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A COMPARATIVE ANALYSIS ON EFFICACY OF PULP, PEEL AND SEED OF *SYZYGIUM CUMINI* AND *MANGIFERA INDICA* AS NUTRACEUTICAL

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ABSTRACT

Antioxidant rich foods particularly of plant origin are considered to be most effective in preventing various diseases caused by reactive oxygen species oxidation. Since composition and hence pharmacological activities vary in different parts of plant, in the present study different parts of fruit - edible pulp and seed of *Syzygium cumini* and non-edible peel of *Mangifera indica* (Fazli) were selected and screened to determine the phytonutrients. Tannin, total phenol content, and antioxidant activities like scavenging, metal chelating, and ferric reducing activity of aqueous and ethanolic extract of samples were determined on day 7, 14 and 21days. Seeds of *S.cumini* appeared to be the most efficient natural antioxidant followed by the pulp. Due to water solubility of the antioxidant molecules aqueous extract of *S.cumini* recorded higher activity whereas the activity of *M.indica* peel was better in alcoholic extract. These findings suggest suitable use of the respective fractions as nutraceutical and functional food.

KEY WORDS

Black plum, Mango peel, Seed, Edible pulp, Antioxidant activity and Phytochemicals.

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INTRODUCTION

Different redox processes generate various forms of activated oxygen radicals, such as superoxide radical (O_2^-), hydroxyl radical (^-OH) as well as non-free radical species, as H_2O_2 and singlet oxygen ($^1O_2$), which may induce some oxidative damage to bio molecules such as carbohydrates, proteins, lipids and DNA[1], thus accelerating aging, cancer, cardiovascular diseases, neurodegenerative diseases and inflammation[2,3].

In order to prevent such oxidation, many substances have been investigated as potential antioxidant. Synthetic antioxidants, such as butylated-

hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ter-butyl hydroquinone (TBHQ) are commonly used as food additives, since they are effective and less expensive than natural antioxidants[4]. Due to some toxic effects and health concerns[5] the uses of natural antioxidant from plant sources are being recommended. Food rich in antioxidants plays an essential role in the prevention of cardiovascular diseases, cancers[6] and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases[7], as well as inflammation and problems caused by cell and cutaneous aging[8].

Antioxidants like flavonoids, tannins, anthocyanins, carotenoids and other phenolic constituents present in food of plant origin are potential antioxidants[9] that can be used both as additives in foods or pharmaceutical supplements and for scavenging reactive oxygen species and thereby protecting against degenerative diseases like cancer, cardiovascular problems.

Diverse natural antioxidants having important biological activities are present in different parts of a plant like leaves, roots, bark, dietary fibres, seeds, fruits etc. A fruit mainly consists of seed and edible pulp with an edible or a non-edible peel. Since nature of antioxidants, their composition and concentration vary in these parts it generates a range of diverse pharmacological activities.

The present study is based on phytochemical screening and study of antioxidant activity of the seed and edible part (pulp with skin) of fruits of wild Indian *Syzygium cumini* (L.) Skeels (Black Plum) and of non-edible peel of fresh ripe *Mangifera indica* (mango), Fazli variety.

Fruits of *Syzygium cumini* are edible and are reported to contain vitamin C, maleic acid as the major acid (0.59% of the weight of fruit), gallic acid, tannins, anthocyanins, such as delphinidin-3-gentiobioside, malvidin-3-laminaribioside, petunidin-3-gentiobioside, cyanidin diglycoside, petunidin and malvidin[10].

Extensive investigations had been carried out with different parts of *Syzygium cumini* to determine their pharmacological properties. The fruits have been used for a wide variety of ailments, including cough, diabetes, inflammation and ringworm[11], chronic diarrhea and other enteric disorders[12]. The peel powder can be employed as a colorant for foods and pharmaceuticals and the anthocyanin pigments from fruit peels were studied for their antioxidant efficacy as extract and in formulations[12].

Seeds of this plant have been reported to be rich in flavonoids along with tannins (19%), ellagic acid, gallic acid (1-2%), starch, jambosine, and glycoside jambolin or antimellin[13].

