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Evaluation Of Antioxidant Activities And Free Radical Scavenging Properties In Mango Leaves, Husks Of Areca And Coconut.

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ABSTRACT

Numerous plant phenolics are identified as potent antioxidants, capable of scavenging deleterious reactive species such as superoxide anions, singlet oxygen, hydroxy radicals, nitric oxide and peroxynitrite. In addition to radical-scavenging and reducing power, plant phenolics are known to induce antioxidant action. In this study, an attempt was made to evaluate the antioxidant activities and free radical scavenging properties in mango leaves, husks of areca and coconut. Hundred grams of the plant powder was extracted in a Soxhlet apparatus with 500 ml of ethanol as solvent and concentrated using a rotor-evaporator. Plant extracts were dissolved in dimethyl sulfoxide (DMSO) and subjected to standard antioxidant assays. The final concentrations used for the study were 10mg/ml, 20mg/ml, 40mg/ml and 80mg/ml. Hydroxyl radical scavenging activity, diphenylpicryl- hydrazyl (DPPH) radical scavenging assay, nitric oxide radical (NO) scavenging assay, superoxide radical scavenging assay and reducing power assay were performed. When analysed, all plant materials exhibited efficient free radical scavenging activity in the order mango leaf > coconut husk > areca husk. Hydroxyl radical and super oxide radical scavenging activity and reducing power of both mango leaf and coconut husk were comparable to each other, in fact, mango leaf exhibited better NO and DPPH scavenging activity than coconut husk. In all assays performed, activity of areca husk was found to be relatively less.

Keywords: Antioxidant; free radical scavenging; mango leaves; coconut husk; areca husk

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INTRODUCTION

Research focusing on role of free radicals and antioxidants has gained significant importance in the recent past. Supra physiological concentration of nitric oxide (NO) radical is cytotoxic and generally accepted that the cytotoxicity is mediated by peroxynitrite ($ONOO^-$), the reaction product of NO and superoxide radical. This $ONOO^-$ may be transported across the cell membrane by passive diffusion or active anionic transport to oxidize the lipid molecules, modify amino acids and proteins, inhibit tyrosine-, thyol- or Fe-S- containing enzyme systems, activate polymerase cell degradation pathways, modify DNA base and induce DAS breaks[1]. It is suggested that the free radical complex causes redox cycling that generates superoxide anion from molecular oxygen and leads to the formation of hydrogen peroxide and hydroxyl radical [2]. The effective detoxification mechanism, the antioxidant defence system works in a sequential manner in the disposal of superoxide radical and the conversion of hydrogen peroxide to water.

Many investigations on free radical scavenging activity have been carried out by researchers and the results indicate efficient free radical scavenging and dose-dependent antioxidant activities of mango leaf extract [3-11]. A few studies are available with regard to coconut husk extract including that of Chakraborty and Mitra and Oliveira *et al.,* who demonstrated antioxidant activity of the methanolic extract of coconut husk[12, 13]. Khonkarn *et al.,* reported that the methanolic extract of coconut peel had trolox equivalent antioxidant activity equivalent to vitamin E [14].

An antioxidant is any substance which delays or inhibits oxidative damage to a target molecule [15]. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [16].

Numerous plant phenolics are identified as potent antioxidants, capable of scavenging deleterious reactive species such as superoxide anions, singlet oxygen, hydroxy radicals, nitric oxide and peroxynitrite [13, 17-19]. In addition to radical-scavenging and reducing power [2], plant phenolics are known to induce antioxidant action through other mechanisms. In the present study, an attempt has been made to evaluate the antioxidant activities and free radical scavenging properties in mango leaves, husks of areca and coconut.

MATERIALS AND METHODS

Preparation of plant extracts

Fresh mango leaves, husk of ripe coconut and areca nut were collected from the native, where those are grown for non-commercial purpose. The plant materials were washed in tap water to remove the dirt, followed by distilled water, cut into smaller pieces and dried under shade. The dried materials were powdered using household electric blender. Hundred grams of the plant powder was extracted in a Soxhlet apparatus with 500 ml of ethanol as solvent and concentrated using a rotor-evaporator. The crude alcoholic extracts thus prepared were used for various analyses.

