CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
http://doi.org/10.5281/zenodo.5112880

Online at: http://www.iajps.com *Research Article*

PHYTOCHEMICAL SCREENING AND IN VITRO ANTIOXIDANT ACTIVITY OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF MUSA ACUMINATA LEAVES

Ch. Madhu*, Vamaraju Upajna, Kontheti Mahesh Pavan, Burreddy Navya Deepika
Avanthi Institute of Pharmaceutical Sciences, Cherukupally, Vizianagaram, Andhra Pradesh, India.

Article Received: June 2021 **Accepted:** June 2021 **Published:** July 2021

Abstract:
This study describes the in-vitro antioxidant activity of aqueous and hydroalcoholic extract of the plant of Musa Acuminata leaves belonging to the family-Musaceace. Folin ciocalteau assaywas used to determine the total Phenolic content while aluminum chloride colorimetric method was used to determine the total flavonoid content. Some of the classical methods to estimate in-vitro antioxidant activity were the Hydrogen peroxide scavenging assay, superoxide radicals scavenging activity, nitric oxide radical scavenging assay, reducing antioxidant power assay, and hydroxyl radical scavenging activity. Less phenolic concentration was identified in aqueous extract (51.01 mg/g of extract) while more phenolic concentration was found in hydroalcoholic extract (103.4 mg/g of extract) which is similar to the case of flavonoid content. Concentration of the content played a major role in determining the reducing power activity and hydrogen peroxide scavenging assay. From the above results, the hydroalcoholic extract of Musa Acuminata shows a significant activity when compared to aqueous extract.

***Key Words**: Musa acuminata., flavonoids, phenols, antioxidant activity, reducing power assay, Hydrogen peroxide scavenging assay.*

Corresponding author:
Chandaka madhu,
Asst. professor,
Dept of Pharmacology,
Avanthi Institute of Pharmaceutical Sciences,
Email id: pharmamadhuphd@gmail.com

QR code

Please cite this article in press Chandaka Madhu et al., ***Phytochemical Screening And In Vitro Antioxidant Activity Of Aqueous And Hydroalcoholic Extract Of Musa Acuminata Leaves****.., Indo Am. J. P. Sci, 2021; 08(07).*

INTRODUCTION:

All the human beings and many of the creatures require oxygen to live. Mitochondria in the cells of living beings is responsible for the production of adenosine triphosphate (ATP) during which free radicals are generated as byproducts. Tissue damage occurs due to oxidative stress at higher concentration of these free radicals which may produce many deleterious effects. As a result, intensive research is being carried out on this aspect since decades. It was found that there are substances called 'antioxidants' which protect the tissues from these harmful effects of the free radicals.

A free radical is a molecule, an ion or an atom that has an unpaired valence electron. These free radicals are highly reactive and immediately initiate chain reaction [1]. Free radicals can be produced from endogenous and exogenous sources. Endogenous free radicals are generated from immune cell activation, auto-oxidation, inflammation whereas exogenous free radicals are generated from external sources such as tobacco smoke, air pollutants, industrial chemicals and organic solvents. Most of the cell organelles are responsible for the production of these radicals which include mitochondria, endoplasmic reticulum, cell membrane, lysosomes and peroxisomes [2]. Subjection to radiation such as x-rays and certain medications which produce free radicals as metabolic byproducts are also responsible for the tissue damage[3].

Musa acuminata., also referred to asKutkihas been used traditionally in the Ayurvedic system of medicine for centuries as a remedy to improve thinking and retaining capacity of the brain and for enhancing concentration and learning capability [4]. It is also being used as an anti-anxiety and anti-epileptic agents [5]. It is also been used in the cure of problems related to heart, gastrointestinal tract and lungs in the countries of India and Pakistan [6]. Advances in the research paid attention regarding the use of Picrorhizain boosting the cognitive activity, its anti-anxiety and anti-epileptic properties, its effectiveness in treating gastric ulcers, and other GIT related problems. The antioxidant property of Picrorhiza gives protection from oxidative damage to the tissues due to free radicals in cardio vascular diseases and few types of malignancies.

