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To Compare and Evaluate the Antioxidant Activity of *Syzygium Cumini* Seeds By different Extracts (Ethanol, Ethyl Acetate, Water)

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ABSTRACT:
Antioxidants are vital substance which poses the ability to protect the body from damage caused by free radical induced oxidative stress. The present study was focused on evaluation of antioxidant activity in ethanolic extract of seeds of Syzygium cumini (L.). by different methods such as DPPH radical scavenging assay, ABTS radical scavenging assay and total antioxidant assay. The quantitative determination of compounds viz., phenolics and flavonoids presupposed to be inhibitors was created and overall antioxidant activity was measured mistreatment normal strategies.TheSyzygiumcuminiextracteffectivelyscavengedfreeradicalsandshoweditspotent antioxidant activity. A linear correlation between total phenolic content and antioxidant activity (r2= 0.99) has been reported. The results were compared to standard antioxidant vitamin c for DPPH radical scavenging assay, ABTS radical scavenging assay and total antioxidant assay.total phenol and Flavanoid contents were expressed as equivalent to Quercetine and gallic acid respectively. In conclusion, Syzygium cumini has strong antioxidant potential. further study validates the therapeutic benefits of the Indian system ofmedicine.

KEY WORDS: *ANTIOXIDANT, FREE RADICALS, SYZYGIUM CUMINI.*

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I. INTRODUCTION:

Antioxidants are important substances, which have the ability to protect the body from damages which are caused by free radical-induced oxidative stress. Much attention has been aimed on the activity of the natural antioxidants present in fruits and vegetables, because potentially these components may decrease the level of oxidative stress. Antioxidants include ascorbate, tocopherols, carotenoids and bioactive plant phenols. Amongst the plant sources Syzygium cumini fruit is one of those which contain a variety of important nutritional compositions [1].

dicot genus cumini (syn. Eugenia jambolana) unremarkably referred to as a Jamun contains varied phytoconstituents like tannins, alkaloids, steroids, flavonoids, terpenoids, fatty acids, phenols, minerals, carbohydrates and vitamins. Jamun, botanically named as dicot genus cumini, belongs to magnoliopsid family. Its fruit is mostly acknowledged to be terribly top quality for its curative perform mainly against polygenic disease thanks to its result on exocrine gland. Some common names are black plum, java plum, jambul and Indian blackberry. Jamun holds anti- hypercholesterolemia properties and helps in regulating the blood lipid profile due to presence of bioactive components. There are large number of phytonutrients such as flavonoids and phenolic acids present in jamun fruit and seed that have been reported for their antiallergic, anti-inflammatory, antidiabetic, anticancer, free radical scavenging (ROS) and antioxidant property.

The Jamun seeds square measure employed in treatment of polygenic disease, allergies, virus infection, inflammation and peptic ulceration. It conjointly has diuretic drug, anti-nociceptive, physiological condition, chemoprotective and cardioprotective result. Jamun seed is generally acknowledged to be very high quality for its curative function chiefly against diabetes because of its effect on pancreas. Jamun seed conjointly contains albumen, fat, glycosides, Associate in Nursing organic compound, jambosine3, resin, ellagic acid, quercetin, acid furthermore as components of metal, vanadium, chromium, atomic number 11 and metallic element. The various extracts of different parts of jamun possess a range of pharmacological properties such as antibacterial, antimicrobial, antifungal, antiviral, antioxidant and free radical scavenging activity, cardioprotective, anti-inflammatory, neuropsychopharmacology antactivitie, radioprotective, chemo preventive, larvicidal, gastroprotective and antiulcerogenic activities [2].

SYZYGIUM CUMINI (TAXONOMY)
Botanical name: Syzygium Cumini Kingdom: Plantae
Order: Myrtales

Family name: Myrtaceace Genus: Syzygium
Common Names: EUGENIA JAMBOLANAPLUM,

BLACK PLUM, JAMAN, JAMBOLAN.

Vernacular name: Alla Neredu [3]

Fig.no:1Fig.no:2

MEDICINAL BENEFITS OF SYGIUM CUMINI:

- May Help ManageDiabetes

Jamuns are well known for managing blood sugar level. dietetics dietician and Health professionalperson,ShilpaArorasays,"Jamunseedscontaincompoundsknownasjambolineand jambosine that scale back the speed at that sugar is free into the blood. Jamun seeds also rise the production ofinsulin.

