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Invitro-Antioxidant activity of the seed and leaf extracts of syzygium cumini

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Abstract : *The present research was subjected to screen invitro antioxidant activity of the seed and leaf extract of Syzygium cumini. The antioxidant activity was determined by in vitro methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, ABTS Assay, Total antioxidant activity (Phosphomolybdic acid method), Nitric oxide radical scavenging, Ferric reducing antioxidant power (FRAP) assay, Hydroxyl radical scavenging activity, Total Reducing antioxidant potential, Reducing power. The extract showed significant antioxidant activity in all antioxidant assays when compared to ascorbic acid. The results of this research work are promising thus indicating the utilisation of the seed and leaf of Syzygium cumini as a significant source of natural antioxidants*

Keywords: *Antioxidant activity, ABTS, DPPH, FRAP, NOS, TRAP, Total antioxidant activity, Reducing power, Hydroxyl radical scavenging activity, Syzygium cumini.*

I. INTRODUCTION

Plants are the potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants (Walton, and Brown, 1999). Antioxidant agents like tannins, flavonoids, phenols, polyphenols, and nitric acid, scavengers of free radicals such as peroxidase, hydrogen peroxidase or lipid peroxyl thus inhibits the oxidative mechanism that lead to degenerative diseases. Antioxidants are found in all parts of plants such as bark, stalks, leaves, fruits, roots, flowers, pods and seeds. The most effective components seem to be flavonoids and phenolic compound of many plant raw materials, particularly in herbs, seeds and fruits. Increasing the antioxidants intake can prevent diseases and lower the health problems. Research is increasingly showing that antioxidant rich foods, herbs reap health benefits.

Syzygium cumini seeds are extensively used for various ailments such as anti-inflammatory, hypolipidaemic, antidiabetic and antioxidant, neuroprotective and recently it has been reported (Patel et al, 2010) for the DNA protection against radiation. Although the syzygium cumini seeds were used for several biological activities; the possible cardioprotective (Oliver, 1980) reported the effect of the syzygium cumini (Jamun) seeds against the DOX induced acute myocardial stress in rats. Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have antiinflammatory effects .Glycosides, flavaloids, tannins and alkaloids have hypoglycemic activities. Syzygium cumini might be responsible for its high antioxidant activity. This activity was observed when an increase in levels of plasma glucose, vitamin-E, ceruloplasmin, lipid peroxides and a decrease in levels of vitamin C and glutathione observed in diabetic rats, recover back to the normal levels after treatment with Syzgium cumi seed kernel extract (Warrier et al, 1996). Histopathological studies also promise its protective effect on pancreatic β-cells. Ethanolic extract of Syzgium cumini seed kernel also lowering the thiobarbituric acid reactive substance (TBARS) and increased in reduced glutathione (GSH), superoxide dismutase (SOD) and catalyse (CAT).

II. MATERIALS AND METHODS

1.1. COLLECTION OF PLANT MATERIALS

The fully mature Syzygium cumini Skeels leaves and seeds were collected in sep 2012 from Saravanampatty in Coimbatore District of Tamil Nadu, India from a single tree. The seed was identified and authenticated wide voucher specimen by the college afflicted to KMCH College of Pharmacy, Coimbatore. (10)

1.2. PREPARATION OF EXTRACTS

The Syzygium cumini Skeels fruits were first washed well and pulp was removed from the seeds. Seeds were washed several times with distilled water to remove the traces of pulp from the seeds. The seeds and leaves were dried at room temperature and coarsely powdered. The powders were taken equally and extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was subjected to successive solvent extraction with Petroleum ether, Ethyl acetate, Methanol and Water using Soxhlet extraction method, aqueous extract was prepared by cold maceration process. The Plant Extracts Were Concentrated Using Rotary Flash Vaporator (Buchi, Switzerland) And Stored In Desicator.

1.3. INVITRO-ANTIOXIDANT ACTIVITY

1.3.1. ABTS ASSAY

The scavenging activity of the test sample was tested using ABTS+ assay. The method was described by Re et al., 1999 with a slight modification. The ABTS+ radical solution was prepared by mixing 14mM ABTS stock solution with 4.9 mM ammonium per sulphate and incubated 16h in the dark at room temperature until the reaction was stable. The absorbance of the ABTS+ solution was equilibrated to 0.70±0.02 by diluting with ethanol at room temperature. To 1ml of the ABTS+ solution various concentration of the test sample (20-100µg/ml) was added. The absorbance was measured at 734nm after 6minutes. The percentage inhibition of absorbance was calculated and plotted as a function of the concentration of standard and sample to determine the antioxidant concentration. Ascorbic acid was used as a standard.