METHODS AND MATERIALS:

COLLECTION OF PLANT MATERIAL:

The plant material Musa Acuminata leaves was collected in the month of August 2020 from Tagarapuvalasa,

Process of extraction:

The plant Musa acuminata leaves was collected, shade dried and made in to a coarse powder which is divided in to two equal halves (1:1). One part was subjected to aqueous extract and the another part was subjected to hydroalcoholic extract (70:30% v/v of water and alcohol). The process of excration selected was maceration for 7 days with regular interval of stirring. After 7 days the extract was filtered and the filterate was subjected to freeze drier. The residue was obtained. The percentage yield was calculated.

PHYTOCHEMICAL EVALUATION:

DETERMINATION OF TOTAL PHENOLIC CONTENT: [7]

Folin ciocalteau method was used to determine the total phenolic content of the extract which uses Gallic acid as standard. This principle was according to Singleton and Rossi. 0.1ml (100 µg) of sample solution was taken in 3ml of distilled water in which 0.5ml of Folin ciocalteau reagent was added and mixed thoroughly. It was then incubated for 3min at room temperature. After incubation, 3ml of 20% sodium carbonate was added and mixed cautiously. It was again incubated in boiling water bath for about 1 min. The absorbance was measured at a wavelength of 650nm. The total phenol concentration was expressed in terms of milli grams of Gallic Acid equivalents per gram of extract.

DETERMINATION OF TOTAL FLAVANOID CONTENT: [8]

Aluminum chloride assay was used to determine the total Flavonoid content. About 1ml of the standard solution of catechin (20, 40, 60, 80 and 100µg/ml) was added to 4ml of distilled water in a 10ml volumetric flask. To this mixture, 0.3ml 5% sodium nitrate was added. After 5 min, 0.3 ml 10% Aluminum chloride was added. At the 6th min, 2 ml of 1M sodium hydroxide was added and the total volume was made up to 10 ml with distilled water and was mixed thoroughly. The absorbance was measured at a wavelength of 510nm against prepared against the blank solution. Total flavonoid content was expressed in terms of milli grams of catechin equivalents per gram of extract.

IN VITRO ANTIOXIDANT ACTIVITY:

1) FERRIC REDUCING POWER ASSAY: [9]

Kenichi Oyaizu determined the process of the reducing power assay. In this assay, Ascorbic acid was used as standard. Concentrations of 50, 100 ,150 ,200, 250 µg/mlmethanol were used. They were

mixed with 2.5ml of 0.2Mphosphate buffer with pH 6.6 and 2.5ml of 1% Potassium ferric cyanide. The mixture was incubated at a temperature of 50°C for about 20 minutes. After incubation 2.5ml of 10% trichloroaceticacid was added to the mixture.Then this mixture was centrifuged at 3000rpm for about 10minutes. The top layer of the mixture was added to the combination of 2.5ml distilled water and 0.5ml of 1% Ferric chloride.The absorbance was measured at a wavelength of 700nm. Absorbance of the reaction mixture is directly proportional to the reducing power.

2) HYDROGEN PEROXIDE SCAVENGING ACTIVITY: [10]

Ruch et al determined the method of the hydrogen peroxide scavenging activity of the extract. Hydrogen peroxide solution was prepared using phosphate buffer of pH 7.4. Concentrations of 100, 200,300,400,500 µg/ml of the extract with 3.4ml of buffer were mixed with 0.6ml of 40mM hydrogen peroxide solution. The absorbance was measuredat a wavelength of 230nm. The percentage of scavenging activity of hydrogen peroxide was calculated by using the following equation:

% Of scavenging = [(A of control – A of sample) / A of Control] X 100

Where, A of control = absorbance of the control reaction (containing all reagents except test compound) and a sample is the absorbance of the test compound. Test was carried out in triplicate.