- Boosts StomachHealth

Jamun seeds may be used to manage a number of stomach-related problems effectively. Jamuns are rich is fiber content that helps improve the functioning of the digestive system. Jamun seeds may also be used as oral medication to combat sores, inflammation and ulcers in the intestines.

- Helps Regulate BloodPressure

Jamunseedscouldswaybeaboonforfolkshandlinghighbloodpressurebecausetheseedextract of the fruit contains a sort of inhibitor known as ellagic acid that will facilitate keep a check on speedy fluctuations of force per unitarea.

- May BoostImmunity

Jamun seeds contain powerful antioxidants like flavonoids and additionally synthetic resin compounds that facilitate keep harmful free radicals cornered.

- Aids Weightloss

Since jamun is rich in fiber, it may help keep tummy sated and propel weight loss process [4].

SIDE EFFECTS OF EXCESS SYGIUM CUMINI:

- Blood sugarImbalance

Generally, according to Ayurveda, the consumption of Jamun is incredibly helpful for patients of high blood pressure. It is simply controlled by together with Jamun fruit or kernel powder within the diet. however, many folks begin ingestion an excessive amount of so as to regulate it because of that there could also be a retardant of

low force per unit area.

- Constipation

Jamun is made in water-soluble vitamin. In such a state of affairs, if you consume it in excess amount, you will have a constipation downside.

- Acne

Ifyou'vegotalotofintakesofberries,itwillcauseissuesforyourskin.skindisordercouldescape.

- Vomiting problem

Many people face vomiting problem after eating Jamun. If you also have a problem, then it is better not to consume it [4].

II. MATERIALS ANDMETHODS:

In this project we used Maceration process. This is an extraction procedure in which coarsely powdered drug material, (*sygium cumini seeds*) is placed inside a container; as we are performing comparative studies, we used three solvents. The solvents (ethanol, ethyl acetate and water) are poured on top until completely covered the drug material. The beaker is then closed and kept for at least three days. The content is stirred periodically, and as it is placed inside bottle it is shaken time to time to ensure complete extraction. At the end of extraction, the micelle is separated from marc by filtration. Subsequently, the micelle is then separated from the solvent. (9,10)

Fig.no.3: Ethyl acetate

Fig.no.4: Ethanol

Fig.no.5: Water

PHYTOCHEMICAL SCREENING TEST:

The extracts obtained were subjected to phytochemical screening for the identification of phytoconstituents such as primary and secondary metabolites.

Detection of Alkaloids:

Wagner's test:

ThefiltratewastreatedwithWagner'sreagent.Theformationoforangereddishbrownprecipitate indicates the presence ofalkali.

Mayer's test:

ThefiltratewastreatedwithMayer'sreagent.Theformationofcreamcolourprecipitateindicates the presence ofalkaloids.

Dragendroffs test (Solution of potassium bismuth iodide):

ThefiltratewastreatedwithDragendroffssreagent.Thedevelopmentofredprecipitateshowsthe presence ofalkaloids.

Detection of Flavonoid's:

The extract is treated with few drops of sodium hydroxide solution formation of intense yellow colour which becomes colorless on addition of dilute acid indicates the presence of flavonoids.

Test for saponins:

Foam Test:

A small quantity of extract was taken in a test tube and few ml of water was added and shaken, saponins will produce froth, which is stable for 15 minutes.

Detection of tannin's:
Lead Acetate Test:
To the test solution, a few drops of 10% lead acetate solution were added. The development of white precipitate shows the presence of phenolic compounds.
Detection of triterpenoids:
5ml extract mixed with 2ml of chloroform and 2ml of acetic anhydride and a few drops of concentrated H_2SO_4 was added formation of reddish violet colour indicates presence of triterpenoids.

Detection of carbohydrates:
Few ml of extract and 2ml of Molisch reagent were shaken well. After that 2ml concentrated H_2SO_4 was poured along the sides of test tube violet colour is produced indicates the presence of carbohydrates.