Plant extracts were dissolved in dimethyl sulfoxide (DMSO) and subjected to standard antioxidant assays. One hundred mg plant extract was first dissolved in 1 ml DMSO. Hundred µl was taken from this and further diluted in 1ml DMSO and different volumes such as 25 µl, 50 µl, 100 µl, 200 µl were taken from the diluted extracts and used for further analysis. Thus the final concentrations used for the study were 10mg/ml, 20mg/ml, 40mg/ml and 80mg/ml. All experiments were carried out using above concentrations, in triplicate and the mean value was considered as the result at that particular concentration. Following assays were performed:

Hydroxyl radical scavenging activity [21]

The radical scavenging activity of extracts was determined using Fenton's reaction on $FeCl_3/H_2O_2$ mixture. Briefly various concentrations of the extract was mixed with 1 ml of reaction buffer (100 µM $FeCl_3$, 104 µM EDTA, 1.5 mM H_2O_2, 2.5 mM deoxyribose and 100 µM L-Ascorbic acid; pH 7.4) and incubated for 1 hour at 37ºC. 1 µl of 0.5% 2- thio barbituric acid in 0.025 M NaOH and 1ml 2.8% TCA (Trichloro acetic acid) was added to the mixture and heated for 30 minutes at 80ºC. The colour developed was measured at 532 nm against a

blank containing the phosphate buffer using a spectrophotometer. Ascorbic acid was used as a positive control. The inhibitory effect on the activity of hydroxyl radical was calculated as:

$$\text{OH radical scavenging (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}}$$

Diphenylpicryl- hydrazyl (DPPH) radical scavenging assay [22]

The amount of extract per ml at which the absorbance at 517 nm decreases to half its initial value was used as the antioxidant value for the extract. 1.0 ml of 500µM DPPH in methanol was mixed with equal volume of extract solution in phosphate buffer (pH 7.4), mixed well and kept in dark for 30 minutes. The absorbance at 517 nm was monitored in the presence of different concentrations of the extracts. Blank experiment was also carried out to determine the absorbance of DPPH before interacting with the extract. In this assay, the positive control was ascorbic acid and the percentage of inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}}$$

Nitric oxide radical (NO) scavenging assay [23]

Sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) was mixed with different concentration of extract and incubated at 25ºC for 180 minutes. The samples from the above were reacted with Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid). The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine dichloride was read at 546 nm. Ascorbic acid was a positive control.

$$\text{NO radical scavenging (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}}$$

Superoxide radical scavenging activity [21]

This activity was measured by the reduction of nitro blue tetrazolium (NBT) according to method reported by Hazra *et al.,*[21]. The nonenzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce NBT to a purple formazan. The 1 ml reaction mixture contained phosphate buffer (20 mM, pH 7.4), NADH (73 µM), NBT (50 µM), PMS (15 µM) and various concentrations of sample solution. After incubation for 5 min at 25ºC temperature, the absorbance at 562 nm was measured against a blank to determine the quantity of formazan generated. Ascorbic acid was used as a positive control.

$$\text{Superoxide scavenging (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}}$$

Reducing power assay [24]

The $Fe3^{+}$-reducing power of the extract was determined by the method of Oyaizu[24] with a slight modification. Different concentrations of the extract was mixed with 0.5 ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml potassium hexacyanoferrate (0.1%), followed by incubation at 50°C in a water bath for 20 minutes. After incubation, 0.5 ml of 10% TCA was added to terminate the reaction. The upper portion of the solution (1 ml) was mixed with 1 ml distilled water, and 0.1 ml $FeCl_3$ solution (0.01%) was added. The reaction mixture was left for 10 minutes at room temperature and the absorbance was measured at 700 nm against a blank solution. A higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as a positive control.