3) NITRIC OXIDE SCAVENGING ACTIVITY: [11]

Garret determined the method of Nitric oxide radical scavenging activity. The aqueous solution of sodium nitroprusside generates nitric oxide at physiological pH. Griess Illosvoy reaction demonstrates the reacting ability of nitric oxide with that of oxygen to generate nitrate ions.2 ml of 10 mM sodium nitroprusside and 0.5 ml phosphate buffer saline of pH 7.4were added to 0.5 ml of extract at different concentrations and it was incubated at a temperature of 25°C for 150 minutes. 0.5 ml of the incubated mixture was removed and added to 1ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and was again incubated at room temperature for about 5 minutes. Lastly, 1ml of naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 minutes.Then absorbance was measured at a wavelength of 540 nm using a spectrophotometer to calculate the nitric oxide radicals scavenging activity.

The percentage inhibition of nitric oxide radicals scavenging activity was calculated according by using the following equation:

% Inhibition = $[(A_0-A_1) / A_0] \times 100$)

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

4.) SUPEROXIDE RADICAL SCAVENGING ACTIVITY: [12]

To the mixture of 0.1 ml of NBT and 0.3 ml of the extract, 1 ml of alkaline dimethyl sulfoxide (DMSO) (1 ml DMSO is made up of 5 mM NaOH in 0.1 ml water) was added and the final volume of 1.4 ml was obtained. The absorbance was measured at a wavelength of 560 nm.

% inhibition = Absorbance of Control – Absorbance of Test/ Standard X 100 Absorbance of Control

5) HYDROXYL RADICAL SCAVENGING ACTIVITY: [13]

Fenton reaction yielded deoxyribose which was degenerated using spectrophotometric method. This was carried out both in the presence and absence of the test compound. 0.3 mlof Deoxyribose (30 mM), 0.3ml of ferric chloride (1mM) , 0.3ml of EDTA (1mM), 0.3ml of Hydrogen peroxide (20mM) are present in the final reaction mixture in the phosphate buffer having pH 7.4 and 0.3 ml of test compound at various concentrations. They were then incubated at a temperature of 37°C for about 30 minutes. After incubation, 0.5ml of 5% trichloro acetic acid and 0.5ml of 1% thiobarbituric acid were added to this mixture and was placed in boiling water bath for 30 minutes. The mixture was then cooled and the absorbance was measured at a wavelength of 532 nm.

6) LIPID PEROXIDATION INHIBITING ACTIVITY: [14]

To 0.1ml of rat liver homogenate (25% w/v) in tris-Hcl buffer (40 mM, pH 7.0), 0.1ml each of KCL (30 mM), ascorbic acid (0.06mM) and ferrous ion (0.16 mM) and various quantities of extracts and ascorbic acid were incubated for 1 hour at a temperature of 37°c. The treatment of the obtained mixture with sodium dodecyl sulfate (0.2 ml, 8.1%), thiobarbituric acid (1.5 ml, 0.8%) and acetic acid (1.5 ml, 20%, pH 3.5) was a key step. Final volume of the reaction mixture was made up to 4ml with the help of distilled water and was placed in oil bath at a temperature of 100°c for about 60 minutes. Then cool the mixture. Finally, add 1 ml distilled water and 5 ml of butanol-pyridine mixture (15:1 v/v). centrifuge the tubes after shaking them vigorously. Measure the absorbance of chromophore containing organic layer at a wavelength of 532nm.

RESULTS AND DISCUSSIONS:

Table: Percentage yeild

S.No	Aqueous extract	Hydro alcoholic extract
Percentage yield of extract	7.81	11.6

PRELIMINARY PHYTOCHEMICAL SCREENING

Table-1: Data showing the preliminary phytochemical screening of aqueous and hydro alcoholic extract of Musa acuminata.