1.3.2. TOTAL ANTIOXIDANT ACTIVITY (PHOSPHOMOLYBDIC ACID METHOD)

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex. An aliquot of 0.2 ml of sample solution (20-100µg/ml) was combined in a vial with 2 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95ºC for 30 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expressed relative to that of ascorbic acid.

1.3.3. FRAP ASSAY

A modified method of Benzie and Strain et al., (1996) was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-Striazine) solution in 40 mM HCl and 20 mMFeCl3. 6H2O. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml FeCl3 .6H2O. The temperature of the solution was raised to 37ºC before using. Plant extracts (20-100µg/ml) were allowed to react with 900µl of FRAP solution and the solution was made upto 1ml with methanol. After 4 minutes readings of the colored product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM FeSO4. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

1.3.4. NITRIC OXIDE RADICAL SCAVENGING

Nitric oxide was generated from sodium nitro prusside in aqueous solution at physiological pH, which interacts with oxygen to produce nitric ions, which may be determined by the Griess Illosvoy reaction. 2ml of 10mM sodium nitro prusside 0.5ml phosphate buffer saline (pH 7.4) was mixed with 0.5ml of sample at different concentrations and the mixture incubated at 25ºC for 150 minutes. From the incubated mixture 1.5ml was taken out and added into 1.5ml of griess reagent (1% sulphanilamide,2% o-phosphoric acid,0.1% napthyl ethylene diamine di Hcl) and incubated at room temperature for 5mins. The absorbance of the mixture at 546nm was measured with a spectrophotometer.

1.3.5. REDUCING POWER

The $Fe3^{+}$-reducing power of the extract was determined by the method of Oyaizu et al., 1986 with a slight modification. Different concentrations (20-100 µg/ml) of the extract (1ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium hexa cyanoferrate (1%), followed by incubation at 50°C in a water bath for 20 min. After incubation, 2.5 ml of TCA (10%) was added to terminate the reaction. The upper portion of the solution (2.5ml) was mixed with 2.5 ml distilled water, and 0.5 ml FeCl3solution (0.1%) was added. The reaction mixture was left for 10 min at room temperature and the absorbance was measured at 700 nm against an appropriate blank solution. A higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as a positive control (Oyaizu,2003)

1.3.6. DPPH ASSAY

This was assayed as described by Elizabeth and Rao (1990).the reaction mixture contained methanol-50ml. DPPH (diphenyl 2-picryl hydrazyl radical) 0.3mM.1ml of 0.3mM DPPH in methanol was added to 100µl of compound with concentrations ranging from 20µg to 100µg.DPPH solution with methanol was used as a positive control and methanol alone acted as a blank. When DPPH reacts with antioxidants in the sample, it was reduced and the color changed from deep violet to light yellow. This was measured at 517nm.

1.3.7. HYDROXYL RADICAL SCAVENGING ACTIVITY

The scavenging activity for hydroxyl radicals recommended with major changes. Reaction mixture contained 0.6ml 1 of 1.0 mM Deoxy ribose, 0.4ml of 0.2mM Phenyl hydrazine, 0.6ml of 10mM phosphate buffer (pH 7.4). It was incubated for one hour at room temperature. Then add 1ml of 2-8% TCA, 1ml of 1%

TBA and 0.4 ml of extract at various concentrations and kept in water bath for 20 minutes. The absorbance of the mixture at 532nm was measured with a spectrophotometer. The hydroxyl radical scavenging activity was calculated

1.3.8. TOTAL REDUCING ANTIOXIDANT POTENTIAL

The reaction mixture contains 0.35 ml of 27, dichloro flurecein diacetate (1mM in ethanol), 1.75ml of 0.01N NaOH incubated for 20 minutes at room temperature. 17.9 ml of 25mM sodium phosphate buffer (pH 7.2). The upper portion of the solution (150µl) different concentration of plant extract was added (20-100µg/ml) and made upto 0.5ml with methanol. 25µl of 56mM was added to initiate the reaction. The absorbance was measured at 490nm against the corresponding blank samples. Ascorbic acid was used as a reference standard.