Test for Steroidal Glycosides:
Keller Kiliani Test: (Test for deoxy sugars)
Smallquantityofsamplewastakentothataddchloroform,glacialaceticacidandferric-Chloride. This mixture is taken in a test tube containing sulfuric acid. It shows reddish brown colour which upon standing produces bluish greencolour.
Legals Test:
Small quantities of sample were taken to that add Pyridine and sodium nitroprusside mixture and add sodium hydroxide solution this shows pink or red colour.
Test for coumarin glycosides:
Small quantity of the extract dissolved in tew ml of methanol. This was made alkaline using aqueous sodium hydroxide. Blue or green fluorescence indicates the presence of coumarin glycosides [11].

ANTIOXIDANT METHODS:
ABTS RADICAL SCAVENGING ASSAY:
The scavenging activity of the test sample was tested using ABTS+ assay. The method was reported by Re et al., 1999 with a slight change. The ABTS+ radical solution was prepared by mixing 14mM ABTS stock solution with 4.9 mM ammonium per sulphate and incubated 16h in thedarkatroomtemperatureuntilthereactionwasstable.TheabsorbanceoftheABTS+solution wasequilibratedto0.70±0.02bydilutingwithethanolatroomtemperature.To1mloftheABTS+ 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 deliberated and planned as a function of the concentration of customary and sample to determine the antioxidant concentration. Ascorbic acid was used as customary[(12)].

DPPH ASSAY METHOD:
TheDPPHscavengingactivityofvariousfractionswasevaluatedinstepwiththetacticofBrand-Williamsetal.1mLof0.1-millimeterDPPHresolutioninMainewasmixedwithonemilliliterof every fraction at varied concentrations. The corresponding blank sample was ready, and ascorbic acid was used as reference customary. Mixture of 1mL ethanol and 1mL DPPH solution was employed as control. The mixture was jolted well and incubated for 30 min in the dark. The reactionwascarriedoutintriplicate,andthedecreaseinabsorbancewasmeasuredat517nmafter incubation using a multiplate reader (Synergy 4 Biotek. USA)[(13)].

TOTAL ANTIOXIDANT ACTIVITY (PHOSPHOMOLYBDIC ACID METHOD):
The antioxidant activity of the sample was determined by the transformation of Mo (VI) to Mo (V)toformphosphomolybdenumcomplex.Analiquotof0.2mlofsamplesolution(20-100µg/ml) was combined in a vial with 2 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphateand4mMammoniummolybdate).Thevialswereclosedandincubatedinawaterbath at 95ºC for thirty min. once the samples had cooled to temperature, the absorbance of the mixture wasmeasuredat695nmagainstablank.Theantioxidantactivitywasexpressedrelativetothatof ascorbic acid[(14)].

TOTAL PHENOL CONTENT:
The TPC make up my mind by Folin–Ciocalteau methodology [12] with slight modifications. Briefly, different concentrations of fractions (20-100 µL) were taken, and 80 µL of Folin–Ciocalteaureagentand200µLsodiumcarbonate(20%)wereadded,madeupto700µLemploying distilled water and incubated at ambient temperature (25-27°C) for ninety min. The colour developed was measured at 760 nm employing a multimode reader (Biotek, USA). The phenolic resin contents were calculated employing a

customary curve for GA, and also the result was expressed as mg GA equivalents (GAE) per gram dry weight of fraction (mg GAE/g). All measurements were performed in triplicates[15].

TOTAL FLAVANOID CONTENT:

The TFC was estimated using customary procedures deliberated by Chang et al. with slight modifications. various concentrations of fractions were diluted with 150 µL of ethanol. Further, 10µLof10%aluminumchloridesolutionand1Mpotassiumacetate(10µL)wasaddedandmade up to 280 µL using distilled water. The resulted solution was mixed well and incubated at room temperature for forty min. The absorbance was measured at 415 nm by employing a multimode reader (Biotek, USA). Quercetin was used as a standard, and results were expressed as mg quercetin equivalents (QE) per gram dry weight of fraction (mg QE/g)[16]

III. RESULT AND DISCUSSION: PHYTOCHEMICALSTUDIES:

Table. No. 1: phytochemical constituents of various extracts of Syzygium cumini seeds

Test	Ethyl acetate	Ethanol	Water
Detection of alkaloids a. Wagner's test	✓ 	X	✓ 
b. Mayer's test	X	X	X
c. Dragendroffs test	✓ 	✓ 	✓ 
Detection of flavonoids	✓ 	✓ 	✓ 