Tests for	Aqueous extract	Hydro alcoholic extract
Carbohydrates	+	+
Glycoside	+	+
Saponins	+	+
Alkaloids	-	-
Phytosterols	-	-
Fixed Oils	+	+
Gums and Mucilage	-	-
Proteins	+	+
Phenolic compounds and Tannins	-	-
Flavonoids	+	+

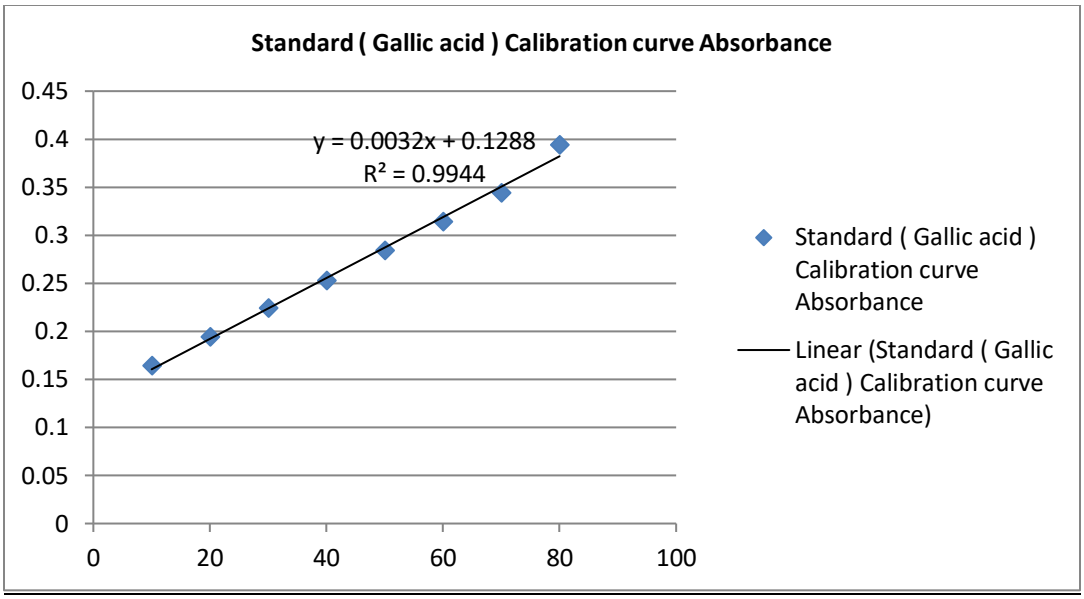
(+ = present - = absent)

ESTIMATION OF TOTAL PHENOLIC CONTENT:

Table 2: Data showing absorbance of various concentration of Gallic acid

Standard (Gallic acid) Calibration curve	
Concentration (µg/ml)	Absorbance
10	0.164
20	0.194
30	0.224
40	0.253
50	0.284
60	0.314
70	0.344
80	0.394

Sample	
Concentration (100µg/ml)	Absorbance
Aqueous Extract	0.146
Hydro alcoholic extract	0.164



