Reprint **ISSN 1997-2571 (Web Version)**

Journal of Innovation & Development Strategy (JIDS)

(J. Innov. Dev. Strategy)

Volume: 10	Issue: 3	December 2016

J. Innov. Dev. Strategy 10(3): 11-17 (December 2016)

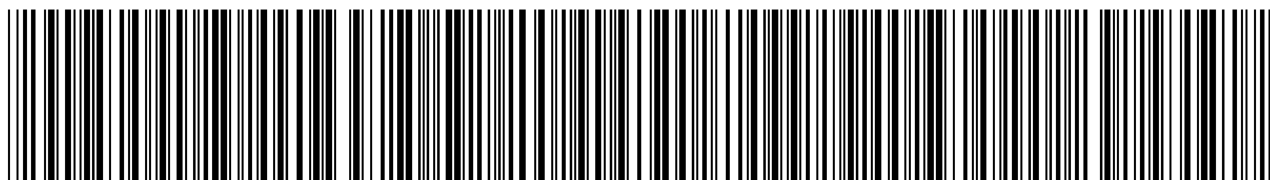
STUDY ON THE ANTIOXIDANT ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *Abroma augusta* LEAVES

M.M. UDDIN, D. TALUKDER, A.B.M.H. HAQUE, I. JAHAN AND N. AKTER

An International Scientific Research Publisher

Green Global Foundation©

Web address: http://ggfjournals.com/e-journals archive
E-mails: editor@ggfjournals.com and editor.int.correspondence@ggfjournals.com



ISSN-1997-2571 (Online)
J. Innov. Dev. Strategy 10(3):11-17(December 2016)

STUDY ON THE ANTIOXIDANT ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *Abroma augusta* LEAVES

M.M. UDDIN[1], D. TALUKDER[1*], A.B.M.H. HAQUE[2], I. JAHAN[2] AND N. AKTER[1]

[1]BCSIR Laboratories, Rajshahi, Rajshahi-6206, Banglasesh;
[2]Department of Chemistry, University of Rajshahi, Rajshahi-6205, Bangladesh.

*Corresponding author & address: Debashish Talukder, E-mail: dtbcsir12@gmail.com
Accepted for publication on 28 November 2016

ABSTRACT

Uddin MM, Talukder D, Haque ABMH, Jahan I, Akter N (2016) Study on the antioxidant activity of different solvent extracts of *Abroma augusta* leaves. *J. Innov. Dev. Strategy.* 10(3), 11-17.

To evaluate the antioxidant activity of *Abroma augusta* leaves, the different fractions of methanolic extracts were taken to determine the total phenolic content, total flavonoid content, reducing power capacity and free radical scavenging activity. Among the fractions, the highest phenolic content was found in ethyl acetate fraction (0.466 ± 0.001 mg gallic acid/g of extract). In chloroform (CF), petroleum ether (PEF) and dia-ion resin absorb fractions (DIRAF) the values were found 0.196 ± 0.001, 0.112 ± 0.001 and 0.111 ± 0.001 mg gallic acid/g of extract respectively followed by methanol fraction 0.255 ± 0.001 mg gallic acid/g of extract. The order of total flavonoid content of different fractions were DIRAF (3.06 ± 0.010) > PEF (2.15 ± 0.001) > CF (0.878 ± 0.001) followed by methanol fraction (1.17 ± 0.001) mg catechin/g of extract. The reducing power capacity exhibited the order of EAF (1.363 ± 0.010) > CLF (0.182 ± 0.014) > PEF (0.065 ± 0.007) > DIRAF (0.047 ± 0.004) nm at 100 mg /ml followed by ascorbic acid standard (3.674 ± 0.008) nm at 100 µg/ml concentration. The total antioxidant activity of the extractives showed the order of EAF (1.935 ± 0.007) > PEF (1.776 ± 0.008) > CLF (0.414 ± 0.007) > DIRAF (0.094 ± 0.012) nm at 100 µg/ml. In case of DPPH radical scavenging activity, DIRAF showed the highest (45.71) µg/ml and EAF showed the lowest (191.17) µg/ml with IC_{50} followed by BHT standard and the order was: BHT > DIRAF > CLF > PEF >EAF.