III. FIGURES AND TABLES

In the present study the extracts prepared from dried parts of the plant *Syzygium cumini* were subjected to Pharmacognostical, Preliminary, Phytochemical anaylsis and Antimicrobial and Invitro-Antioxidant Acitivity study was done.

Pharmacognostical Studies

Table 1 reveals the % extractability with the various solvents used for the study. Ethyl acetate extended higher % extractability (1.2%). The other chemical constituents, such as total ash, acid insoluble and water soluble ash value and loss of moisture content are also depicted in the same table. Table 2 reveals the physical nature of the ethyl acetate extract of *S.cumini*.

Phytochemical Studies

Table 3 depicts the result of qualitative analysis of Ethyl acetate extract of *S.cumini*. The study confirms the presence of Alkaloids and Glycosides to a larger extent. Table 4 reveals the R_f value of the phytochemical constituents with respective solvent system.

Invitro-Antioxidant Activity

In the present work, the Ethyl acetate extract of *Syzygium cumini* was evaluated for their DPPH Radical scavenging activity. It was observed that the DPPH Radical scavenging activity of *S.cumini* was as comparable to that of standard ascorbic acid (Table 05 and Figure 1).

Total antioxidant capacity of the Ethyl acetate extract of *Syzygium cumini*, expressed as the number of gram equivalents of ascorbic acid. Ethyl acetate extract of *S.cumini* showed higher total antioxidant capacity when compare to standard Ascorbic acid (Table 6 and Fig 2).

Table 7 and Figure 3 shows that the FRAP values of the Ethyl acetate extract of *Syzygium cumini* are significantly lower than that of ascorbic acid.Table 8 reveals the % of reducing power assay of Ethyl acetate extract of *S.cumini* against standard ascorbic acid (Table 8 and Fig 4).

Hydroxyl radical scavenging activity was comparatively lower than that of standard ascorbic acid (Table 9 and Figure 5).The scavenging activity of ABTS radical assay showed that Ethyl acetate extract had higher activity when compared to ascorbic acid (Table 10 and Fig 6).

Percent inhibition of the nitrite oxide generated is measured by comparing the absorbance values of control and test preparations and ascorbic acid can be used as a positive control. The % inhibition was increased with increasing concentration of the extract. The *syzygium cumini* Ethyl acetate extract had better reducing power (Table 11 and Fig 7).The Total reducing antioxidant potential activity was evaluated. It was observed that Ethyl acetate extract had higher activity when compared to ascorbic acid (Table 12 and Fig 8).

Table-1- Physicochemical Parameters Value of S.cumini Ethyl acetate extract.

Parameters	**% of w/w**
Extractive	
Petroleum ether	0.6%
Chloroform	1%
Ethyl acetate	1.2%
Methanol	0.07%
Water	1.16%
Ash value	
Total ash	25%
Acid insoluble ash value	2.5%
Water soluble ash value	5.25%
Moisture content Loss of moisture content	0.1gram

Table-2-The physical characters for Ethyl acetate *S.cumini* extract.

Parameters	Ethyl acetate extract
Color	Dark greenish
Odor	No odor
Taste	Characteristic
Consistency	Viscous

Table 3 The qualitative analysis of the Ethyl acetate extract of *S.cumini*

S.No	Phytoconsituents	Ethyl acetate extract of *S.cumini*
1	Alkaloids	++
2	Glycosides	++
3	Sterols	+
4	Tannin	+
5	Resins	-
6	Carbohydrates	+
7	Protein & Amino acids	+
8	Saponins	+
9	Gum &mucilage	-
10	Flavaloids	+
11	Vitamin-C	-

+=Present; ++=Moderately present; +++=Appreciable amount; - =Absent.

Table- 4-TLC phytoconstituents analysis of Ethyl acetate extract of *S.cumini*.

S.No	Compounds	Solvent system	Detecting agent	Solvent front	No of spots	Rf value
1	Alkaloids	Tolune:Ethyl acetate : Diethyl amine(7:2:1)	Ultra violet Spectroscopy	3.9	1 2 3	1.20 2.00 2.30
2	Glycosides	Benzene: Ethanol(19:2)	Ultra violet Spectroscopy	4.8	1 2 3	2.2. 3.67 4.49
3	Flavaloids	Tolune:Ethyl acetate (93:7)	Ultra violet Spectroscopy	5.8	1 2 3	2.41 3.44 4.56
4	Steroids	Petroleum Ether: Ethyl acetate(7:3)	Ultra violet Spectroscopy	4.3	1 2	0.94 1.54
5	Triterprinoids	Chloroform:Ethyl acetate(9:1)	Ultra violet Spectroscopy	4.5	1	2.47