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RESULTS

Antioxidant activities and free radical scavenging properties of plant materials

When the antioxidant potentials of the ethanolic extracts of selected plant materials were evaluated, all the tested plants showed free radical scavenging effect in the form of DPPH scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging and superoxide radical scavenging. When different concentrations ranging from 10 to 80 mg/ml of mango leaf extracts were subjected to various standard biochemical tests to detect free radical scavenging activities, IC_{50} value of 14.94, 35.25, 48.25 and 68mg/ml for DPPH scavenging, hydroxyl radical scavenging, nitric oxide radical scavenging and superoxide radical scavenging respectively. Similarly, when coconut extracts were tested the IC_{50} values obtained were 39.01, 45.5, 55 and 66.5 mg/ml and when areca nut husk were tested 51.5, 65.5, 76.5 and 70 mg/ml (Table 1). When the IC_{50} values of different radical scavenging assays were compared, it shows that the values are relatively closer in case of mango leaves and coconut husk and is comparable to the test standard sample used while all values were slightly less in areca nut husk extract.

Table 1: Free radical scavenging and scavenging capacity of biologically relevant oxidants of selected plant extracts with IC_{50} value (mg/ml)

Extract/positive control	DPPH	OH◦ radical	NO◦ radical	O2-- scavenging
Mango leaf extract	14.94	35.25	48.25	68.0
Coconut husk extract	39.01	45.5	55.0	66.5
Areca husk extract	51.5	65.5	76.5	70.0

Hydroxyl radical scavenging activity

The percentage of inhibition (hydroxyl radical scavenging ability) at concentrations of mango leaves, coconut husk and areca nut husk are shown in (Table 2a). There was statistical significance in hydroxyl radical scavenging activity between the different concentrations of different plants and between different plant materials studied while analyzing the results statistically with two-way ANOVA (Table 2b).

Table 2a: Hydroxyl radical scavenging activity of different plant extracts with various concentrations

Concentration (mg/ml)	Experiments							
Samples	Mango leaf		Coconut husk		Areca husk		Total	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
10	42.810	1.829	15.480	2.548	13.907	1.392	24.066	14.179
20	55.353	.451	54.083	3.608	26.050	1.438	45.162	14.477
40	57.390	.104	58.217	2.872	46.347	2.212	53.984	6.019
80	66.357	.192	58.523	2.689	52.360	2.953	59.080	6.396
Total	55.478	8.815	46.576	19.009	34.666	16.235	45.573	17.193

Dependent Variable: Activity level, Test: Hydroxyl radical scavenging, Result expressed as % inhibition, n=3

Table 2b: Comparison of hydroxyl radical scavenging activity of plant extracts in various concentrations

Source	F value	df	P value	Significance
Expt. A	279.349	2.24	.000	HS
Concentration	458.554	3.24	.000	HS
Expt. A* Concentration	41.736	6.24	.000	HS

Two-way ANOVA results**,** Dependent Variable: Activity level, HS – highly significant ($p < 0.01$)

When the activity of different plant materials was compared using Tukey HSD, it was observed that at a concentration of 10mg/ml mango leaves are exhibiting significantly high activity than other two plant materials. At the same time no significant difference in activity was observed between coconut husk and areca nut husk at 10mg/ml concentration. At 20 and 40 mg/ml concentrations no significant difference was observed between mango leaves and coconut husk, while other experimental groups showed highly significant difference. Hydroxyl radical scavenging activity at concentration of 80mg/ml was found to be significantly higher in mango leaves than other materials and in coconut husk more than areca nut husk (Table 2c).