Detection of Tannins	✓ 	✓ 	✓ 
Detection of Saponins	✓ 	✓ 	✓ 
Detection of Carbohydrates	✓ 	✓ 	✓ 
Detection of Triterpenoids	✓ 	✓ 	✓ 
Detection of glycosides *Test for Steroidal Glycosides:* a. Kelari killani test	x	✓ 	x

DPPH RADICAL SCAVENGING ASSAY:

It is an extensively used, relatively rapid and accurate method for the assessment of free radical scavenging activity. DPPH is a solid free radical and gain an electron or hydrogen radical to become a solid diamagnetic molecule. Antioxidant donates the electron or hydrogen atom after interactionwithDPPHradicalandthusneutralizingfreeradicalcharacteroftheDPPHandconvert it to 1-1, diphenyl-2-picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug. The reduction capacity of DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant and radical progress which results in the scavenging of the radical by hydrogen donation. It is Visually evident as change in colorfrompurpletoyellow.HenceDPPHisusuallyusedasasubstancetoevaluatetheantioxidant activity. The $1C_{50}$ values of the sygium cumini ethanol extracts were found to be 49 µg/ml respectively. IC_{50} Value for the ascorbic acid (Standard) was found to be 6.8µg/ml and also presented in TableNo.2.

EXTRACT /STANDARD	CONCENTRATION µg/ml	%INHIBITION	IC_{50}VALUE
Ethanolic extract of *sygium cumini seeds*	**10**	**2.91±0.25**	**14µg/ml**
	20	**10.77±0.91**	
	40	**21.86±1.32**	
	60	**35.40±0.78**	
	80	**42.88±1.24**	
	100	**54.15±0.64**	
Ascorbic acid	**2**	**8.98±0.27**	**6.8µg/ml**
	4	**18.22±2.09**	
	6	**30.98±3.95**	
	8	**44.95±2.96**	
	10	**57.02±3.98**	
	12	**66.1±2.76**	

TABLE.NO.2: DPPH RADICAL SCAVENGING ASSAY OF *SYGIUM CUMINI*

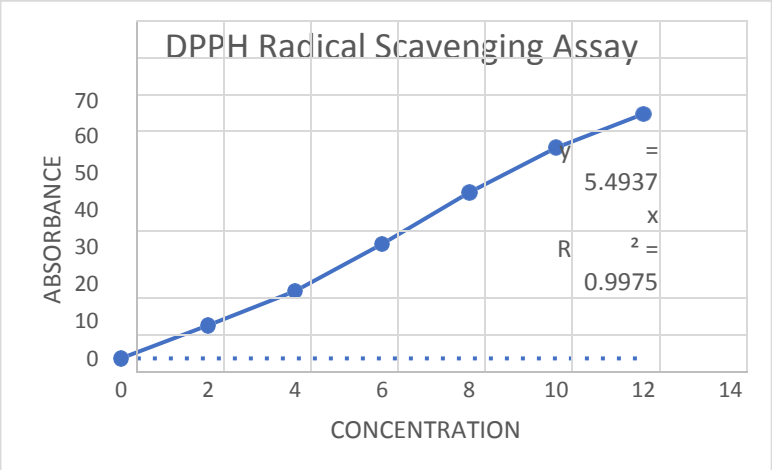

Fig.6: IC50 determination of standard ascorbic acid for DPPH assay

ABTS RADICAL SCAVENGING ASSAY:

It is one of the most commonly used assays in food industry for the measurement of antioxidant abilityoffoods.lnthis,ABTSisconvertedtoitsradicalcationbyadditionofpotassiumpersulfate. Thisradicalcationisblueincolorandabsorbslightat734nm.TheABTSradicalcationisreactive towards most antioxidants including polyphenols. Thiols and ascorbic acid. During this reaction, the blue ABTS radical cation is converted rear to its colorless neutral form. The IC_{50} values ofthe sygium cumini ethanol extracts were found to be 126.19µg/ml respectively. $1C_{50}$ value for the ascorbic acid (Standard) was found to be 14.1µg/ml and also presented in TableNo.3

EXTRACT /STANDARD	CONCENTRATION µg/ml	%INHIBITION	IC_{50}VALUE
Ethanolic extract of *sygium cumini seeds*	**100**	**13.59±1.14**	**126.19 µg/ml**
	200	**17.73±1.87**	
	300	**28.64±4.11**	
	400	**35.97±1.02**	

