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RESEARCH ARTICLE

CYTOPROTECTIVE ACTIVITY OF *COCOS NUCIFERA* AND *MANGIFERA INDICA* FLOWER EXTRACTS

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Abstract: *Cocos nucifera* and *Mangifera indica* are being exploited largely for its wide therapeutic applications around the world. Present study investigated antioxidant and antihemolytic activities of flower extracts of *Cocos nucifera* and *Mangifera indica*. Aqueous, methanol, acetone, diethyl ether and hexane extracts were used for the study. Antioxidant competence of the five extracts was assessed by DPPH and TBARS method. Antihemolytic assay was determined using erythrocytes model and the extent of membrane damage was determined by quantifying Malondialdehyde. Among all the five extracts studied, methanolic extract of *Cocos nucifera* exhibited significant antioxidant as well as antihemolytic activities.

Key words: Cocos nucifera, Mangifera indica, Antioxidant, Antihemolytic, Erythrocytes, Malondialdehyde

INTRODUCTION:

Reactive oxygen species (ROS) generated in cells, are fundamental in modulating various physiological functions and represent an essential part of aerobic life and metabolism. However excessive generation of ROS disrupts the antioxidant defense system of the body which may lead to oxidative stress[1]. Oxidative stress have been implicated in the etiology of several degenerative disorders including cancer, diabetes, rheumatoid arthritis, atherosclerosis, liver cirrhosis, Alzheimer's disease and other neurodegenerative disorders[2].

Antioxidants, the compounds that can scavenge free radicals play a significant role as they prevent damage of cell proteins, lipids, carbohydrates, nucleic acids as well as biomembranes caused by reactive oxygen species[3]. Synthetic antioxidants such as Butyl hydroxy anisole (BHA) or Butyl hydroxy toluene (BHT) are used to decelerate these processes. However, due to their unstable and highly volatile nature, they have frequently raised some questions about their safety and efficacy ever since their first introduction to the food industry. Consequently, the need to identify alternative natural and safe sources of food antioxidants arose, and hence the search for natural antioxidants, especially of plant origin, has increased in recent years[4].

Various model membrane systems such as low density lipoprotein (LDL) and red blood cell (RBC) membrane comprising physiologically important membrane protein components, offer a physiologically relevant and a relatively simple system for studying lipid peroxidation[5]. Erythrocytes are being used to study oxidant/antioxidant interaction since they serve as good models for oxidative damage of cell membranes[6].

Hemolysis is caused due to the destruction of erythrocytes membrane causing the release of hemoglobin and other internal components into the surrounding fluid. Due to the predominance of polyunsaturated fatty acids in the erythrocyte membranes, they are highly susceptible to oxidative damage whose consequences are lipid peroxidation and hemolysis.

In recent years due to the considerable attention directed towards the identification of plants with antioxidant activity, current study aims at the screening of antioxidant and antimemolytic potential of *Cocos nucifera* and *Mangifera indica* flower extracts. *Cocos nucifera* and *Mangifera indica* are known for their therapeutic values like antibacterial, antifungal, hypoglycemic, hepatoprotective, anticancer, as well as antihemorrhoid properties in human body.

Materials and Methods

Plant material

Cocos nucifera and *Mangifera indica* flowers were collected from herbal garden maintained by Garden City College of

Science and Management studies, Bangalore, Karnataka, India in the month of May and authenticated from the Department of Botany, University of Bangalore, Karnataka, India.

Preparation of plant extracts

Fresh flowers were collected and dried at room temperature. The dried samples were powdered separately. 100gm each of the samples were extracted separately with different solvents starting with non polar to polar solvents in the order of hexane, diethyl ether, acetone, methanol and water. The crude residues were obtained by removing the solvents in rotary evaporator and each of the extracts were resuspended in the respective solvents for further study.

Proximate analysis of the solvent extracts

The proximate composition of flower extracts were carried out to determine the content of ascorbic acid, tannins, saponins, glycosides, proteins, total phenols, flavanoids as well as alkaloids. The protein content was estimated by Bradford method[7]. Total phenolic content was estimated using Folin–Ciocalteue reagent[8]. Flavanoids were estimated following the method of Woiskey and Salatino[9]. Ascorbic acid content was estimated using DNPH reagent[10]. Qualitative analysis of tannins, alkaloids, Saponins, steroids and glycosides were performed for the different extracts[11].