Extract of the seed, is traditionally used to treat diabetes[10], cold, cough, fever and skin problems such as rashes and the mouth, throat, intestines and genitourinary tract ulcers (infected by Candida albicans)[14].

Mango peel is a waste or by-product from pulp processing unit, and an important source of high quality antioxidant dietary fibre, pectin, polyphenols and carotenoids. The peel of mango has a significant potential benefit due to its powerful antioxidant properties and high content of phenolic compound[15]. Content of total phenolics are higher in peels than in flesh at all stages[16].

Mango peel is rich in pectin, cellulose, hemicelluloses, lipids, proteins, triterpene, lupeol along with antioxidants like carotenoids[17] such as the pro-vitamin. A compound, beta carotene, lutein and alpha carotene[18] and polyphenols, such as syringic acid, quercetin, mangiferin pentoside and ellagic acid, kaempferol, gallic acid, caffeic acid, catechins and tannins, a unique xanthonoid called mangiferin[19], which is a heat-stable and pharmacologically active phytochemical.

These phenolic compounds can be a good source of natural antioxidant and can be used in food. Polyphenol rich fractions of peel extract could be used as natural antioxidants and functional food or feed supplements[20] in pharmaceutical and cosmetics industries[21].

Though a flurry of activity has been done on *S.cumini* fruit and seed their comparative antioxidant efficacy has not been studied. Moreover comparison of antioxidant activity between an anthocyanin and a xanthonoid rich fruit peel needs an investigation. Hence this study was done to investigate the comparative antioxidant activity of these natural

product resources over a period of time to understand their stability.

MATERIALS AND METHODS

Chemicals

DPPH, trichloroacetic acid, folin ciocalteu reagent, gallic acid, ferrozine, potassium iodide, ninhydrin, indigo carmine indicator, all other solvents and reagents were procured from Merck (India). All chemicals used were of analytical grade.

Sample

Black Plum (Syzygium cumini) and mango (*Mangifera indica*) (Fazli) were brought from local market of Howrah.

Sample Preparation

Pulp portion (200 gram) of black plum (*S.cumini*) was separated from the seed and lyophilized in Eyela FDU 1200 machine at -40^0 C.

Seeds of *S.cumini* were separately grounded in vortex grinder (Bajaj GX8).

Peels were removed from the ripe mangoes (*M.indica*) (fajli variety) and lyophilized in Eyela FDU 1200 machine at -40^0 C.

Preparation of Sample Extract

Ethanol extract

Three batches each of the three samples (1 gram) were soaked in 10 mL of ethanol and stored in refrigerator (Whirlpool) at 4^0 C. After 7 days each batch of three samples were taken out, centrifuged and the supernatant was collected for antioxidant assay. Same thing was done on 14^{th} and 21^{st} days.

Water extract

Three batches each of the three samples (1 gram) were soaked in 10 mL of water and stored in refrigerator (Whirlpool) at 4^0 C. After 7 days each batch of three samples were taken out, centrifuged and the supernatant was collected for antioxidant assay. Same thing was done on 14^{th} and 21^{st} days.

The entire operation was done on a triplicate basis.

PRELIMINARY PHYTOCHEMICAL SCREENING

Phytochemical screening was done with the ethanolic extract of each sample.

Test for Tannin

1 mL of the sample was taken in a test tube and 1 mL of 0.008M Potassium ferricyanide was added. To it was added 1 mL of 0.02M ferric chloride containing 0.1(N) HCl and observed for blue-black colouration.

Test for Phlobatannin

Crude extract of each sample was boiled with 2% aqueous hydrochloric acid (HCl) and the deposition of red precipitation was observed.

Test for Saponin

Crude extract of each sample was mixed with 5 mL of distilled water in a test tube and shaken vigorously. Few drops of oil were added in it. The formation of stable foam was taken as an indication for presence of saponin.

