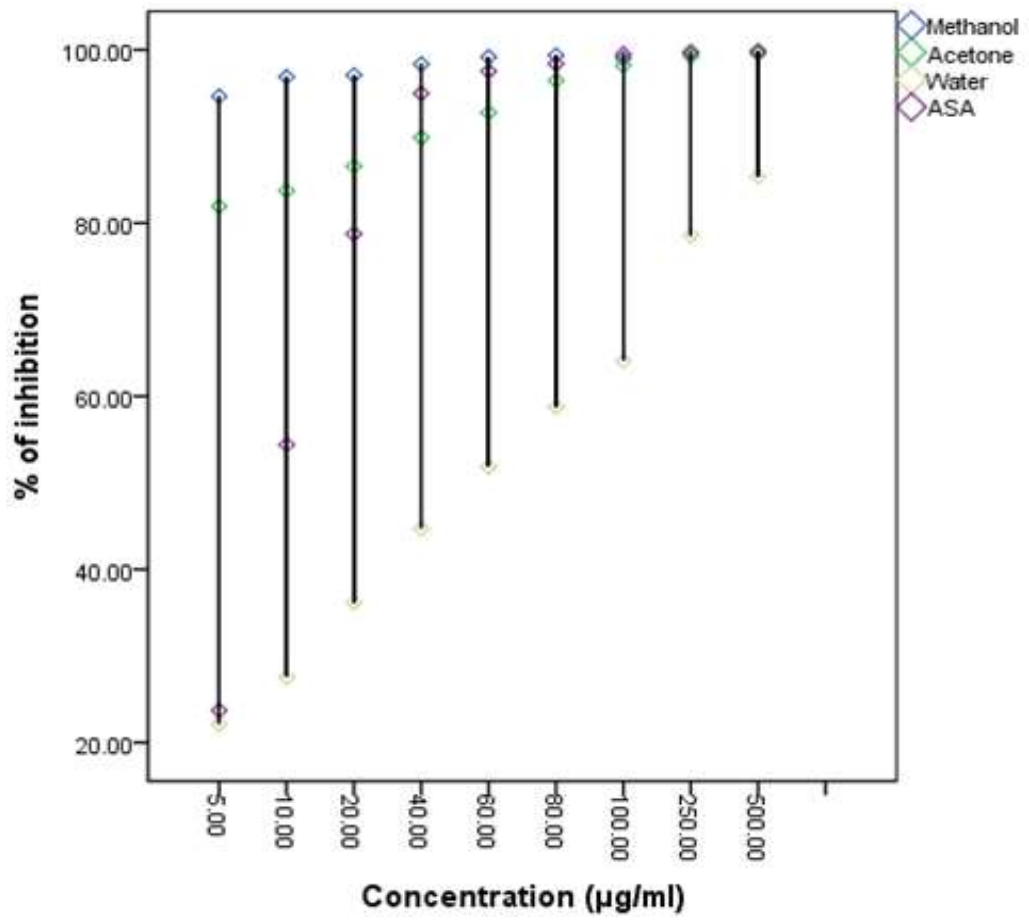

Figure 3. Comparative analysis of different extract and standard sample for ABTS scavenging activity.

Table 3. Comparison of IC_{50} among the extracts for ABTS scavenging of *A. paniculata.*

Extract	IC_{50} (µg/ml)
Methanol extract	02.41 ± 0.02[a]
Acetone extract	02.74 ± 0.03[a]
Water extract	53.13 ± 0.03[c]
Ascorbic acid (ASA	08.81 ± 0.67[b]

Values are expressed as mean ± SD (n=3) and same column with a different letter are significantly y different (P < 0.05).

evidence that flavonoids being phenolic acids are accountable for the antioxidant potential of various medicinal plants (Esmaeili et al., 2015) and viable scavengers of the majority of oxidizing molecules, for instance, a wide range of free radicals linked with numerous ailments, and singlet oxygen (Bravo, 1998). Total phenolic content and flavonoids of various extracts were also determined. Acetone extracts of *A. paniculata* were observed to contain the maximum amount of total phenolic content (80.66% mg Gallic acid equivalent per gram of dry extract) than methanol and water extracts. Methanol extracts of *A. paniculata* samples were reported to contain the maximum amount of flavonoid content (592.34% mg Quercetin equivalent per gram of dry extract) than acetone and water extracts. Figure 5 illustrates the amount of total phenolic content and total flavonoids content in different solvent extracts of the three samples.