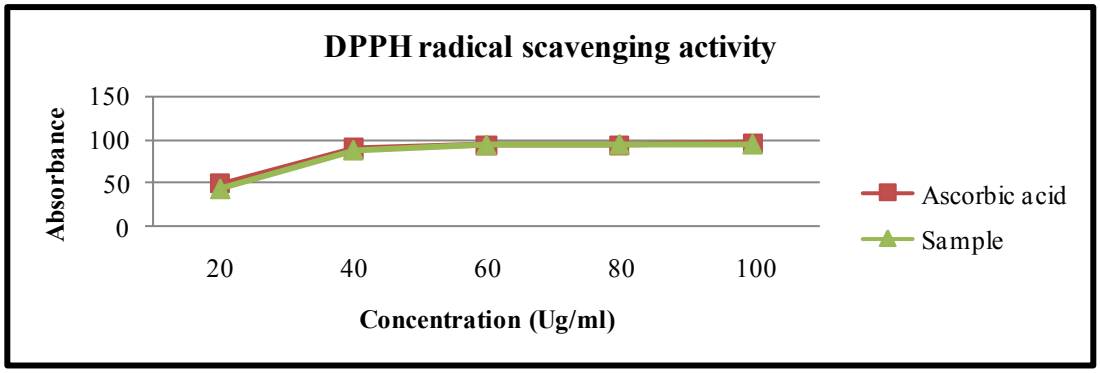

Figure 1-DPPH radical scavenging activity of Ethyl acetate extract of *S.cumini*

Table 5- Percentage of DPPH radical scavenging activity of Ethyl acetate extract of S.cumini

S.no	Concentration (ug/ml)	% of inhibion	
		Ascorbic acid	sample
1	20	49.4	44.2
2	40	90.2	87.5
3	60	93.3	93.4
4	80	93.9	94.0
5	100	95.8	94.2

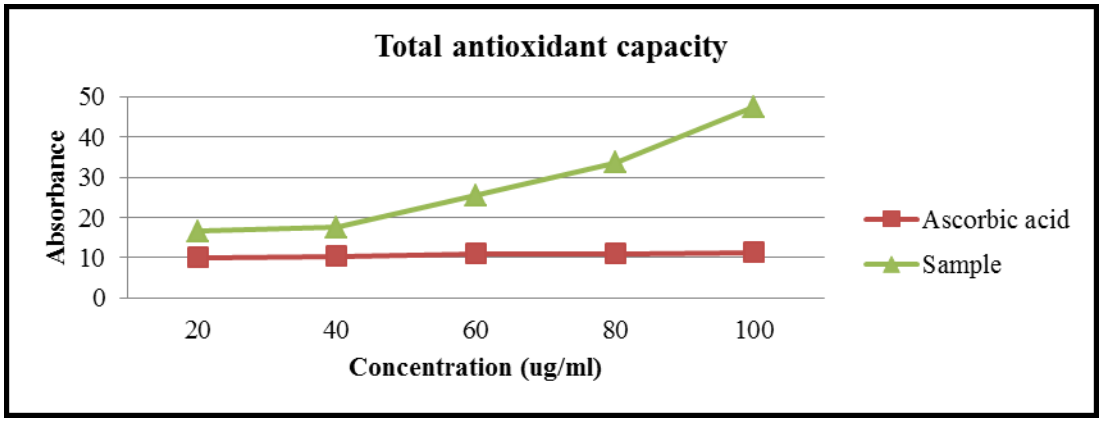

Figure 2-Total antioxidant capacity of Ethyl acetate extract of S.cumini

Table 6- Percentage of Total antioxidant capacity of Ethyl acetate extract of S.cumini

S.No	Concentration (ug/ml)	% total antioxidant capacity	
		Ascorbic acid	sample
1	20	10.0	16.6
2	40	10.4	17.5
3	60	11.0	25.6
4	80	11.1	33.6
5	100	11.4	47.5