Key words: *Abroma augusta, antioxidant, flavonoid, phenolic compound, reducing power capacity, free radical scavenging*

INTRODUCTION

Abroma augusta Linn is commonly known as "Ulotkombal" which is used as a well-known remedy for the treatment of various types of diseases. The root parts are traditionally used as a uterine tonic, dysmenorrhea, sterility and menstrual disorder. Leaves are used in treating rheumatic pain of joints, headache with sinusitis, diabetes, uterine disorder and many other health protective agents. Extracts of fresh leaves and stem in cold water is very efficacious in gonorrhea.

The experimental study showed that the methanolic extracts are effective in diabetic rats at a dose of 300 mg/Kg body weight (Mishra *et al.* 2010). The ethanolic extract of the roots of *Abroma augusta* also exhibited the hypoglycemic effect in alloxan 100 mg/Kg induced diabatic rats (Rao *et al.* 2010; Kar *et al*. 2003). *Abroma augusta* also effective in combined doses for the treatment of diabetes (Halim 2003).

The methanolic extract of *Abroma augusta* showed the strongest antioxidant activity and combination with *C. longa* also possess antioxidant activity by inhibiting thiobarbuteric acid reactive substances (TBARS) and increases in reduced glutathione (GHS), superoxide dismustase (SOD) and catalase (CAT) A. (Farhana *et al.* 2009). The petroleum extracts of the roots of *Abroma augusta* is used for its anti-inflammatory activity (Mishra *et al.* 2008). Antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (Soares *et al.* 1997). In this respect, polyphenolic compounds, like flavinoids and phenolic acids commonly found in plants have been reported to have multiple biological effects, including antioxidants activity (Brown and Rice-Evans, 1998). Flavinoids and phenolic compound have also been reported to be associated with antioxidant effects in biological systems acting as scavengers of singlet oxygen and free radicals (Jorgensen *et al.* 1999; Rice-Evans *et al.* 1997). Hance an effort has been made here to investigate the different fraction of methanolic extracts of *Abroma augusta* leaves for its antioxidant activity.

MATERIALS AND METHODS

The experiment was carried out in BCSIR Laboratories, Rajshahi, Bangladesh during the period from March, 2015 to December 2015.

Expermental materials

Abroma augusta leaves were collected from Bonpara nursery, Natore, Bangladesh.

The main chemicals used in the study were – Flocin- ciocaltue reagent, gallic acid (reagent grade) and 7.5% sodium carbonate for determining the total phenolic content; aluminium chloride($AlCl_3$), potassium acetate, methanol and catechin for total flavonoid; potassium ferricianide [$K_3Fe(CN)_6$], trichloro acetic acid, ferric chloride, ascorbic acid (reagent grade) for reducing power capacity; concentrated sulfuric acid (98%), sodium phosphate (Na_3PO_4), ammonium molybdate, ascorbic acid, methanol for total antioxidant and DPPH-(1,1-diphenyl-2-picrylhydrazyl radical), methanol, butylated hydroxyl toluene (BHT-reagent grade) for DPPH radical scavenging activity respectively. In all cases, the absorbance of the solution was measured by UV-spectro photometer (Shimadzu).

Copyright© 2016 Green Global Foundation
www.ggfjournals.com

Process of extraction

The collected *A. augusta* leaves were washed thoroughly in water, chopped, air dried for a week at 35-40^{0}C and pulverized in electric grinder. Dried ground leaves were exhaustively extracted with methanol (MeOH, analytical grade) in soxhlets apparatus. The resulting juicy extract was filtered and concentrated to obtain a crude residue (23.5%) by using the Buchi Rotavapor R-200. The process was repeated for several times to increase the crude extract. Then sufficient water was added to the crude residue and water triturate part was collected from crude extract. The water triturate fraction was passed through a previously well packed dia-ion resin column which had selectivity to 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.