Test for Flavonoid

To the crude extract of each sample 5mL of dilute ammonia solution were added followed by addition of concentrated sulphuric acid (H_2SO_4). A yellow coloration indicates the presence of flavonoid.

Test for Quinine

Dilute ammonium hydroxide (NH_4OH) was added to 1 mL of crude extract of each sample. Blue green or red coloration indicates the presence of quinine.

Ninhydrin Test

To 5 mL of n-butanol 0.05 gram ninhydrin powder was added. Then 1 mL of this solution was added to the crude extract of each sample. Purple coloration indicates the presence of protein

Glycoside Test

Little amount of crude extract of each sample was boiled with sulphuric acid (H_2SO_4), filtered and benzene ether chloroform was added. The organic layer was separated and ammonia was added resulting red coloration which indicates presence of glycoside.

Molisch Test

In 100 mL ethanol 10 gm of α-napthol was added. Crude sample was mixed with it and then 1 mL sulphuric acid (H_2SO_4) was added with it. Dark pink colouration indicates the presence of carbohydrate**.**

Phenol Test

Few drops of ferric chloride solution were added to the test solution. Turbidity indicates the presence of phenol.

Steroid Test

In minimum volume of chloroform sample solution was added. Then 3-4 drops of glacial acetic acid was

added and 3 drops of concentrated sulphuric acid (H_2SO_4) was added to it. Red coloration is the indication of steroid.

QUANTITATIVE ESTIMATION OF TANNIN

An amount of 0.10 gm of sample was weighed accurately and dissolved it in 50 mL of distilled water; 750 mL of distilled water was added and shaken well. A volume of 25 mL of indigo carmine solution was added and shaken well and titrated against N/10 $KMnO_4$ till golden yellow colour end point. The experiment was repeated with the same quantity of reagents and in the same manner without the sample. The difference between the two titre values represents the indigo carmine solution required to neutralize the tannin. Each mL of 0.1N $KMnO_4$ is equivalent to 0.004157 gm of tannin (as gall tannic acid).

Percentage of tannin (as gallotannic acid) (w/w) was calculated as:

$$\frac{(A - B) \times 0.004157 \times 100 \times N}{W \times 0.1}$$

A = Volume of 0.1N $KMnO_4$ consumed in titration (Test)

B = Volume of 0.1N $KMnO_4$ consumed in titration (Blank)

W = Weight of material taken in gm

N = Normality of potassium permanganate

ESTIMATION OF ANTIOXIDANT ACTIVITY

Ferric Reducing Power Assay

The ferric reducing antioxidant power (FRAP) method was employed with minor modification[22] (Wolfe *et al.* 2003). Briefly, different concentrations of extracts (10-80 µg/mL) in 0.75 mL of distilled water were mixed with 1.25 mL of 0.2 M sodium phosphate, pH 6.6 and 1.25 mL of 1% potassium ferricyanide. After incubation at 50^0 C for 20 min, the reaction mixture was acidified with 1.25 mL trichloroacetic acid (10%). Finally, 0.5 mL of $FeCl_3$ (0.1%) was added to the reaction mixture and the absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicates greater reduction capability.

Scavenging Activity of DPPH Radicals

The samples were reacted with the stable DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) radical in an ethanol solution. The reaction mixture consisted of 50 µl of sample, 300 µl of absolute ethanol and 30 µl of DPPH radical solution (0.5 mM) in ethanol. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UV-Vis spectrophotometer. The mixture of ethanol (330 µl) and sample (50 µl) serve as blank. The control solution was prepared by mixing ethanol (350 µl) and DPPH radical solution (30 µl). The scavenging activity percentage (AA %) was determined according to Mensor *et al*[23].