Table 2c: Comparison of hydroxyl radical scavenging activity between plant extracts

Concentration (mg/ml)	(I)Experiments	(J)Experiments	Mean Diff (I-J)	SE	P	Significance
10	Mango leaf	Coconut husk	27.33000	1.617827	.000	HS
		Areca husk	28.90333	1.617827	.000	HS
	Coconut husk	Areca husk	1.57333	1.617827	.619	
20	Mango leaf	Coconut husk	1.27000	1.843467	.778	
		Areca husk	29.30333	1.843467	.000	HS
	Coconut husk	Areca husk	28.03333	1.843467	.000	HS
40	Mango leaf	Coconut husk	-.82667	1.709592	.881	
		Areca husk	11.04333	1.709592	.002	HS
	Coconut husk	Areca husk	11.87000	1.709592	.001	HS
80	Mango leaf	Coconut husk	7.83333	1.884620	.014	HS
		Areca husk	13.99667	1.884620	.001	HS
	Coconut husk	Areca husk	6.16333	1.884620	.039	HS

Test: OH radical scavenging, Dependent Variable: Activity level, Tukey HSD, Based on observed means. HS-Highly significant ($p<0.01$)

DPPH radical scavenging assay

DPPH radical scavenging properties of mango leaves, coconut husk and areca nut husk are shown in (Table 3a). When the values obtained for DPPH scavenging activity at different concentrations for coconut husk and areca nut husk was statistically analyzed using two-way ANOVA, highly significant difference was obtained ($p\leq0.05$) between different groups and different concentrations (Table 3b). Similarly, when DPPH scavenging activity between the plant materials were compared using Tukey HSD it was observed that at all concentrations studied, the values obtained in case of mango leaf was significantly greater than that of coconut husk and areca nut husk. When similar comparison was done between coconut husk and areca nut husk, coconut husk was found more effective with higher values with high difference ($p \leq 0.05$, Table 3c).

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Table 3a: DPPH activity of different plant extracts in various concentrations

Concentration (mg/ml)	Experiments							
Samples	Mango leaf		Coconut husk		Areca husk		Total	
	Mean	S.D.	Mean	S.D.	Mean	S.D	Mean	S.D.
10	92.810	1.829	27.370	1.794	18.600	.792	46.260	35.144
20	95.353	.451	56.200	1.983	45.257	1.591	65.603	22.847
40	97.390	.104	67.653	1.900	62.280	1.418	75.774	16.421
80	96.357	.192	73.163	2.603	61.337	3.050	76.952	15.558
Total	95.478	1.951	56.097	18.550	46.868	18.524	66.148	25.965

Test: DPPH scavenging, Dependent Variable: Activity level

Table 3b: Comparison of DPPH activity of plant extracts between concentrations

Source	F value	Df	P value	Significance
Expt. A	2695.106	2.24	.000	HS
Concentration	611.857	3.24	.000	HS
Expt. A* Concentration	117.015	6.24	.000	HS

Two-way ANOVA results, Dependent variable: Activity level, HS – highly significant (p<0.01)

Table 3c: Comparison of DPPH activity between plant extracts (Multiple Comparisons)

Dependent Variable: Activity level
Tukey HSD
Test : DPPH Scavenging

Concentration (mg/ml)	(I)Experiments	(J)Experiments	Mean Difference (I-J)	Std. Error	P	Significance
10	Mango Leaf	Coconut husk	65.44000	1.264744	.000	HS
		Areca husk	74.21000	1.264744	.000	HS
	Coconut husk	Areca husk	8.77000	1.264744	.001	HS
20	Mango Leaf	Coconut husk	39.15333	1.216970	.000	HS
		Areca husk	50.09667	1.216970	.000	HS
	Coconut husk	Areca husk	10.94333	1.216970	.000	HS
40	Mango Leaf	Coconut husk	29.73667	1.118723	.000	HS
		Areca husk	35.11000	1.118723	.000	HS
	Coconut husk	Areca husk	5.37333	1.118723	.007	HS
80	Mango Leaf	Coconut husk	23.19333	1.892406	.000	HS
		Areca husk	35.02000	1.892406	.000	HS
	Coconut husk	Areca husk	11.82667	1.892406	.002	HS