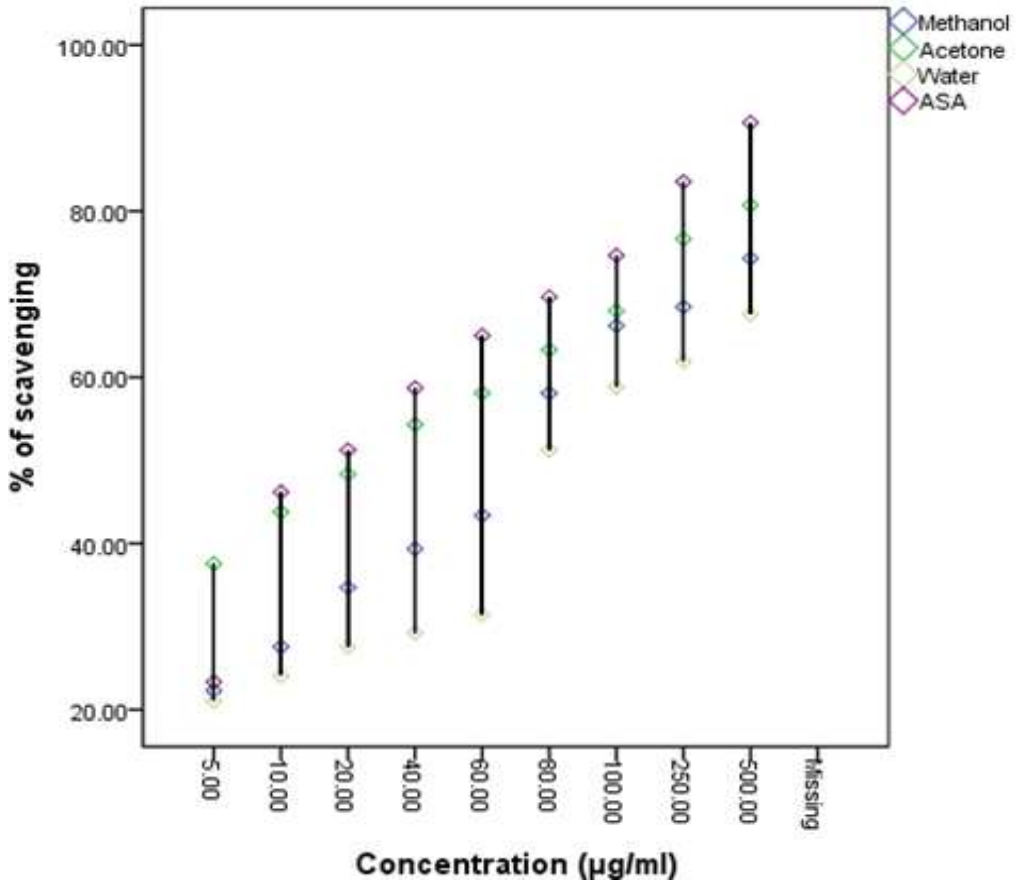

Figure 4. Comparative analysis of different solvent extracts and standard sample for NO scavenging activity.

Table 4. Comparison of IC_{50} among the extracts for NO scavenging of *A. paniculata.*

Extract	IC_{50} (µg/ml)
Methanol extract	68.94 ± 0.06^{c}
Acetone extract	50.89 ± 0.20^{b}
Water extract	78.86 ± 0.22^{d}
Ascorbic acid (ASA)	46.86 ± 0.02^{a}

Values are expressed as mean ± SD (n=3) and same column with a different letter are significantly different (P < 0.05).