$$AA\% = 100 - (Abs_{sample} - Abs_{blank}) \times 100 / Abs_{control}$$

Estimation of Total Phenol

To measure the total phenolic compounds of the extracts the modified version of the FCR assay (Matthäus, 2002)[24] was used. To a volume of 100 µl of aliquot was added 2 mL of 2% Na_2CO_3 in a tube and incubated for 2 minutes; afterwards 100 µl of Folin-Ciocalteu Phenol-Reagent (diluted with distilled water 1:1) was added. The mixtures were vortexed well and incubated in the dark for 90 minutes at 25^0 C. Hereafter the absorbance was measured at 725 nm wavelength by using a Varian Cary 50 UV-Vis spectrophotometer. A blank sample was prepared by adding 100 µl of distilled water instead of the aliquots. Gallic acid was used as a standard and a serial aqueous dilution of gallic acid solution was prepared. The standard curve was fitted by plotting absorbance versus the corresponding concentration of gallic acid solutions. The results were expressed in mg gallic acid equivalent/100 g dry matter (mg GAE/ 100 g DM).

Metal Chelating Activity

The chelation of ferrous ions by the extracts and standards was estimated by the method of Dinis *et al.* (1994)[25] to 0.5 mL extract,1.6 mL deionised water was added and 0.05 mo of $FeCl_2$ (2 Mm)was added. After 30 seconds 0.1 mL of ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex that were soluble in water. After 10 minutes of room temperature, the

absorbance of Fe^{2+}- ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated as

$$PI = \frac{A_{(control)} - A_{(sample)} \times 100}{A_{(control)}}$$

A (control) = Absorbance of control reaction
A (sample or standard) = Absorbance of sample or standard

Statistical Analysis

The experiment was performed in triplicate and data from three different experiments were subjected to analysis of variance (ANOVA) ($P < 0.05$). Statistical analysis was performed using the statistical package for social science (SPSS 16.0 for windows, SpssInc).

RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical screening of ethanolic extract of *S.cumini* pulp, *S.cumini* seed and *M.indica* peels revealed that tannin, saponin, flavonoids, quinine, glycoside, carbohydrate and phenol are present in all the samples. Phlobatanin is present in the *S.cumini* pulp and *M.indica* peel. Steroid is present only in the black plum (*S.cumini*) pulp.

Since tannins are astringent in nature these three natural antioxidant sources can be used for the treatment of gastrointestinal disorders such as diarrhoea and dysentery[26]. This may therefore explain the use of *S.cumini* as a remedy for gastrointestinal ailments.

Saponins exhibit numerous pharmacological properties such as hypocholesteromic, antidiabetic, anticarcinogenic[27], expectorant, cough suppressant and haemolytic activities[28]. These pharmacological activities of saponin explain the uses of *S.cumini* in treatment of cough, cold, fever and diabetes.

The antioxidant phytochemicals from plants, particularly flavonoids and other polyphenols, are reported to inhibit the propagation of free radical reactions, protect the human body from disease, inhibit peroxidation of polyunsaturated fatty acids in cell membranes and are antimicrobial in nature[29]. Phlobatannins are reported to possess astringent or styptic properties[30] which justify the use of *S.cumini* fruit to cure gastrointestinal disorder. Since phenolic compounds undergo non-enzymatic oxidation to quinine on exposure to air quinine was obtained in all the three samples.

Seeds of *S.cumini* was found to contain highest tannin concentration of 42% (Figure No.1) followed by *M.indica* peel (16%). Edible pulp of black plum contain the least tanin concentration of only 5%.

Estimation of Total Phenol

Phenolic compounds are considered to be important plant materials because they have both primary and secondary antioxidant activities like radical scavenging and metal chelating properties due to the structural features[31]. Therefore, it is important to determine the total phenol of an antioxidant. Phenolic concentration as depicted in Figure No.2 is found to be highest in aqueous extract of *S.cumini* seed and minimum in the pulp of the same fruit. Higher concentration of phenolic compounds was recorded in the aqueous extracts which indicate the higher solubility of these compounds in water. Concentration of these phenolic compounds was recorded to be least in ethanolic extract of the mango peel. Though the phenolic concentration declined significantly ($P<0.05$) with time *S.cumini* fruit pulp in ethanol could successfully maintain almost linear nature till the end.