Determination of total phenolic content

Total phenolic compounds of different fractions of *A. augusta* leaves were determined employing the method as described by Singleton and Rossi, (1965) involving 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 against blank. The total content of phenolic compounds in plant methanol extract and in different fractionates in gallic acid equivalents (GAE) was calculated by the formula

$$C = (c \times V) / m$$

Where

C = Total content of phenolic compounds mg/g extract in GAE
c = the concentration of Gallic acid
V = the volume of extract, ml
m = the weight of different plant extracts, g

Determination of total flavonoids

The content of total flavonoids in fractionates of plant extracts was determined by the well-known aluminium chloride colorimetric method. In this method, aluminium chloride complex with groups of flavonoid presents in the sample. The complex has the maximum absorbance at 420 nm. The total content of flavonoid compounds in plant extracts in catechin equivalents was calculated by the following formula

$$C = (c \times V) / m$$

Where

C = total content of flavonoid compounds, mg/g plant extract in catechin equivalent (Cat.E)
c = the concentration of Catechin established from the curve, mg/ml
V = the volume of extract, ml
m = the weight of pure plant extracts, g

Determination of reducing power capacity

The reducing power of different extract of *A. augusta* was evaluated by the method of Oyaizu (1986) using potassium ferricianide [$K_3Fe(CN)_6$], (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 reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substances in the samples causes the reduction of Fe^{3+}- ferricianide complex to the ferrous form by donating an electron. The amount of Fe^{2+}- ferricianide complex can then be monitored by measuring the formulation of Perl's Prussiun blue at 700 nm.

Fe^{3+}- ferricianide + e^- Fe^{2+}- ferrocianide

Increased absorbance of the reaction mixture indicated increased reducing power.

Determination of total antioxidant activity

Total antioxidant activity of different extractives of *A. augusta* were determined by the method of Prieto *et al.* (1999) with some modifications. The phospho molybdenum 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 extract solution, incubated at 95°C for 10 minutes to complete the reaction and measured the absorbance of the solution at 695 nm.

Determination of DPPH radical scavenging activity

The 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) has been widely used to evaluate the free radical scavenging capacity of antioxidants (Choi *et al.* 2000). With this method dia antiradical power of antioxidant activity of different extracts of *A. augusta* was determined by measurement of the decrease in the absorbance of DPPH at 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH was

scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. Butylated hydroxy toluene (BHT, Reagent grade) was added to the solution. The percentage (%) of scavenging was calculated from the following equation

% of scavenging = {Ao- A_1/ Ao} ×100

Where,

Ao is the absorbance of the control and

A_1 is the absorbance of extract/ standard

Then % of scavenging was plotted against concentration and from the graph IC_{50} value was calculated.

RESULTS AND DISCUSSION

Total phenolic content of different fraction of *A. augusta* were determined by Folin –ciocalteu regeant as described in materials and method section and the values were shown in Table 2. Four different fractions such as petroleum ether, chloroform, etheyl acetate and dia-ion regin of same concentration (100 µg/ml) were taken and the absorbance were measursed by using UVspectrometer. Among the fractions, the highest phenolic content was found in ethyl acetate fraction (0.466 ± 0.001 mg gallic acid/g of extract) followed by methanol fraction (0.255 ± 0.001 mg gallic acid/g of extract), chloroform fraction (0.196 ± 0.001 mg gallic acid/g of extract), petroleum ether (0.112 ± 0.000 mg gallic acid/g of extract) and dia- ion resin fractions (0.111 ± 0.001 mg gallic acid/g of extract) respectively. Gallic acid was used as standard (Table 1, Fig. 1) in the assay.

Total flavonoid content were shown in Table 4 and the highest total flavonoid content was found in dia-ion resin absorbed fraction 3.06 ± 0.01 mg catechin/g of extract, while petroleum ether fraction 2.15 ± 0.006 mg catechin/g of extract, methanol fraction 1.17 ± 0.006 mg catechin/g of extract, chloroform fraction 0.878 ± 0.0006 mg catechin/g of extract and ethyl acetate fraction 0.840 ± 0.00 mg catechin/g of extract respectively. Catechin was used as standard (Table 3, Fig. 2).

The iron reducing capacity of the four different fractions of *A. augusta* were investigated and results were dipictate in Table 5. Among the four different extracts, ethyl acetate fraction showed the highest iron reducing capacity with absorbance of 1.363 ± 0.010 nm at 100 µg/ml concentration, followed by chloroform fraction with absorbance of 0.182 ± 0.014 nm while petroleum fraction showed iron reducing capacity with absorbance of 0.065 ± 0.007 nm and dia-ion resin showed 0.047 ± 0.004 nm. In this investigation ascorbic acid was used as standard.