Conclusion

In this study, the antioxidant potential of different extracts of *A. paniculata* was determined with three different solvents. Different solvent extracts showed potential antioxidant activity through the evaluation of antioxidant tests. Methanol is identified as the most appropriate extraction solvent for *A. paniculata* because it showed significant antioxidant power and the highest flavonoid contents than acetone and water solvent extracts. Further scientific studies are suggested to identify the exact compounds exploiting antioxidant activity and to understand its mechanism for such activity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Pharmacy, Noakhali Science and Technology University and Pratyasha Health Biomedical Research Center for cordial

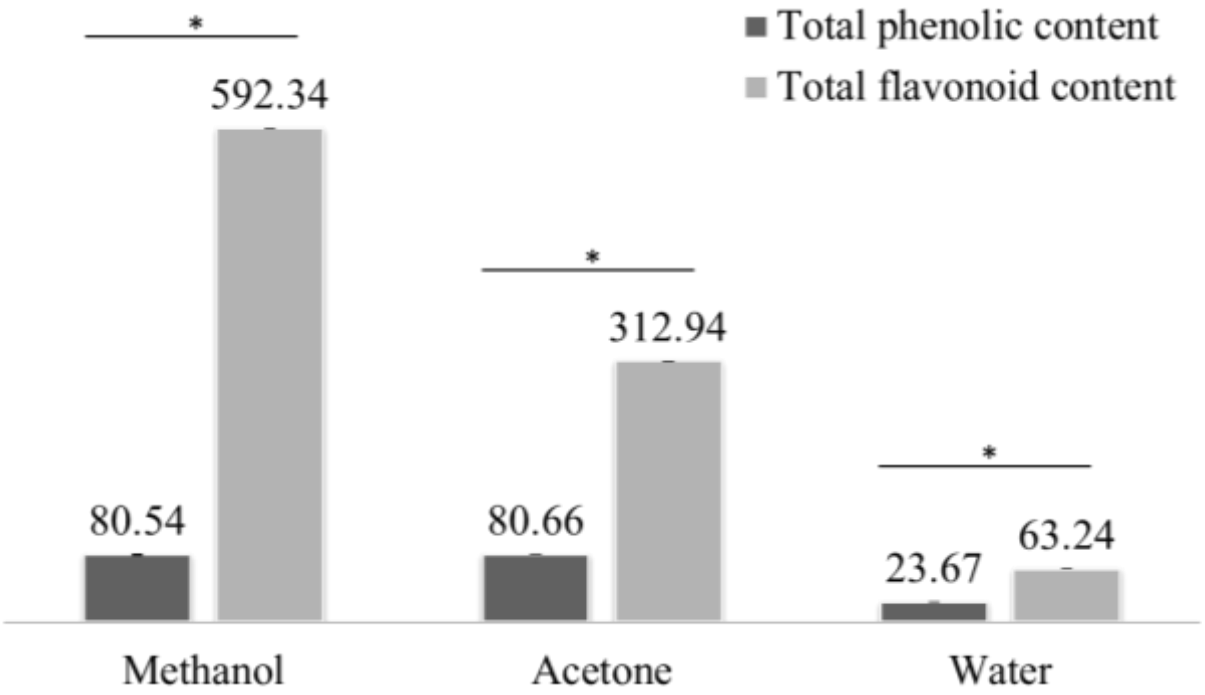

Figure 5. Comparison between phenolic and flavonoid content of different extract of Andrographis paniculata.* means values are statistically significant.

co-operation to conduct the research work.

REFERENCES

Adnan MT, Amin MN, Uddin MG, Hussain MS, Sarwar MS, Hossain MK, Uddin SMN, Islam MS (2019). Increased concentration of serum MDA, decreased antioxidants and altered trace elements and macro-minerals are linked to obesity among Bangladeshi population. Diabetes and Metabolic Syndrome: Clinical Research and Reviews 13(2):933-938.

Ali I, Suhail M, Naqshbandi M, Fazil M, Ahmad B, Sayeed A (2019). Role of unani medicines in cancer control and management. Current Drug Therapy 14(2):92-113.

Amin MN, Siddiqui SA, Uddin MG, Ibrahim M, Uddin SMN, Adnan MT, Rahaman MZ, Kar A, Islam MS (2020). Increased Oxidative stress, altered trace elements, and macro-minerals are associated with female obesity. Biological Trace Element Research. https://link.springer.com/article/10.1007/s12011-019-02002-z

Awah FM, Verla AW (2010). Antioxidant activity, nitric oxide scavenging activity and phenolic contents of *Ocimum gratissimum* leaf extract. Journal of Medicinal Plants Research 4(24):2479-2487.

Balakrishnan N, Panda A, Raj N, Shrivastava A, Prathani R (2009). The evaluation of nitric oxide scavenging activity of *Acalypha indica* Linn root. Asian Journal of Research in Chemestry 2(2):148-150.