Phenolic compounds are compartmentalized in the cell matrix, but once the cell matrix is broken, the compounds become prone to degradation. It has been observed that factors like temperature, light, oxygen, enzymes and pH[32] affect stability of phenolic compounds. The changes that occur may in turn affect the antioxidant properties of the phenolic compounds.

Ferric Reducing Power Assay

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action[33]. The iron (III) to iron (II)-reducing activity is expressed as gallic acid equivalents (mmol gallic acid/g sample). Figure No.3 shows that the best ferric reducing activities were found in water extract of the samples. Highest reducing activity was recorded in case of *S.cumini* seed followed by mango peel. Lowest reducing activity was exhibited by the black plum (*S.cumini*) fruit pulp with skin. A variation in

reducing nature of the samples was observed in ethanolic extract. Compared to *S.cumini* seed and edible pulp, mango peel recorded higher antioxidant value on day 7th and day 14th. Moreover *S.cumini* pulp revealed a better reduction potential in its ethanolic extract. Though pulp of *S.cumini* and mango peel delineated a comparable ferric reducing activity on day 14, the mango peel has suffered 50% loss in activity whereas the black plum pulp has only reduced by 30%. All the antioxidants had undergone a significant ($P<0.05$) fall in their reducing potential with time. Phenolics, carotenoids and anthocyanins are good electron donors and could reduce Fe^{3+}/ferricyanide complex to ferrous form, which indicates the antioxidant activity[34]. Presence of flavonoids, tannins in seed of black plum, carotenoids, mangiferrin, tannin and other polyphenols in mango peel and tannins and anthocyanins in the *S.cumini* pulp are responsible for respective ferric reducing power. High reduction potential of *S.cumini* seed in water extract can be attributed to its high tannin content (Figure No.1) followed by mango peel. Correlation study between total phenol and ferric reducing power has shown very positive correlation (+0.991) which perfectly explain the reducing behaviour of the antioxidants. Mangiferrin present in mango peel has higher solubility in alcohol which might have increased its reducing power in ethanolic extract.

Scavenging Activity of DPPH Radicals

DPPH is a stable free radical with characteristic absorption at 517 nm. Antioxidants, on interaction with DPPH, either transfer electrons or hydrogen atoms to DPPH, or convert it to 2, 2-diphenyl-1-picrylhydrazine thus neutralising the free radical[35]. The colour of the reaction mixture changes from purple to yellow and its absorbance at wavelength 517 nm decreases. The degree of discoloration indicates the scavenging potential of the antioxidant extract, which is due to the hydrogen donating ability[36]. It is evident from Figure No.4 that maximum scavenging activity was recorded by *S.cumini* seed in both the extracts. The fruit pulp extracts of *S.cumini* quenched DPPH free radicals quite effectively whereas scavenging activity for mango peel was much less than these two samples. Radical scavenging activity of all these antioxidants decreased significantly ($P<0.05$) with time. Particularly after day 14 the values declined sharply. Greater scavenging activity was recorded in ethanolic extract of the samples with an exception in case of *M.indica* peel. The activity was higher in aqueous extract of mango peel and it was noted that activity increased (25% in aqueous extract and 100% in ethanolic extract) on day 14 and finally declined on 21st day. Polymeric polyphenols are more potent antioxidants than simple monomeric phenolics. Hagerman[37] (1998) demonstrated the higher antioxidant ability of condensed and hydrolysable tannins at quenching peroxyl radicals over simple phenols. Figure No.1 demonstrates higher concentration of tannin in the seeds of black plum which might have attributed to its higher scavenging activity.