Total antioxidant activity of different fractions of methanolic extract of *A. augusta* such as dia-ion resin absorbed fraction, chloroform fraction, ethyl acetate fraction and petroleum ether fraction were investigated (Table 6). Among the fractions, ethyl acetate fraction showed the highest total anti-oxidant activity with absorbance 1.935 ± 0.007 nm at 100 µg/ml. Petroleum ether fraction and chloroform fraction showed 1.776 ± 0.008 and 0.414 ± 0.007 nm at 100 µg/ml respectively. Dia-ion resin absorbed fraction showed the lowest total antioxidant activity with absorbance of 0.094 ± 0.012 nm at the same concentration.

DPPH radical scavenging activity of different fractions of methanolic extract was investigated and the results were shown in Table 7. Four different fractions such as dia-ion resin absorbed, chloroform, petroleum ether and ethyl acetate fractions were taken while butylated hydroxy toluene (BHT) was used as standard. Among the fractions, the highest value of IC_{50} was found in dia-ion resin absorbed fraction (45.71 µg/ml). On the other hand, CLF, PEF and EAF showed the value 88.24, 96.76 and 191.17 µg/ml respectively. From the results of Table 7, it is clear that all the extractives posses DPPH radical scavenging activity and EAF showed the lowest. From the study it is observed that, ethyl acetate fraction showed the highest activity in total phenolic content, reducing capacity and total antioxidant. On the other hand, dia-ion resin absorbed fraction showed total flavonoid and DPPH radical scavenging. Ethyl acetate and dia-ion resins are good adsorbent for separating polyphenolic compounds (Soto *et al.* 2012). Ogawa *et al.* (2008) also obtained highly purified poly phenols fractions from the seed shells of *Aesculus turbinate* using dia-ion HP-20. Yoshida *et al.* (1989) also reported that plant polyphenols inhibit scavenge DPPH free radicals. Total polyphenols are significantly negatively correlated to IC_{50} values of DPPH radicals scavenging which is established by Shan Zhao *et al.* (2011) and some other workers. Plant flavonoids are health promoting and disease preventing dietary antioxidant compounds which have been shown in numerous *in vitro* and *in vivo* experiments to have antioxidant activity (Middletone 1996).

Data for total phenolic content

Table 1. Absorbance of gallic acid at different concentrations for determination of total phenolic content

Concentration (µg/ml)	Absorbance (nm)			Absorbance (nm) Mean ± STD
	a	b	c	
10	0.521	0.519	0.522	0.521±0.002
15	1.435	1.433	1.436	1.435±0.002
20	1.782	1.780	1.784	1.782±0.002
25	2.033	2.034	2.034	2.034±0.001
30	3.025	3.025	3.027	3.026±0.001

a= $Replication_1$, b= $Repliction_2$ and c= $Replication_3$

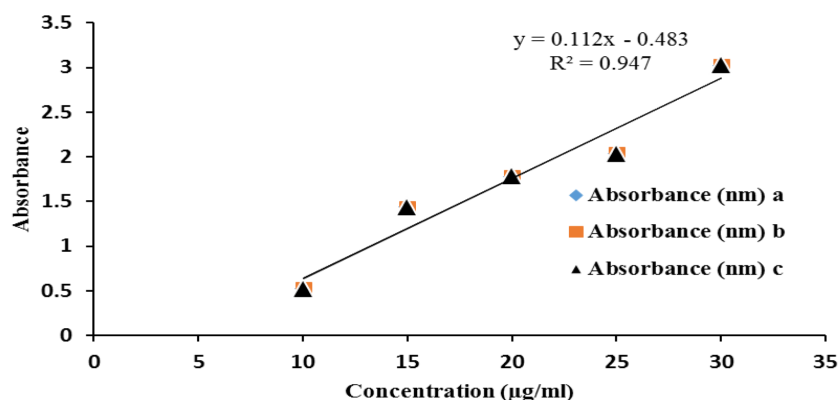


Fig. 1. Standard curve of gallic acid for the determination of total Phenolic content

Table 2. Determination of total phenolic content of different fractions of methanolic extract of *Abroma augusta*