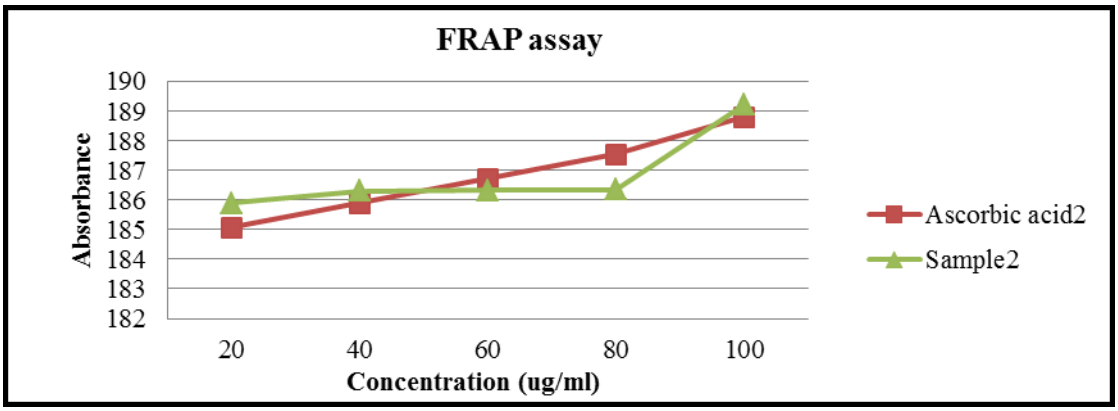

Figure 3-FRAP assay activity of Ethyl acetate extract of S.cumini

Table 7- Percentage of FRAP assay activity of Ethyl acetate extract of *S.cumini*

S.No	Concentration (ug/ml)	% of FRAP assay activity	
		Ascorbic acid	sample
1	20	185.06	185.89
2	40	185.89	186.3
3	60	186.72	186.32
4	80	187.55	186.35
5	100	188.79	189.21

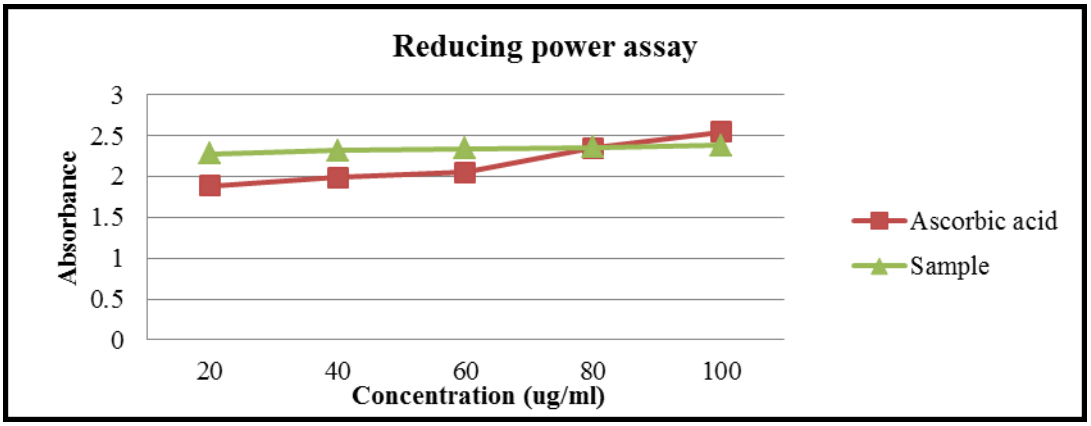

Figure 4-Reducing power assay of Ethyl acetate extract of S.cumini

Table 8-Percentage of Reducing power assay of Ethyl acetate extract of *S.cumini*

S.No	Concentration (ug/ml)	% of reducing power	
		Ascorbic acid	sample
1	20	1.89	2.28
2	40	1.99	2.32
3	60	2.05	2.34
4	80	2.35	2.36
5	100	2.55	2.38