Metal Chelating Activity

Ferrozine can quantitatively form complexes with Fe^{2+}. However, in presence of other chelating agents this complex formation is disrupted. This results in the decrease in red colour intensity of the solution. Measurement of colour reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. The ferrous ions possess the ability to move single electrons by virtue of which it triggers propagation of many radical reactions, even with relatively non-reactive radicals[38]. Main strategy to avoid the reactive oxygen species generation is through chelating metal ions. Phenolic compounds, flavonoids in particular, are able to chelate metals due to the presence of hydroxyl groups attached to their ring structures[31]. In the present study Figure No.5 delineates the metal chelating activity of the selected natural antioxidants. From the graph it is evident that highest chelating activity was observed in *S.cumini* fruit. Seeds of black plum recorded a higher activity than mango peel. Antioxidants in aqueous extract recorded a higher activity than ethanolic solutions. Metal chelating activity is found to increase significantly ($P<0.05$) with time. Maximum increase in activity (590%) occurred in case of ethanolic extract of mango peel from day 7 to 21. The order of activity is similar in both the

extracts which are as follows: pulp of *S.cumini*> seed of *S.cumini*> peel of *M.indica*.

Table No.1: Phytochemical screening report of the antioxidants

S.No	Phytochemicals	*S.cumini* pulp	*S.cumini* seed	*M.indica* peel
1	Tannin	+	+	+
2	Phlobatannin	+	-	+
3	Saponin	+	+	+
4	Flavonoid	+	+	+
5	Quinine	+	+	+
6	Amino acid	-	-	-
7	Glycoside	+	+	+
8	Carbohydrate	+	+	+
9	Phenol	+	+	+
10	Steroid	+	-	-

(+ indicates present and - indicates absent)