Bandaranayake WM (2006). Quality control, screening, toxicity, and regulation of herbal drugs. in. modern phytomedicine: Turning Medicinal Plants into Drugs. Wiley-VCH Verlag GmbH & Co. KGaA. Available at: https://onlinelibrary.wiley.com/doi/abs/10.1002/9783527609987.ch2

Bodeker G, Ong C, Grundy C, Burford G, Shein K (2005). WHO global atlas of traditional, complementary and alternative medicine. Available at: https://apps.who.int/iris/handle/10665/43108

Bravo L (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews 56(11):317-333.

Chang CC, Yang MH, Wen HM, Chern JC (2002). Estimation of total flavonoid content in propolis by two complementary colometric methods. Journal of Food and Drug Analysis 10(3):178-182.

Chang S, Wu J, Wang S, Kang P, Yang N, Shyur L (2001). Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. Journal of Agricultural and Food Chemistry 49:3420-3424.

Das DC, Jahan I, Uddin MG, Hossain MM, Chowdhury MA, Fardous Z, Rahman MM, Kabir AH, Deb SR, Siddique MA, Das A (2020). Serum CRP, MDA, vitamin C, and trace elements in Bangladeshi patients with rheumatoid arthritis. Biological Trace Element Research. https://link.springer.com/article/10.1007%2Fs12011-020-02142-7

Demiray S, Pintado M, Castro P (2009). Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: *Tilia argentea*, *Crataegi folium* leaves and *Polygonum bistorta* roots. World Academy of Science, Engineering and Technology 3(6):312-317.

Dua VK, Ojha VP, Roy R, Joshi BC, Valecha N, Devi CU, Bhatnagar MC, Sharma VP, Subbarao SK (2004). Anti-malarial activity of some xanthones isolated from the roots of *Andrographis paniculata*. Journal of Ethnopharmacology 95(2-3):247-51.

Ekor M (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Pharmacology 4:177.

Esmaeili AK, Taha RM, Mohajer S, Banisalam B (2015). Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from *in vivo* and *in vitro* grown *Trifolium pratense* L. (Red Clover). BioMedical Research International 2015:1-11.

Fan Y, He X, Zhou S, Luo A, He T, Chun Z (2009). Composition analysis and antioxidant activity of polysaccharide from *Dendrobium denneanum*. International Journal of Biological Macromolecules 45(2):169-173.

Gupta M, Mazumdar U, Sivahkumar T, Vamis MLM, Karki S, Sambathkumar R, Manikandan L (2004). Antioxidant and anti-inflammatory activities of *Acalypha fruticosa*. Nigerian Journal of Natural Product and Medicines 7(1):25-29.

Handa SS, Sharma A (1990). Hepatoprotective activity of andrographolide against galactosamine and paracetamol intoxication in rats. Indian Journal of Medical Research - Secton B Biomedical Research Other Than Infectous Diseases 92(AUG.):284-292.

Hasan MA (2003) . Shaystha o shoundarja paricharjai vesoz udvid (in Bengali). Al Hakim 13:11-16.

Hasanuzzaman M, Ali MR, Hossain M, Kuri S, Islam MS (2013).