	500	**47.26±4.04**	
Ascorbic acid	**10**	**28.1±2.19**	**14.1 µg/ml**
	20	**36.22±3.91**	
	30	**50.55±2.85**	
	40	**69.77±1.02**	
	50	**79.99±0.97**	

TABLE.NO.3: ABTS RADICAL SCAVENGING ASSAY OF *SYGIUM CUMINI*

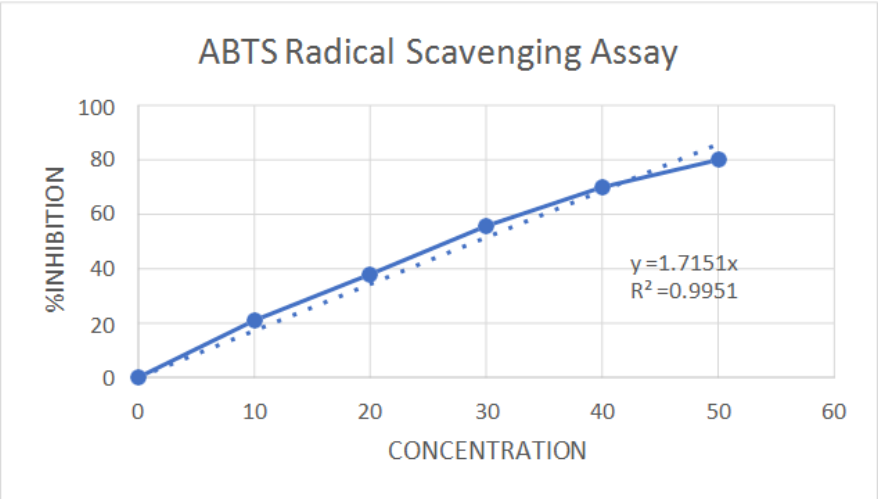

Fig.7: IC50 determination of standard ascorbic acid for ABTS assay

TOTAL ANTIOXIDANT ACTIVITY:

Theassaywasbasedonthereductionof MO(VI)-MO(V)bytheextractsandsubquetentformation of a green phosphate/ Mo (V) complex at acidic ph. Total antioxidant activity of the ethanolic extract of sygium cumini were found to be 0.256±0.03 µg vitamin c equivalence per mg of plant extract total antioxidant activity of the std vitamin c was specified infig.no.12

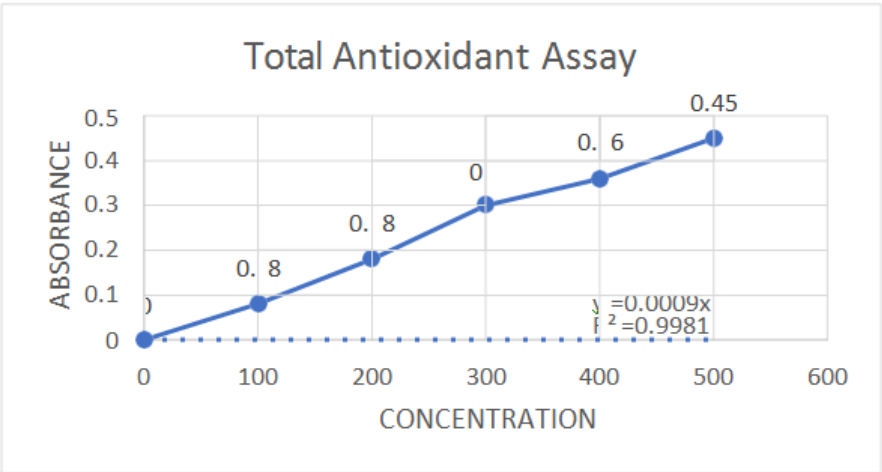

Fig.8: Total antioxidant activity of standard ascorbic acid

TOTAL PHENOL CONTENT

The antioxidant activity of phenolics is mainly due to their redox properties, which can play an importantroleinabsorbingandneutralizingfreeradicals.quenchingSingletandtripletOxygenor decomposing peroxides. Total phenol content of the ethanolic extracts of Syzygium cumini were found to be 189±0.04µg Gallic acid equivalents per mg of plant extract. The total phenol content of Standard Gallie acid was shown in the Fig.No. 13