TANIN ESTIMATION

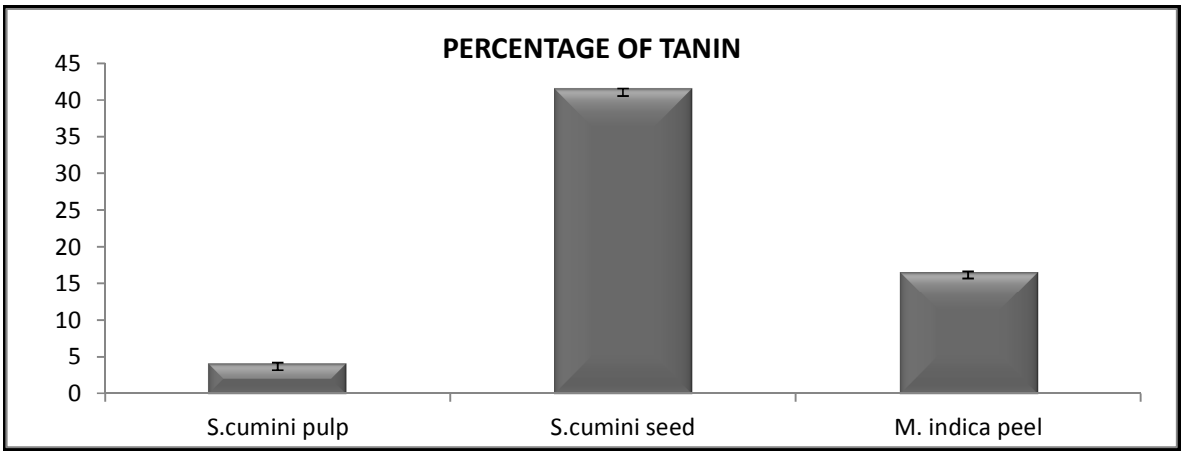

Figure No.1: Graph showing the percentage of tanin

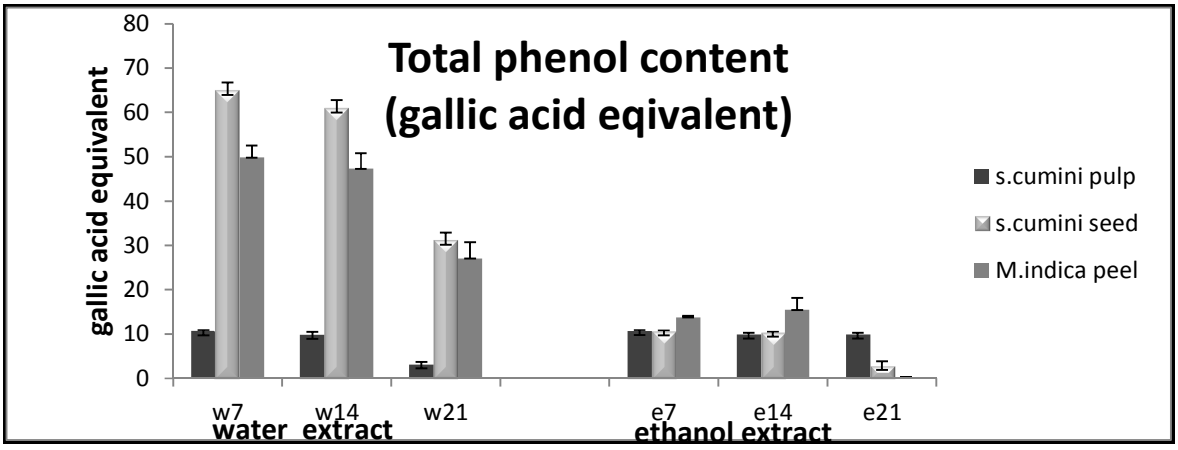

Figure No.2: Graph showing the total phenol content in gallic acid equivalent/gm of the aqueous and ethanolic extract of antioxdants on 7th, 14th, 21st day.

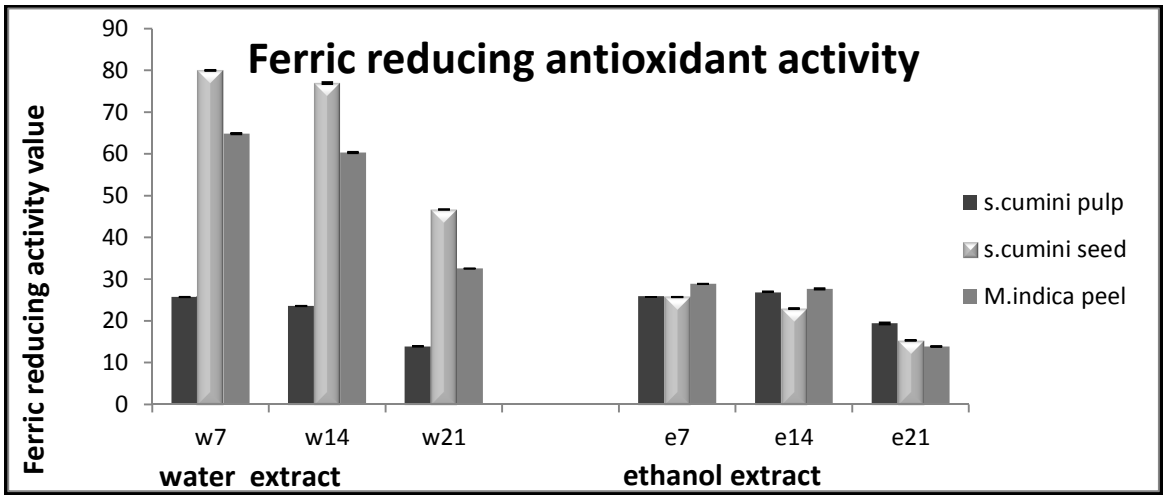

Figure No.3: Graph showing the Ferric reducing activity of the aqueous and ethanolic extract of antioxdants on 7th, 14th, 21st day.