Graph -1: graph depicting absorbance curve of gallic acid

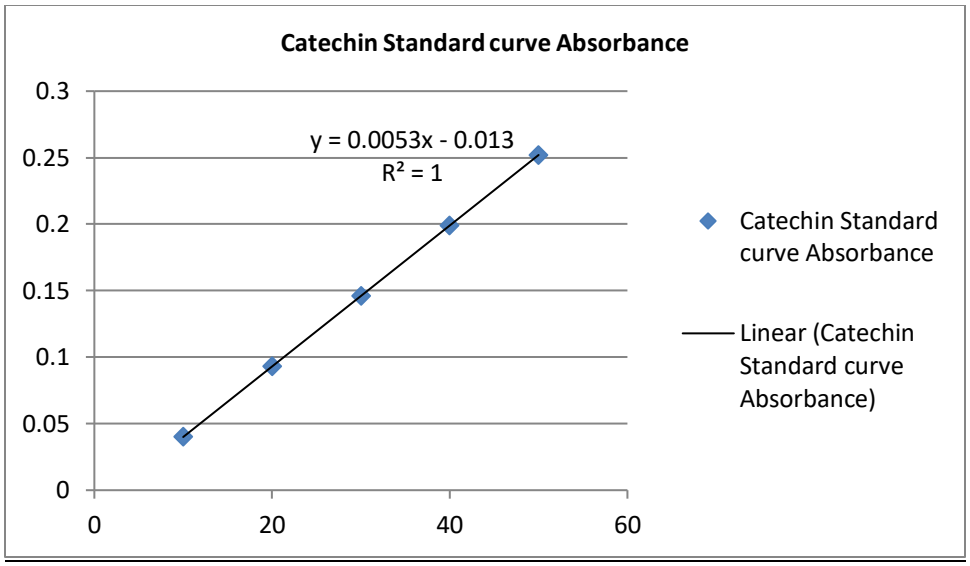
From the Standard Graph of Gallic Acid, the total phenol concentration present in the Aqueous and Hydro alcoholic extract was found to be:
Aqueous Extract: 51.01 mg GAE/ g of extract
Hydro Alcoholic Extract: 103.4 mg GAE / g of extract

TOTAL FLAVANOID CONTENT:

Table 3: Data showing absorbance of various concentration of Catechin.

Catechin Standard curve	
Concentration (µg/ml)	Absorbance
10	0.040
20	0.093
30	0.146
40	0.199
50	0.252

Sample Solution	
Aqueous Extract (100 µg/ml)	0.118
Hydro Alcoholic Extract (100 µg/ml)	0.095



Graph -2: graph showing absorbance curve of catechin

From the Standard Graph of Catechin, the total flavonoid concentration present in the Aqueous and Hydro alcoholic extract was found to be:

Aqueous Extract: 172.72 mg of CE/ g of extract

Hydro alcoholic Extract: 200.4 mg of CE / g of extract

1)FERRIC REDUCING POWER:

Table:4: data showing ferric reducing power activity

Extract	50	100	150	200	250
Ascorbic acid(std)	0.743±0.001	0.787±0.002	0.811±0.0015	0.817±0.0015	0.826±0.0025
AQUEOUS	0.143±0.0014	0.152±0.0025	0.164±0.0015	0.171±0.003	0.222±0.003
HYDROALCHLC	0.202±0.002	O.244±0.004	0.296±0.006	0.323±0.003	0.340±0.004

Results expresses as mean±sem for six observations

2) HYDROGEN PEROXIDE SCAVENGING ACTIVITY:

Table 5: data showing hydrogen peroxide scavenging activity

EXTRACT	100	200	300	400	500
ASCROBIC ACID	0.769±0.001	0.783±0.001	0.823±0.002	0.830±0.002	0.835±0.003
AQUEOUS	0.392±0.002	0.502±0.003	0.653±0.002	0.679±0.004	0.770±0.005
HYDROALCHOLIC	0.627±0.002	0.711±0.004	0.756±0.003	0.785±0.002	0.817±0.002

Results expresses as mean±sem for six observations

3) NITRIC OXIDE SCAVENGING ACTIVITY:

Table 6: data showing nitric oxide scavenging activity

EXTRACT	25	50	75	100	125	IC50(µg/ml)
Ascorbic acid	9.4±0.1	12.6±0.3	14.3±0.6	24.8±0.4	33.2±0.6	206.6
Aqueous	15.4±0.7	27.4±0.7	28.9±0.3	29.1±0.3	31.6±0.3	248.16
Hydroalcoholic	12.7±0.4	19.6±0.7	29.4±0.6	36.1±0.9	37.1±0.6	163.25