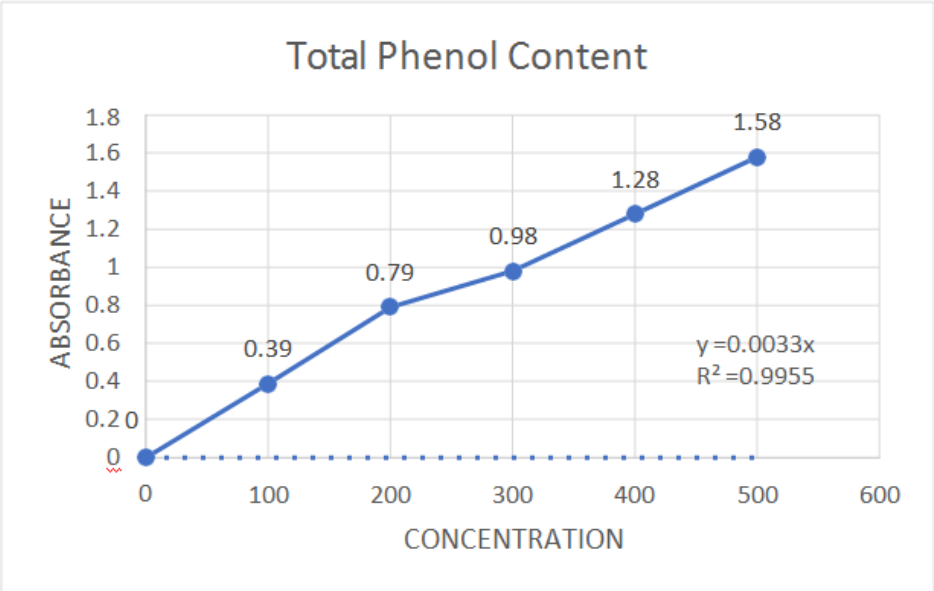

Fig.9: Total Phenol Content of standard gallic acid

Total flavonoid content

TotalflavonoidcontentofstandardQuercetinwasspecifiedintheFigureNo.14.Theprincipleof thismethodisthataluminumchlorideformsacidstablecomplexeswithC-4ketogroupandeither the C-3 or C-5 hydroxyl groups of flavones and flavonols. In addition, aluminum chloride forms acid stable complex with ortho- di hydroxyl groups in the A or Brings of the flavonoids. Total flavonoid content of the ethanolic extracts of Syzygium cumini was found to be 36±0.02µg Quercetin equivalents per mg of plant extract shown in the Fig.No.14

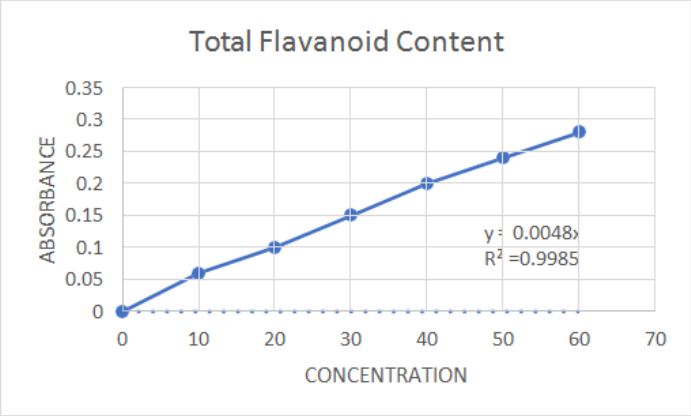

Fig.10: Total Flavanoid Content of standard Quercetine

IV. CONCLUSION:

Freeradicalsareoftenraisedasbyproductsofbiologicalreactionsorfromexogenousfactors.The participationoffreeradicalsinthepathogenesisofamanydiseasesarewelldocumented.Apotent scavenger of free radicals can serve as a possible preventive intervention for the diseases. The present study demonstrated that the ethanolic extract of *SYZYGIUM CUMINI SEEDS* showed prominent antioxidant activity. It is concluded that *SYZYGIUM CUMINI SEEDS* is a good source ofnaturalantioxidantandmightbeusefulintreatingthediseasesassociatedwithOxidativestress.

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