Based on observed means, HS – Highly significant P< 0.01

Nitric oxide radical (NO) scavenging assay

Nitric oxide radical scavenging properties (percentage of inhibition) of mango leaf, coconut husk and areca nut husk are shown in (Table 4a). When the nitric oxide scavenging activity at different concentrations for mango leaf, coconut husk and areca nut husk were analyzed using Two way ANOVA a statistically significant difference between groups and concentrations were noted($p<0.05$, Table 4b). When the activity of different plant materials were compared, it was observed that at all concentration of mango leaves are exhibiting significantly high activity than other two plant materials except at concentration of 200 where the activities of all plant materials are comparable without any significant difference. While comparing the activities of coconut

husk and areca nut husk significant difference was noted at 10 & 20mg/ml concentrations while no significant difference in activity was observed at 20 & 40mg/ml concentrations (Table 4c).

Table 4a: Nitric oxide scavenging activity of different plant extracts in various concentrations

Concentration (mg/ml)	Experiments							
Samples	Mango leaf		Coconut husk		Areca husk		Total	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
10	32.810	1.829	18.733	2.065	12.800	1.051	21.448	9.022
20	45.353	.451	25.593	1.435	26.253	.577	32.400	9.753
40	47.767	.104	37.393	1.488	29.963	2.375	38.374	7.875
80	56.357	.192	60.277	2.097	62.530	4.437	59.721	3.654
Total	45.478	8.842	35.499	16.559	32.887	19.206	37.986	16.045

Test: Nitric oxide scavenging, Dependent Variable: Activity level

Table 4b: Comparison of nitric oxide scavenging activity of plant extracts in various concentrations (Two-way ANOVA results)

Source	F value	Df	P value	Significance
Expt. A	148.228	2.24	.000	HS
Concentration	642.009	3.24	.000	HS
Expt. A* Concentration	39.015	6.24	.000	HS

Test:Nitric oxide scavenging, Dependent Variable: Activity level, HS – Highly significant (p<0.01)

Table 4c: Comparison of nitric oxide scavenging activity between plant extracts

Concentration (mg/ml)	(I)Experiments	J)Experiments	Mean Diff (I-J)	SE	P	Significance
10	Mango leaf	Coconut husk	14.07667	1.391644	.000	HS
		Areca husk	20.01000	1.391644	.000	HS
	Coconut husk	Areca husk	5.93333	1.391644	.013	Sig.
20	Mango leaf	Coconut husk	19.76000	.759576	.000	HS
		Areca husk	19.10000	.759576	.000	HS
	Coconut husk	Areca husk	-.66000	.759576	.678	
40	Mango leaf	Coconut husk	10.37333	1.346246	.001	HS
		Areca husk	17.80333	1.346246	.000	HS
	Coconut husk	Areca husk	7.43000	1.346246	.004	HS
80	Mango leaf	Coconut husk	-3.92000	2.315084	.282	
		Areca husk	-6.17333	2.315084	.083	
	Coconut husk	Areca husk	-2.25333	2.315084	.618	

Test: Nitric oxide scavenging, Dependent Variable: Activity level, Tukey HSD, Based on observed means. HS-Highly significant (p<0.01), Sig. Significant (p<0.05)

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Superoxide radical scavenging

Superoxide scavenging properties (percentage of inhibition) of mango leaf, coconut husk and areca nut husk are shown in (Table 5a). When the superoxide scavenging activity between different concentrations were statistically analyzed using two-way ANOVA, there was statistical significance between different concentrations for all plants ($p<0.05$ Table 5b). When the activity of different plant materials was compared, activity of all plant materials at concentration of 10 & 80mg/ml were comparable to each other. At concentrations 20 & 40 mg/ml activity of areca catechu was significantly lower than other two plants which were comparable to each other (Table 5c).