Results expresses as mean±sem for six observations

4)SUPEROXIDE RADICAL SCAVENGING ACTIVITY:

Table7: data showing superoxide radical scavenging activity

Extract	100	200	300	400	500	Ic50(µg/ml)
Ascorbic	26.49±0.6	34.82±0.6	40.38±0.6	45.94±0.6	56.36±1.39	435.14
Aqueous	23.02±0.6	30.18±0.3	32.13±0.6	35.06±0.3	37.02±0.53	887.18
Hydroalcoholic	27.88±2.5	34.36±1.0	39.46±1.74	44.32±2.23	52.97±6.27	470.83

Results expresses as mean±sem for six observations

5) HYDROXYL RADICAL SCAVENGING ACTIVITY:

Table 8: data showing hydroxyl radical scavenging activity

Extract	100	200	300	400	500	Ic50(µg/ml)
Ascorbic	30.6±0.4	35.6±2.2	41.4±0.5	46.8±0.9	55.8±1.9	433.44
Aqueous	16.4±1.26	22.6±1.26	26.6±0.6	43.4±1.2	52.68±0.4	491.18
Hydroalcoholic	24.2±0.5	25.4±0.5	30.6±1.1	44.6±1.2	55.8±1.5	470.73

Results expresses as mean±sem for six observations

6) LIPID PEROXIDATION INHIBITING ACTIVITY:

Table 9: data showing lipid peroxidation inhibiting activity

Extract	100	200	300	400	500
Ascorbic	0.134±0.001	0.144±0.004	0.190±0..3	0.237±0.002	0.285±0.002
Aqueous	0.87±0.001	0.102±0.002	0.126±0.001	0.139±0.001	0165±0.002
Hydroalcoholic	0.092±0.002	0.123±0.001	0.170±0.002	0.222±0.2	0.243±0.002

Results expresses as mean±sem for six observations

Table-1 describes about the phytochemical screening of aqueous and hydro-alcoholic extract of Musa acuminata., we can observe that both aqueous and hydroalcoholic extracts contain glycosides, carbohydrates, alkaloids, flavonoids and phenols but not proteins, tannins or steroids.

Table-2 and graph -1 depict that the aqueous extract contains 58mg GAE/g of total phenol concentration and hydroalcoholic extract contains 116.1 mg GAE/g of total phenol concentration.

Table-3 and graph-2depict that the aqueous extract contains202.8mg CE/g of total flavonoid concentration and hydroalcoholic extract contains 242.6 mg GAE/g of total flavonoid concentration.

Table:4 describes that the reducing power plays a vital role in depicting the antioxidant capacity of the plant extract. The reducing power of Aqueous and Hydroalcoholic extract of Musa acuminata were majorly dose-dependent. The absorbance is directly proportional to the concentration. From Table-3, we can infer that reducing activity of hydroalcoholic extract was more when compared to the aqueous extract.

Table-5 describes the hydrogen peroxide scavenging activity. The decomposition activity of hydrogen peroxide results from its antioxidant and free radical scavenging activity.

Table-6 describes the nitric oxide scavenging activity. IC_{50} value of ascorbic acid is 206.6 µg/ml. The IC_{50}value of Aqueous and Hydroalcoholic extract of Musa acuminata are 248.16 µg/ml and 163.25 µg/ml respectively.

Table-7 describes the superoxide radicals scavenging activity. IC_{50} value of ascorbic acid is 435.14 µg/ml. The IC_{50}value of Aqueous and Hydroalcoholic extract of Musa acuminata are 887.18 µg/ml and 470.83 µg/ml respectively. It can be inferred that hydroalcoholic extract has more superoxide activity than aqueous extract.

Table-8 describes the hydroxyl radical scavenging activity. Hydroalcoholic extract has better scavenging activity when compared to aqueous extract. The IC_{50} value of ascorbic acid is 433.44 µg/ml. the IC_{50} value of aqueous and hydroalcoholic extract is 491.18 µg/ml and 470.73 µg/ml respectively.