Evaluation of total phenolic content, free radical scavenging activity and phytochemical screening of different extracts of *Averrhoa bilimbi* (fruits). International Current Pharmaceutical Journal 2(4):92-96.
Hussain MS, Irahim M, Hasan MM, Aziz MT, Suchi SA, Uddin MG (2018). An in vivo study of the pharmacological activities of a methanolic acetate fraction of *Pistia stratiotes* L.: An ethno-medicinal plant used in Bangladesh. Animal Models and Experimental Medicine 1(3):221-227.
Li H, Zhang D, Tan L-H, Yu B, Zhao S-P, Cao W-G (2017). Comparison of the antioxidant properties of various solvent extracts from *Dipsacus asperoides* and identification of phenolic compounds by LC-ESI-QTOF-MS–MS. South African Journal of Botany 109:1-8.
Madav S, Tripathi HC, Mishra SK (1995). Analgesic, antipyretic and antiulcerogenic effects of andrographolide. Indian Journal of Pharmaceutical Sciences 57(3):121.
Mamatha A (2011). Quantitative HPTLC analysis of Andrographolide in *Andrographis paniculata* obtained from different geographical sources (India). International Journal of Pharmacy and Pharmaceutical Sciences 3(2):42-44.
Marcocci L, Packer L, Droy-Lefaix M-T, Sekaki A, Gardès-Albert M (1994). Antioxidant action of *Ginkgo biloba* extract EGb 761. Methods in Enzymology (Academic Press) 234:462-475.
Menon V, Bhat S (2010). Anticancer activity of andrographolide semisynthetic derivatives. Natural Product Communications 5(5):1934578X1000500508.
Mukherji PK (2008). Quality control of herbal drugs: An approach to evaluation of botanicals. New Delhi: Business Horizons.
Nagalekshmi R, Menon A, Chandrasekharan DK, Nair CK (2011). Hepatoprotective activity of *Andrographis paniculata* and *Swertia chirayita*. Food and Chemical Toxicology 49(12):3367-3373.
Nugroho AE, Andrie M, Warditiani NK, Siswanto E, Pramono S, Lukitaningsih E (2012). Antidiabetic and antihiperlipidemic effect of *Andrographis paniculata* (Burm. f.) Nees and andrographolide in high-fructose-fat-fed rats. Indian Journal of Pharmacology 44(3):377.
Oyaizu M (1986). Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition and Dietetics 44(6):307-315.
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(2):337-331.
Purohit SS, Sharma AK, Prajapati ND, Kumar T (2006). A handbook of medicinal plants: a complete source book. Vol. 2. Jodhpur: Agrobios (India). Available at: https://agris.fao.org/agris-search/search.do?recordID=XF2015047664
Roy S, Rao K, Bhuvaneswari CH, Giri A, Mangamoori LN (2010). Phytochemical analysis of *Andrographis paniculata* extract and its antimicrobial activity. World Journal of Microbiology and Biotechnology 26(1):85.
Sharma P (1983). Charaka Samhita Vol. 2. Chaukhamba Orientalia. Available at: https://www.narayana-verlag.de/homeopathy/pdf/07926_3Contents_Volume2.pdf
Sheeja K, Shihab PK, Kuttan G (2006). Antioxidant and anti-inflammatory activities of the plant *Andrographis paniculata* Nees. Immunopharmacology and Immunotoxicology 28(1):129-140.
Singha PK, Roy S, Dey S (2003). Antimicrobial activity of Andrographis paniculata. Fitoterapia 74(7-8):692-694.
Singha PK, Roy S, Dey S (2007). Protective activity of andrographolide and arabinogalactan proteins from *Andrographis paniculata* Nees. against ethanol-induced toxicity in mice. Journal of Ethnopharmacology 111(1):13-21.
Subhan N, Alam MA, Ahmed F, Awal MA, Nahar L, Sarker SD (2008). In vitro antioxidant property of the extract of *Excoecaria agallocha* (Euphorbiaceae). DARU-Journal of Faculty of Pharmacy 16(3):149-154.
Tatiya AU, Surana SJ, Sutar MP, Gamit NH (2012). Hepatoprotective effect of poly herbal formulation against various hepatotoxic agents in rats. Pharmacognosy Research 4(1):50.
Uddin MG, Hossain MS, Rahman MA, Uddin AHMM, Bhuiyan MS (2017). Elemental zinc Is inversely associated with C-reactive protein and oxidative stress in chronic liver disease. Biological Trace Element Research 178(2):189-193.
Uddin MG, Islam MM, Ahmed A, Siddiqui SA, Debnath A (2020). Evaluation of anthelmintic, antioxidant and anti-inflammatory potential of methanolic extract of *Artocarpus lacucha* leaves. Discovery Phytomedicine 7(1):27-32.
Valdiani A, Talei D, Tan SG, Kadir MA, Maziah M, Rafii MY, Sagineedu SR (2014). A classical genetic solution to enhance the biosynthesis of anticancer phytochemicals in *Andrographis paniculata* Nees. PLoS One 9(2):e87034.
Welz AN, Emberger-Klein A, Menrad K (2018). Why people use herbal medicine: Insights from a focus-group study in Germany. BMC Complementary and Alternative Medicine 18(1):92.
WHO (2011). Quality control methods for herbal materials. Available at: ps://apps.who.int/iris/bitstream/handle/10665/44479/9789241500739_eng.pdf?sequence=1&isAllowed=y
Wolfe K, Wu X, Liu RH (2003). Antioxidant activity of apple peels. Journal of Agricultural and Food Chemestry 51(3):609-614.