Sample	No. of sample	Concentration (µg/ml)	Absorbance (nm)	GAE/gm of dried sample	GAE/gm of dried sample Mean ± STD
Methanol fraction	1	100	2.372	0.255	0.255 ± 0.001
	2	100	2.370	0.254	
	3	100	2.374	0.255	
Petroleum ether fraction	1	100	0.778	0.112	0.112 ± 0.000
	2	100	0.778	0.112	
	3	100	0.778	0.112	
Chloroform fraction	1	100	1.710	0.196	0.196 ± 0.001
	2	100	1.711	0.196	
	3	100	1.712	0.197	
Ethyl acetate fraction	1	100	4.742	0.466	0.466 ± 0.001
	2	100	4.742	0.466	
	3	100	4.743	0.467	
Dia-ion resin adsorbed fraction	1	100	0.750	0.111	0.111± 0.001
	2	100	0.754	0.112	
	3	100	0.752	0.111	

Data for total flavonoid content

Table 3. Absorbance of catechin at different concentration for the determination of total flavonoids

Concentration (µg/ml)	Absorbance (nm)			Absorbance (nm) Mean ± STD
	a	b	c	
50	0.022	0.019	0.019	0.019 ±0.000
100	0.043	0.044	0.043	0.043 ± 0.001
150	0.156	0.158	0.157	0.157 ±0.002
200	0.269	0.269	0.267	0.268 ±0.001
250	0.363	0.365	0.365	0.364 ±0.002

a= Replication_1, b= Repliction_2 and c= Replication_3

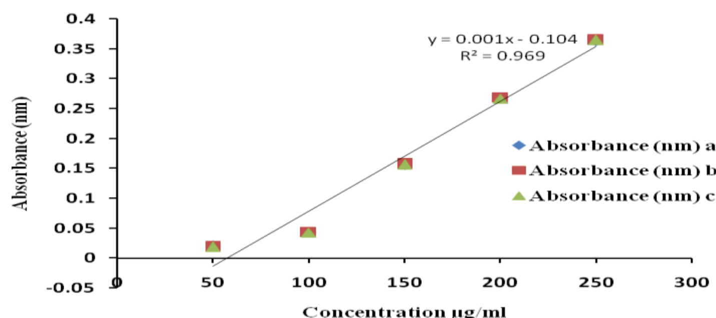


Fig. 2. Standard curve of catechin for the determination of total flavonoids

Table 4. Determination of total flavonoid content of different fractions of methanolic extract of *Abroma augusta*

Sample	No. of sample	Concentration (µg/ml)	Absorbance (nm)	Cat.E/g of dried sample	Cat.E/g of dried sample Mean ± STD
Methanol fraction	1	100	0.105	1.16	1.17 ± 0.006
	2	100	0.107	1.17	
	3	100	0.107	1.17	
Petroleum ether fraction	1	100	0.282	2.15	2.15 ± 0.006
	2	100	0.282	2.15	
	3	100	0.284	2.16	
Chloroform fraction	1	100	0.054	0.879	0.878 ± 0.006
	2	100	0.056	0.880	
	3	100	0.052	0.877	
Ethyl acetate fraction	1	100	0.047	0.840	0.840 ± 0.00
	2	100	0.047	0.840	
	3	100	0.447	0.840	
Dia-ion resin adsorbed fraction	1	100	0.446	3.06	3.06± 0.01
	2	100	0.447	3.07	
	3	100	0.445	3.05	

Data for reducing power capacity content

Table 5. Reducing power capacity of different fractions of methanolic extract of *Abroma augusta* and ascorbic acid (standard) at different concentrations

Name of sample	Concentration (µg/ml)	Absorbance (nm)			Absorbance (nm) Mean ± STD
		a	b	c	
ascorbic acid (standard)	20	0.1903	0.1901	0.1904	0.1903±0.0002
	40	1.926	1.921	1.930	1.925±0.007
	60	2.194	2.190	2.192	2.192±0.106
	80	2.604	2.610	2.612	2.608±0.001
	100	3.678	3.670	3.675	3.674±0.008
Chloroform fraction	20	0.002	0.002	0.002	0.002±0.004
	40	0.0004	0.0038	0.0044	0.0041±0.007
	60	0.016	0.0156	0.0163	0.0159±0.012
	80	0.0250	0.025	0.025	0.025±0.012
	100	0.182	0.183	0.182	0.182±0.014
Petroleum ether fraction	20	0.006	0.006	0.006	0.006±0.004
	40	0.010	0.012	0.012	0.011±0.003
	60	0.022	0.023	0.023	0.023±0.005
	80	0.031	0.033	0.033	0.033±0.011
	100	0.065	0.065	0.064	0.065±0.007
Ethyl acetate fraction	20	0.192	0.199	0.196	0.196±0.004
	40	0.400	0.403	0.410	0.404±0.003
	60	0.568	0.560	0.566	0.565±0.010
	80	0.992	0.991	0.995	0.993±0.002
	100	1.360	1.365	1.362	1.353±0.010
Dia-ion resin absorbed fraction	20	0.014	0.012	0.012	0.013±0.005
	40	0.023	0.022	0.023	0.023±0.006
	60	0.032	0.031	0.031	0.031±0.004
	80	0.044	0.043	0.042	0.043±0.007
	100	0.046	0.047	0.048	0.047±0.004