Table-9 describes lipid peroxidation inhibiting activity. Lipid peroxidation can be described as a chain reaction that is caused by free radicals that are subjected to oxidative stress.

CONCLUSION:

Discovery of antioxidants was a hope to cure many disorders. They still have a lot of importance even after the development of various other remedies after intensive researches.

The phytochemical screening of aqueous and hydroalcoholic extracts of Musa acuminata showed the presence of compounds such as flavonoids and phenols which have antioxidant activity. As a result, Musa acuminata was considered to be having antioxidant activity.

In order to evaluate the medicinal uses of Musa acuminata., in-vitro antioxidant activity of aqueous and hydroalcoholic extracts of Musa acuminata. were performed.

These tests revealed the evidence of antioxidant activity in Musa acuminata. plant extracts. The dose dependent activity of Musa acuminata was obtained from reducing power, hydrogen peroxide radical assay, nitric oxide radical scavenging, superoxide radical scavenging and microsomal lipid peroxidation assay. It was also found that the extract of Musa acuminata. was found to scavenge the free radicals such as peroxides, superoxide and hydroxyl radicals. From the various tests, it was concluded that the hydroalcoholic extract of Musa acuminata. plant is found to possess more antioxidant properties when compared to that of aqueous extract.

Finally, our study concluded that Musa acuminata. has antioxidant activity and can also be used in the therapy of various cardiac diseases, GIT related disorders and cognitive impairments.

ACKNOWLEDGEMENT:
We acknowledge the continuous support of Dr. M.B.V Raju(principal) for successfully completing the research.

REFERENCES:

1. Sen S, Chakraborty R, Sridhar C, Reddy Y S R, De B. Free radicals, antioxidants, Diseases and phytomedicines: Current status and Future prospect. International Journal of Pharmaceutical Sciences Review and Research. 2010; 3(1): 91-100.
2. Machlin L J and Bendich A. Free radical tissue damage: protective role of antioxidant nutrients. Symposium presented by the American Institute of nutrition at the 71 Annual meeting of the Federation of American Societies for Experimental Biology; 1987 April 2; Washington, DC.
3. Valko M, Leibfritz D, Moncol J, Cronin M T D, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry and Cell Biology. 2007(39) 44-84.
4. Mukherjee DG, Dey CD. Clinical trial on Brahmi.I. J Exper Med Sci 1966; 10:5-11.
5. Chopra RN. Indigenous Drugs of India. 2nd ed.Calcutta, India: U.N. Dhur and Sons; 1958:341.
6. Nadkarni KM. The Indian Materia Medica.Columbia, MO: South Asia Books; 1988:624-625.
7. Singleton VL, Rosi JA, Am. J. Enol. Vitic 1965; 10: 144-158
8. J.Zhishen, T.Mengcheng, W.Jianming, Food Chemistry, 64, 1999, 555-559.
9. Oyaizu M. studies on products of browning reaction. Antioxidant activities of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986; 44: 307-315.
10. Ruch RJ, cheng SJ, Klaunig JE , Carcinogenesis 1989; 10: 1003-1008
11. Garrat D C, the Quantitative Analysis of Drugs, Japan, Chapman and Hall, 1964, 456
12. Elizabeth K, Rao MNA (1990) Oxygen radical scavenging activity of curcumin. Inter J Pharm 58:237–240
13. Patra J, Rath S, Karmabeer J. Evaluation of antioxidant and antimicrobial activity of seaweed (Sargassun sp.) Extract : A study on inhibition of glucathione-s transferase activity. Turk J Biol 2008; 32: 119-125
14. Fraga CG, Leibovitz BE , Toppel Al. Lipid peroxidation measured as TBARS in tissue characterization and comparison with homogenates and microsomes. Free Radic Biol Med 1981 ; 4: 155-161