Table 5a: Superoxide scavenging activity of different plant extracts in various concentrations

Dependent Variable: Activity level
Est: Superoxide Scavenging

Concentration (mg/ml)	Experiments							
	Mango leaf		Coconut husk		Areca husk		Total	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
10	22.913	1.829	25.277	1.218	22.510	1.506	23.567	1.857
20	35.363	.437	33.493	2.190	28.687	1.007	32.514	3.225
40	57.390	.104	55.503	3.484	37.947	1.304	50.280	9.471
80	62.043	1.187	64.693	1.705	52.330	13.527	59.689	8.866
Total	44.428	16.733	44.472	16.789	35.368	13.104	41.513	15.811

Table 5b: Comparison of superoxide scavenging activity of plant extracts in various concentrations (Two-way ANOVA results)

Source	F value	df	P value	Significance
Expt. A	19.047	2.24	.000	HS
Concentration	136.156	3.24	.000	HS
Expt.A *Concentration	3.254	6.24	.017	Sig

Dependent Variable: Activity level
Est: Superoxide Scavenging

Table 5c: Comparison of superoxide scavenging activity between plant extracts in various concentrations (Multiple Comparisons)

Concentration (mg/ml)	(I)Experiments	(J)Experiments	Mean Difference (I-J)	Std. Error	.P	Significance
10	Mango Leaf	Coconut husk	-2.36333	1.255899	.224	
		Areca husk	.40333	1.255899	.945	
	Coconut husk	Areca husk	2.76667	1.255899	.149	
20	Mango Leaf	Coconut husk	1.87000	1.155018	.309	
		Areca husk	6.67667	1.155018	.003	HS
	Coconut husk	Areca husk	4.80667	1.155018	.014	Sig
40	Mango Leaf	Coconut husk	1.88667	1.754218	.562	
		Areca husk	19.44333	1.754218	.000	HS
	Coconut husk	Areca husk	17.55667	1.754218	.000	HS
80	Mango Leaf	Coconut husk	-2.65000	6.451321	.912	
		Areca husk	9.71333	6.451321	.353	
	Coconut husk	Areca husk	12.36333	6.451321	.214	

Test: superoxide scavenging, Dependent Variable: Activity level, Tukey HSD, Based on observed means.
HS – Highly significant $p<0.01$, sig. significant ($p<0.05$)

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Reducing power

Reducing power (percentage of inhibition) of mango leaf, coconut husk and areca nut husk are shown in (Table 6a). When the superoxide scavenging activity between different concentrations were statistically analyzed using two-way ANOVA, there was statistical significance between different concentrations for all plants ($p<0.05$, Table 6b.)

Table 6a: Reducing power of plant extracts in various concentrations

Concentration (mg/ml)	Experiments							
Samples	Mango leaf		Coconut husk		Areca husk		Total	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
10	.246	.001	.226	.001	.096	.001	.190	.07
20	.266	.003	.252	.001	.124	.001	.21	.07
40	.280	.002	.272	.001	.138	.001	.23	.07
80	.327	.006	.331	.001	.156	.003	.27	.09
Total	.280	.031	.270	.041	.129	.023	.23	.08

Test: Reducing power, Dependent Variable: Activity level

Table 6b -Comparison of reducing power of plant extracts in various concentrations

Two way ANOVA results
Dependent Variable: Activity
Level Est: Superoxide Scavenging

Source	F value	df	P value	Significance
Expt. A	17320.173	2.24	.000	HS
Concentration	2141.667	3.24	.000	HS
Expt. A * Concentration	81.164	6.24	.000	HS

Comparison of different plant extract showed a highly significant difference in reducing power between the plant extracts at every concentration except mango leaf and coconut husk extracts in concentration of 80mg/ml (Table 6c).