a= $Replication_1$, b= $Repliction_2$ and c= $Replication_3$

Data for total antioxidant activity

Table 6. Total antioxidant activity of different fractions of *Abroma augusta* and ascorbic acid (standard) at different concentrations

Name of sample	Concentration (µg/ml)	Absorbance (nm)			Absorbance (nm) Mean ± STD
		a	b	c	
Ascorbic acid (standard)	20	0.735	0.736	0.730	0.734±0.008
	40	1.355	1.351	1.355	1.354±0.024
	60	1.684	1.680	1.681	1.682±0.039
	80	1.928	1.928	1.928	1.928±0.036
	100	3.192	3.202	3.200	3.198±0.106
Petroleum ether fraction	20	0.172	0.167	0.181	0.173±0.007
	40	0.207	0.271	0.210	0.211±0.004
	60	0.287	0.291	0.288	0.288±0.008
	80	1.581	1.578	1.581	1.579±0.007
	100	1.781	1.777	1.770	1.776±0.008
Ethyl acetate fraction	20	0.006	0.0065	0.0056	0.0057±0.005
	40	0.010	0.012	0.014	0.012±0.007
	60	0.400	0.411	0.407	0.406±0.008
	80	1.188	1.172	1.180	1.170±0.005
	100	1.943	1.930	1.1932	1.935±0.007
Chloroform fraction	20	0.294	0.283	0.298	0.289±0.007
	40	0.023	0.025	0.029	0.026±0.003
	60	0.098	0.092	0.099	0.098±0.010
	80	0.277	0288	0.279	0.281±0.004
	100	0.412	0.420	0.410	0.414±0.007
Dia-ion resin absorbed fraction	20	0.338	0.334	0.341	0.338±0.004
	40	0.024	0.019	0.020	0.024±0.007
	60	0.042	0.427	0.426	0.432±0.005
	80	0.067	0.060	0.061	0.063±0.007
	100	0.096	0.097	0.990	0.094±0.012

a= $Replication_1$, b= $Repliction_2$ and c= $Replication_3$

Data for DPPH Radical Scavenging Activity

Table 7. DPPH radial scavenging activity of different fraction of methanolic extract of *Abroma augusta* and BHT (standard) at different concentrations

Name of sample	Concentration (µg/ml)	% of scavenging			Absorbance (nm) Mean ± STD	IC_{50} (µg/ml)
		a	b	c		
BHT (standard)	25	36.45	36.37	36.71	36.51±0.18	37.5
	50	63.69	63.97	63.67	63.77±0.17	
	100	88.50	88.73	88.92	88.71±0.21	
	150	95.83	95.89	95.65	95.79±0.12	
	200	96.35	96.57	96.27	96.39±0.15	
Chloroform fraction	25	26.07	26.34	26.22	26.21±0.14	88.24
	50	39.02	39.54	39.10	39.22±0.28	
	100	53.24	53.84	53.09	53.39±0.40	
	150	68.09	68.03	68.50	68.20±0.26	
	200	70.33	70.44	70.91	70.56±0.31	
Petroleum ether fraction	25	29.86	29.94	29.54	29.78±0.21	96.76
	50	34.28	34.13	34.82	34.41±0.36	
	100	52.03	52.29	52.48	52.26±0.22	
	150	65.45	65.64	64.97	65.35±0.34	
	200	76.30	76.12	76.21	76.21±0.09	
Ethyl acetate fraction	25	23.80	23.71	23.84	23.78±0.07	191.17
	50	31.47	31.39	31.55	31.47±0.08	
	100	46.24	46.34	46.41	46.33±0.08	
	150	49.90	49.88	48.87	49.55±0.59	
	200	50.20	50.31	50.15	50.22±0.08	
Dia-ion resin absorbed fraction	25	26.56	26.75	26.64	26.65±0.09	45.71
	50	55.84	56.04	55.96	55.95±0.10	
	100	62.35	62.52	62.64	62.50±0.14	
	150	75.21	75.47	75.64	75.44±0.22	
	200	82.39	83.12	82.89	82.80±0.37	