Determination of Antioxidant activity

DPPH radical scavenging assay

1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity was determined as per the method of Habila[12]. 0.1mL of each of the solvent extract was taken in different test tubes and volume in each of the test tube was made up to 100µL using methanol. 3mL of 0.1mM DPPH in methanol was added to each of the test tube and the mixture was shaken vigorously and allowed to stand for 20 minutes. Absorbance of the solution was measured at 517ηm using spectrophotometer (Shimadzu UV-2550). Ascorbic acid (0.1mg/mL) was used as positive control for the assay.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the extracts was estimated as per the method of Shang[13]. 0.1mL each of the plant extract was added to the reaction mixture containing 0.1mL of Deoxyribose (3mM), 0.5mL of $FeCl_3$(0.1mM), 0.5mL of EDTA (0.1mM), 0.5mL of Ascorbic acid (0.1mM), 0.5mL of H_2O_2 (1mM) and 0.8mL of Phosphate buffer (20mM pH 7.4). The reaction mixture was incubated at 37°C for 1hour. Then 1mL of Thiobarbutiric acid (TBA) as well as 1mL of 2.8% Trichloro acetic acid (TCA) were added and incubated at 100°C for 20 minutes. Thiobarbutiric acid reactive substances formed were measured after cooling the mixture and measuring the absorbance at 532ηm.

Superoxide radical scavenging assay

The assay was done following the method of Khanna[14]. 0.1mL each of the plant extract was added to the reaction mixture containing 50mM phosphate buffer (pH-7.6), 20µg/ml riboflavin, 12mM EDTA and 0.1mM NBT. The reaction was initiated by illuminating the reaction mixture for 5 minutes and the absorbance was measured at 590ηm. Inhibition of blue formazone formation was considered for scavenging activity. Quercitin (0.1mg/mL) was used as positive control.

Reducing power assay

The reducing power of extract was determined by the method of Yen and Duh[15]. 100 µl of sample was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and Potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 minutes. 2.5 ml of trichloro acetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The supernatant solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ solution (0.5 ml, 0.1%), and the absorbance (OD) was measured at 700nm. Increased absorbance of the reaction mixture indicates increase in reducing power.

***In vitro* Antihemolytic activity**

The inhibition of human erythrocyte hemolysis by the plant extract was evaluated according to the procedure described by Ebrahimzadeh[16]. In this experiment, venous blood samples were collected from healthy well nourished human adults into glass centrifuge tube containing a known amount of anticoagulant. The tube was centrifuged at 1500rpm for 10 minutes and supernatant was discarded. The resulting pellet was washed carefully with phosphate buffered saline (0.1M) to get buff colored cells called packed RBCs. To study H_2O_2 induced oxidative damage 0.1mL of 1mM H_2O_2, 0.1mL of 0.1mM $FeCl_3$ and 100µL of plant extracts were incubated for 15minutes. Then 500µL of packed RBC was added and incubated at 37oC for 1 hr. Ascorbic acid (0.1mg/mL) was used as positive control. Thereafter the content of the tube was centrifuged at 1500rpm for 10 minutes and absorbance was measured at 540ηm using spectrophotometer. Further the extent of erythrocyte damage due to lipid peroxidation of cell membrane was measured in terms of Malondialdehyde content.

Lipid peroxidation

Malondialdehyde content was estimated according to the methods described by Hodges[17]. To 100µL of the sample, 0.5mL of 20% TCA, 0.5ml of 0.5% Butylated hydroxy toluene (BHT) and 1.0ml of 0.65% TBA were added. Then the test tubes were kept in boiling water bath for 25 minutes, cooled to room temperature and centrifuged at 12,000rpm for 10 minutes. Absorbance of the supernatant was read at 532ηm.

Solvent extract	Proteins	Phenols	Flavonoids	Tannins	Alkaloids	Saponins	Ascorbic acid	Glycosides	Steroids
Aqueous (*M indica*)	++	++	++	+	-	+	+	+	-
Aqueous (*C.nucifera*)	++	++	++	+	-	+	++	+	-
Methanol (*M.indica*)	-	+++	+++	+++	+	++	-	-	+
Methanol (*C.nucifera*)	-	+++	+++	+++	+	+	-	-	-
Acetone (*M.indica*)	-	+	+	++	+	++	-	+	+
Acetone (*C.nucifera*)	-	+	+	++	+	+	-	+	+
Diethyl Ether (*M.indica*)	-	+	+	+	+	-	-	++	+
Diethyl Ether (*C.nucifera*)	-	+	-	+	+	-	-	++	+
Hexane (*M.indica*)	-	-	-	-	++	-	-	+	++
Hexane (*C.nucifera*)	-	-	-	-	++	-	-	+	++