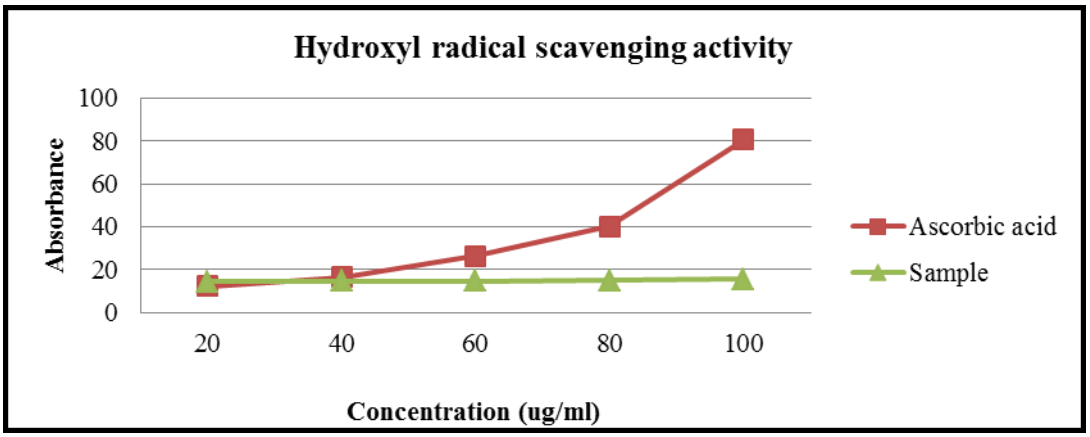

Figure 5-Hydroxyl radical scavenging activity of Ethyl acetate extract of S.cumini

Table 9- Percentage of Hydroxyl radical scavenging activity of Ethyl acetate extract of *S.cumini*

S.No	Concentration (ug/ml)	% of - Hydroxyl radical scavenging activity	
		Ascorbic acid	sample
1	20	12.2	14.2
2	40	16.3	14.5
3	60	26.2	14.7
4	80	40.0	14.9
5	100	80.5	15.4

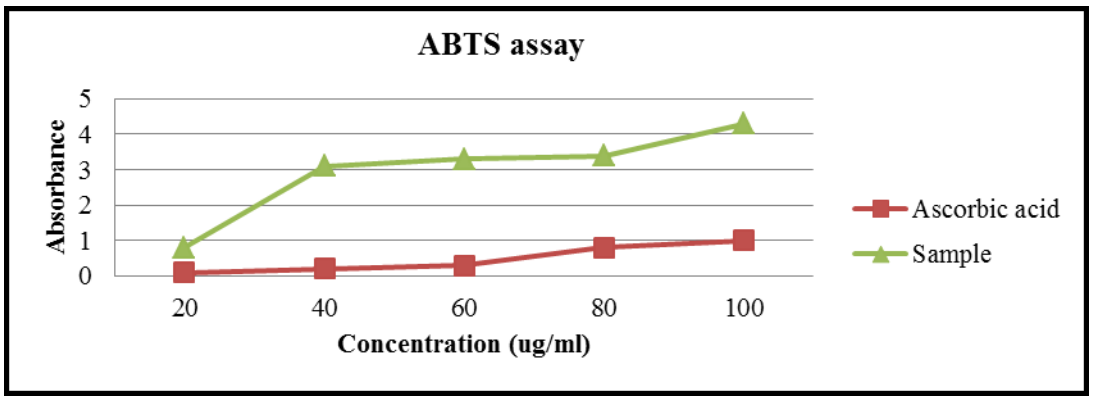

Figure 6-ABTS radical scavenging activity of Ethyl acetate extract of S.cumini

Table 10- Percentage of ABTS radical scavenging activity of Ethyl acetate extract of *S.cumini*

S.No	Concentration (ug/ml)	% of ABTS radical scavenging activity	
		Ascorbic acid	Sample
1	20	0. 1	0. 8
2	40	0. 2	3.1
3	60	0. 3	3.3
4	80	0. 8	3.4
5	100	1.0	4.3

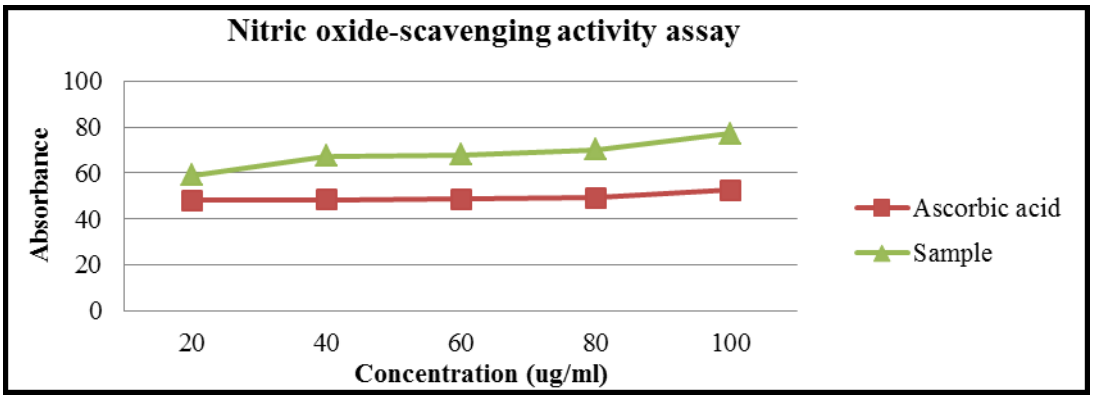

Figure 7-Nitric oxide scavenging activity of Ethyl acetate extract of S.cumini

Table 11- Percentage of Nitric oxide scavenging activity of Ethyl acetate extract of *S.cumini*