a= $Replication_1$, b= $Repliction_2$ and c= $Replication_3$

CONCLUSION

From the present study, it can be concluded that *Abroma augusta* leaves are rich in polyphenols and flavonoids which were successfully extracted with ethyl acetate and dia-ion resin, might be helpful in preventing various oxidative stress related diseases. The present work also provided the evidance for presence of bioactive compounds in *Abroma augusta* leaves. Hence, it may be expected that the present stludy will stimulate the further work of indentification and isolation of bioactive compounds which are responsible for the antioxidant activity.

ACKNOWLEDGEMENT

The authors express their gratitude to Mrs. Aeysha Akter, Director (in-charge), BCSIR Laboratories, Rajshahi, Bangladesh for providing laboratory facilities to carry out the research work.

REFERENCES

Brown JE, Rice-Evans CA (1998) Luteolin-rich artichoke extract protects low-density lipoprotein from oxidation *in vitro Free RidicalResearch*, 29(3), 247-255.

Choi YJ, Kim SK, Kim SH, Lee KS, Choi KY (2000) *Saccharomyces cerevisiae* Ste5 is important for induction and substrate specificity of Fus3 MAP kinase in the pheromone signaling pathway. *Mol Cells* 10(3), 301-308.

Farhana M, Hussain I, Haroon TS (2009) Hepatitis C: the dermatologic profile *J. Pak. Assoc. Derm.* 18: 171-181.

Halim E (2003) Effect of *Coccinia indica* L. and *Abroma augusta* L. on glycemia, lipid profile and on indication of end organ damage in streprozoticin induced diabetic rats. *Indian Journal of chemical Biochemistry.* 18(2), 54-63.

Jorgensen K, Kiebler T, Hylander I, Vermehren C (1999) Interaction of a lipid-membrane destabilizing enzyme with PEG-liposomes. *Int. J. Pharm*. 183, 21-24.

Kar A, Choudhary BK, Bandyopadhyay NG (2003) Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. *J. Ethnopharmacol*., 84, 105-108.

Middleton E (1996) Biological properties of plant flavonoid: an overview Int. *J. Pharmacognosy.* 34: 344-348.

Mishra M, Gosh G, Mishra D, Bascik B (2008) Advance in pharmacology and toxicology. 9(3), 147-150.

Mishra SB, Rao CV, Ojha SK, Vijayakumar M, Verma A (2010) An analytical review of plants for antidiabetic activity with their phytoconstituents & mechanism of action. IJPSR 1(1), 30-48.

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. *J.Agric. Food Chem*. 56.12046-12051.

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

Prieto P, Pineda N, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E, Anal-Biochem.269:, 337-341.

Rao L, Atmakuri, Suneetha Dathi (2010) Current Trends in Herbal Medicines. *Journal of Pharmacy Research,* 3(1), 109-113.

Rice-Evans CA, Sampson J, Bramley PM, Holloway DE (1997) Why do we expect carotenoids to be antioxidants in vivo? Free Rad. Res. 26: 381-398.

Shan Zhao, Lie Yan Liu, Si Yu Chen, Ling Ling Shi, Yu Juu Liu, Chao Ma (2011) Antioxidant potential of polyphenols and Tannins from Burs of castenea mollissima Blume. Molecules.16, 8590-8600.

Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am J Enol Viticult* 16: 144-158.

Soares JR, Dinis TCP, Cunha AP, Almeida LM (1997) Antioxidant Activities of some Extracts of *Thymnus zygis. Free Radical Research,* 26: 469-478.

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.

Yoshida T, Morik, Hatauo T, Okomura T, Uehara I, Kamagoe K, Fujita Y, Okuda T (1989) Studies on inhibition mechanism of autooxidation by tannins and flavonoids.Radical scavenging effects of tannins and related polyphenols on 1,1-diphenyl-2-picrylhydrazyl radical. Chem. Pharma. Bull. 37(7), 1919-1921.