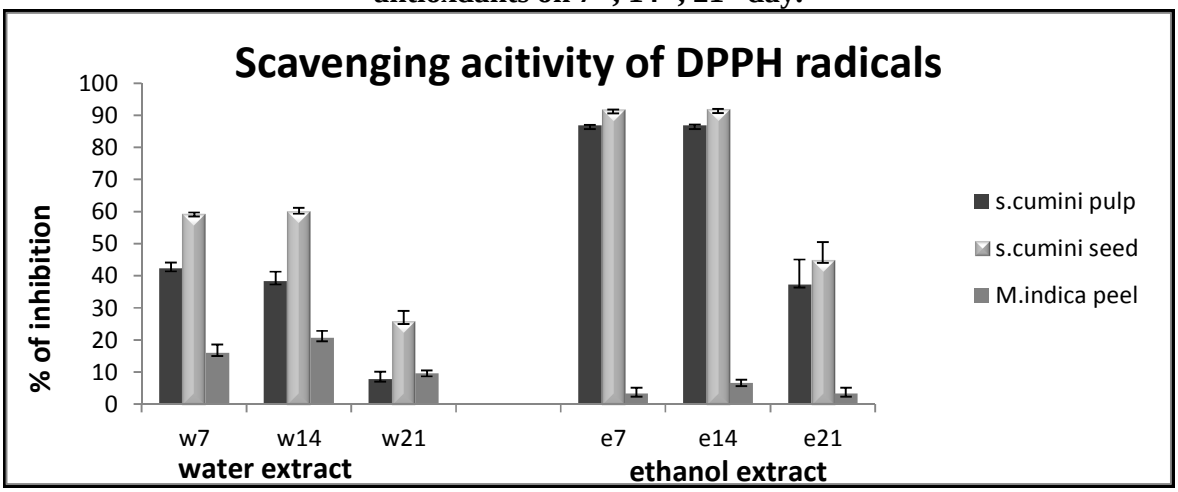

Figure No.4: Graph showing the DPPH scavanging activity of the aqueous and ethanolic extract of antioxdants on 7th, 14th, 21st day.

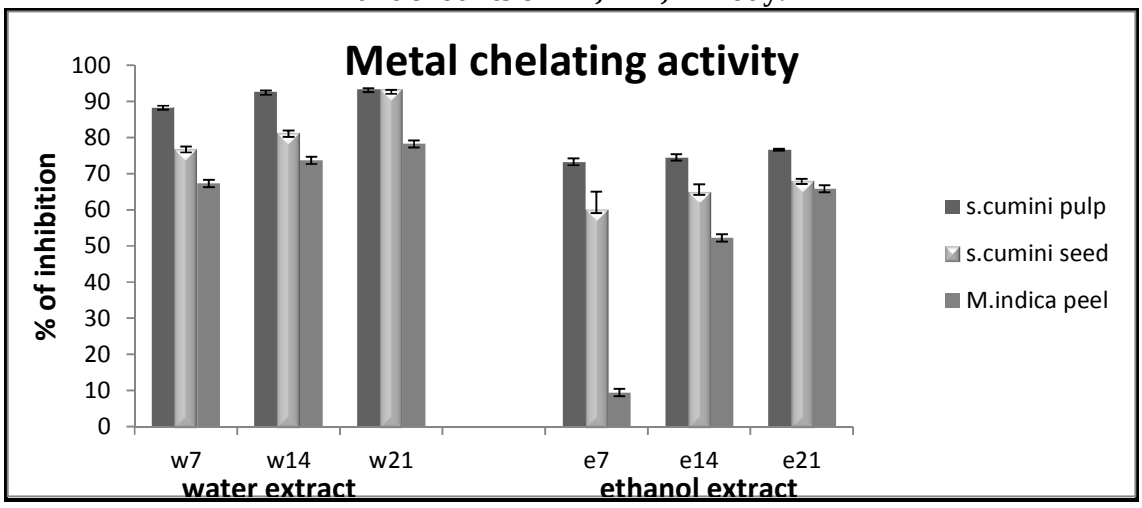

Figure No.5: Graph showing the metal chelating activity of the aqueous and ethanolic extract of antioxdants on 7th, 14th, 21st day.

CONCLUSION

This study revealed that maximum antioxidant activity (except metal chelating) was exhibited by the black plum (*S.cumini*) seed. The edible pulp and skin of *S.cumini* also recorded a high scavenging activity and highest metal chelating activity. Total phenol and hence ferric reducing power of mango peel was comparable with the activity of *S.cumini* seed. Since most of the antioxidant compounds present in the samples are water soluble metal chelating activity, total phenol content and ferric reducing power was found to be higher in aqueous extract of the antioxidants but DPPH radical scavenging activity was higher in ethanolic extract. Thus it can be finally concluded that *S.cumini* seed was found to be most effective antioxidant.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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