Table 6c: Comparison of reducing power between different plant extracts (Multiple Comparisons)

Concentration (mg/ml)	(I)Experiments	(J)Experiments	Mean Difference (I-J)	Std. Error	P	Significance
10	Mango Leaf	Coconut husk	.02033	.000770	.000	HS
		Areca husk	.15000	.000770	.000	HS
	Coconut husk	Areca husk	.12967	.000770	.000	HS
20	Mango Leaf	Coconut husk	.01433	.001656	.000	HS
		Areca husk	.14233	.001656	.000	HS
	Coconut husk	Areca husk	.12800	.001656	.000	HS
40	Mango Leaf	Coconut husk	.00767	.001186	.002	HS
		Areca husk	.14167	.001186	.000	HS
	Coconut husk	Areca husk	.13400	.001186	.000	HS
80	Mango Leaf	Coconut husk	-.00433	.002919	.362	
		Areca husk	.17100	.002919	.000	HS
	Coconut husk	Areca husk	.17533	.002919	.000	HS

Test: Reducing power, Dependent Variable: Activity level, Tukey HSD, Based on observed means.
HS- Highly significant ($p<0.01$)

DISCUSSION

Several biochemical assays were used to screen the antioxidant properties of mango leaves, husk of coconut and areca, such as scavenging activity on DPPH radicals (measuring the decrease in DPPH radical absorption after exposure to radical scavengers), scavenging activity on hydroxyl radicals, nitric oxide radical and superoxide radical (measuring the decrease in radical absorption after exposure to radical scavengers) and reducing power (measuring the conversion of a Fe^{3+}/ferri cyanide complex to the ferrous form). The assays were performed for each extract separately using whole extracts instead of individual compounds. Whole extract was used in this study keeping in mind the additive and synergistic effects of phytochemicals in plants responsible for their potent bioactive properties and the fact that it is not single but the combination of natural phytochemicals in a complex mixture sometimes has more antioxidant potential [25]. In complex systems, various different mechanisms may contribute to oxidative processes and different reactive oxygen species might be generated targeting various structures such as lipids, proteins and carbohydrates. Therefore, the extracts were subjected to variety of antioxidant assays to elucidate their potential to scavenge wide variety of free radicals [26].

One of the assays carried out was hydroxyl radical scavenging assay and generally a concentration dependent increase in activity was noted in case of all three plant materials studied. The percentage inhibition noted at highest concentration studied was comparable to standard ascorbic acid. Hydroxyl radical is one of the potent reactive oxygen species in the biological system which reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. The activity of the studied plant material mainly can be ascribed to phenolic hydroxyls in flavonoids which are the main active groups capable of scavenging hydroxyl radical.

Another assay carried out was DPPH assay which is a widely used model to evaluate the antioxidant property of plant extracts. DPPH is a stable nitrogen-centered free radical, the color of which- changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers. Our analysis showed concentration dependent excellent DPPH radical scavenging activity of tested plant materials. The ability of the extracts to scavenge $DPPH^{\cdot}$ radicals was in the order of mango leaf > coconut husk>areca husk and the IC_{50} values were 51.5, 39.01 and 14.94 respectively.

Likewise, all extracts revealed a good scavenging activity on superoxide radical and NO radical and in all assays the scavenging activity was found to be increasing with increasing concentration of extracts. Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress [27]. Numerous biological reactions generate superoxide anions and NO radicals which are highly toxic species and contribute to significant levels of oxidative stress. As all the three plant extracts studied exhibited significant inhibition of these radicals, it can be stated that they are efficient scavengers of superoxide radicals and NO radicals.

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [28]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [29]. From the analysis we can conclude that the reducing power of all extracts increased with the concentration increase and were excellent, especially in the case of mango leaves and coconut husk compared to areca husk. With regards to reducing power, higher reducing activities can be attributed to higher amounts of polyphenolics and the reducing capacity of a compound may reflect its antioxidant potential [30]. It has been reported that the reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [31]. Hence, it can be presumed that mango leaves and coconut husk may have the highest amounts of reductones and polyphenolics compared to areca husk.