S.No	Concentration (ug/ml)	% of nitric oxide scavenging activity	
		Ascorbic acid	Sample
1	20	48.1	59.0
2	40	48.2	67.2
3	60	48.6	68.0
4	80	49.1	70.1
5	100	52.6	77.1

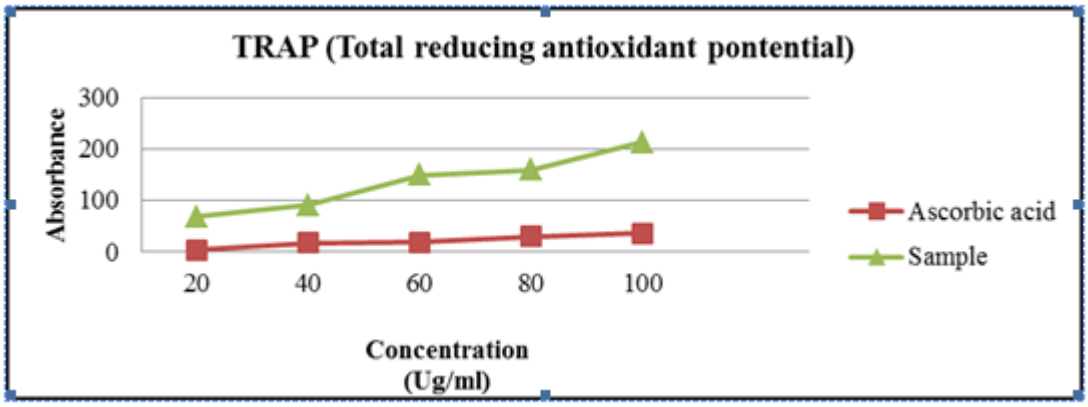

Figure 8-Total reducing antioxidant potential activity of Ethyl acetate extract of S.cumini

Table 12- Percentage of TRAP activity assay of Ethyl acetate extract of *S.cumini*

S.No	Concentration (ug/ml)	% of inhibition	
		Ascorbic acid	sample
1.	20	3.125	68.75
2.	40	17.18	90.62
3.	60	18.75	150
4.	80	29.68	159.3
5.	100	35.93	212.5

IV. CONCLUSION

In the present study, the extracts prepared from dried seeds and leaves of the plant *S.cumini* were subjected to phytochemical screening , antibacterial activity and invitro antioxidant activity.

It was observed that the Ethyl acetate extract of S.cumini extended higher extractive value, when compared with Petroleum ether, Chloroform, Methanol and Water. Therefore Ethyl acetate extract was chosen for in depth study.

The Ethyl acetate extract of *S.cumini* was subjected to Thin layer chromotograpy using silica gel-G. The R_f value were calculated and the study reveals the presence of Flavaloids, glycosides, Alkaloids, Steroids and Triterprioids .

The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences.The flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities(Aiyelaagbe and Osamudiamen, 2009). The steroids are known to be important for their cardiotonic activities and also insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics(Callow,1936).The Tannins were reported to exhibit antiviral, antibacterial and anti-tumour activities. It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic(Callow,1936). The Saponin is used as mild detergents and in intracellular histochemistal staining. It is also used to allow antibody access in intracellular proteins. In medicine, it is used in hypercholestrolaemia, hyperglycaemia, antioxidant, anticancer, antiinflammatory, weight loss, etc. It is also known to have antifungal properties(Haslem,1989).

In vitro antioxidant activity of the seed and leaf extract of *Syzygium cumini was performed* .The results of different assays potrays that the extract has maximum antioxidant activity and reducing power. Hydroxyl radical scavenging activity of ethyl acetate extract of Syzygium cumini shows a steady state increase when compared to the standard ascorbic acid. All the other assays confirms the free radical scavenging activity of the extract.

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