Our observation of significant free radical scavenging and dose-dependent antioxidant activities of mango leaf extract is consistent with earlier reports [3-11]. Kawpoomhae *et al.,* reported IC_{50} values for the DPPH

scavenging activity of the methanol extracts of mango leaf as 6.18 ± 0.15, while we have recorded the value 14.94 [6]. The difference in observation could be due to different cultivar used or the influence of local environment in which it was grown. In contrast to our observation, in a study conducted by Olabinri *et al.,* it was reported that polyphenol extract from mango leaves failed to scavenge hydroxyl radical at all the concentrations [11]. DPPH scavenging of mango leaf alcoholic extracts reported by Maha-ard et *al*., is closely similar to our observation [8]. The authors after comparing alcoholic and water extracts concluded that the type of extraction solvent and mango varieties plays the major role on quantity of phenolic compounds and the antioxidant activity [8]. Four major phenolic compounds detected in mango leaves were mangiferin, penta- O-galloyl-glucoside gallic acid, and methyl gallate conferring these plant material a good antioxidant activity [9].

With regard to coconut husk extract only a few studies were found after thorough search in the literature. And our observations were consistent with earlier reports [10,11,]. Khonkarn *et al*., reported that the methanolic extract of coconut peel had trolox equivalent antioxidant activity equivalent to vitamin E [14]. Antioxidant properties of coconut husk extract can be ascribed to its phenolic components. The major phenolic compound identified in coconut husk is 4-hydroxybenzoic acid and other phenolic acids such as ferulic acid, 4-coumaric acid, 4-hydroxybenzaldehyde and vanillic acid are also detected [32]. In addition, earlier reports indicate presence of catechins and epicatechin in *Cocos nucifera* husk extract [33].

In connection with antioxidant activities of areca husk only two previous reports could be obtained from the literature. Wetwitayaklung *et al.,* studied the antioxidant activities in various ages of seeds and various parts of areca including the fruit peel and reported presence of tannin and phenolic components in all parts studied and also antioxidant activity. This study had highlighted the activity of areca seed rather than husk [34]. An extensive investigation on antioxidant activity of *Areca catechu* flower, husk and seed extracts was done by Zhang, who reported efficient antioxidant activities of areca husk [35]. Although the actual values noted by Zhang are different from our study, we also have observed efficient free radical scavenging activity of areca husk. As studied before, the *Areca catechu* L. plant contains a diverse group of phenolic compounds with antioxidant activity, including flavonoids, lignans and stilbenes, and simple phenolic acids, such as hydroxybenzoic acids and hydroxycinnamic acids, syringic acid and epicatechin which may be attributed to antioxidant activity [36].

Overall, phenolic compounds in plant extracts contribute significantly to their antioxidant potential because of their unique structure. Phenolics are composed of one or more aromatic rings bearing single or multiple hydroxyl groups and are therefore potentially able to quench free radicals by forming stabilized phenoxyl radical [37,38]. It is generally believed that plants which are having more phenolic content show good antioxidant activity and scientific evidences indicate that there is a direct correlation between total phenol content and antioxidant activity [39-42]. However, there are reports which do not show this correlation [43, 44]. In the same way we also have observed a correlation with phenolic component and antioxidant activity. Mango leaf which showed higher phenolic content showed comparatively better scavenging activity followed by coconut husk and areca husk.

In conclusion, as all three studied plant materials revealed free radical scavenging activity and reducing power, it can be suggested that these are useful antioxidants for the nutraceutical industry. Therefore, further studies are required for isolation and identification of antioxidant active compounds from ethanol extracts of these materials before making it available for benefits for human health.

CONCLUSION

When analysed, all plant materials exhibited efficient free radical scavenging activity in the order mango leaf > coconut husk > areca husk. Hydroxyl radical and super oxide radical scavenging activity and reducing power of both mango leaf and coconut husk were comparable to each other, in fact, mango leaf exhibited better NO and DPPH scavenging activity than coconut husk. In all assays performed, activity of areca husk was found to be relatively less.

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