Table 1: Proximate analysis of different solvent extracts of *M. indica and C. nucifera* flowers

Table 2A: DPPH radical scavenging assay

Sample	Aqueous extract	Methanol extract	Acetone extract	Diethyl Ether extract	Hexane extract
Ascorbic acid (Positive control)	95.01±0.120	-	-	-	-
M.indica	57.26±0.038	63.60±0.056	53.06±0.045	44.5±0.098	38.87±0.075
C.nucifera	40.01±0.090	42.95±0.105	52.69±0.087	37.21±0.069	28.03±0.064

Values are means ± SEM; n = 3

Table 2B: Hydroxyl radical scavenging assay

Sample	Aqueous extract	Methanol extract	Acetone Extract	Diethyl ether extract	Hexane extract
Ascorbic acid (Positive control)	94.45±0.120	-	-	-	-
M.indica	67.26±0.038	76.25±0.056	46.72±0.045	64.5±0.098	38.87±0.075
C.nucifera	70.01±0.090	97.15±0.105	95.45±0.087	67.21±0.069	68.03±0.064

Values are means ± SEM; n = 3

Table 2C: Superoxide radical scavenging assay

Sample	Aqueous extract	Methanol extract	Acetone extract	Diethyl ether extract	Hexane extract
Quercitin (Positive control)	94.78±0.120	82.27±0.087	86.03±0.067	86.43±0.092	84.01±0.071
M.indica	67.26±0.038	76.25±0.056	46.72±0.045	64.5±0.098	38.87±0.075
C.nucifera	60.20±0.090	62.95±0.105	70.01±0.090	47.21±0.069	37.03±0.064

Values are means ± SEM; n = 3

Table 2D: Reducing power assay

Sample	Aqueous extract	Methanol extract	Acetone extract	Diethyl ether extract	Hexane extract
BHT (Positive control)	-	83.84±0.087	-	-	-
M.indica	54.26±0.038	68.26±0.056	55.50±0.045	44.5±0.098	38.87±0.075
C.nucifera	50.01±0.090	52.95±0.105	44.13±0.087	37.21±0.069	36.03±0.064

Values are means ± SEM; n = 3

Table 3: Antihemolytic activity of *M.indica* and *C.nucifera* solvent extract

Sample	% Hemolysis		Malondialdehyde (µgm/ml)	
	M.indica	*C.nucifera*	*M.indica*	*C.nucifera*
H_2O_2 + RBC (Negative control)	100	100	2.330	2.010
Ascorbic acid + RBC (Positive control)	6.38	6.38	0.148	0.148
H_2O_2 + Ascorbic acid + RBC	10.72	10.72	0.249	0.249
H_2O_2 + Aqueous extract + RBC	37.45	32.76	0.872	0.763
H_2O_2 + Methanol extract + RBC	29.14	14.57	0.786	0.456
H_2O_2 + Acetone extract + RBC	45.71	39.69	1.041	0.924
H_2O_2 + Diethyl Ether extract + RBC	73.32	70.87	1.685	1.651
H_2O_2 + Hexane extract + RBC	68.18	61.43	1.611	1.431

RESULTS

Proximate analysis

Table 1, Shows the results of proximate analysis of *Cocos nucifera* and *Mangifera indica* flower extracts. Considerable amount of protein, saponins and glycosides were reported in the aqueous extract of *Mangifera indica* flowers. Higher levels of flavanoids and polyphenols were present in the methanol as well as acetone extracts of Cocos and Mangifera in comparison to hexane, ethyl acetate and aqueous extracts.

Antioxidant activity

Table 2A, refers to DPPH radical scavenging assay. DPPH, a nitrogen centered free radical was reduced in the presence of different solvent extracts of fruit and spike. Ascorbic acid in water, used as positive control showed 95% scavenging activity which is followed by methanol extract of M. indica showing 63.6% activity. Acetone extract of *M. indica* as well as *C. nucifera* exhibited considerable scavenging activity of 53.06% and 52.69% respectively.

Table 2B, refers to Hydroxyl radical scavenging assay. Hydroxyl radicals were generated by the Fenton reaction and the TBA reacting substance was estimated photo metrically. Methanol and Acetone extracts of *C. nucifera* showed higher scavenging activities of 97.15% and 95.45% respectively in comparison to the positive control ascorbic acid(94.45%).

Table 2C, refers to Superoxide radical scavenging activity. The superoxide radical generated from Riboflavin and NBT in the presence of light were scavenged by the different solvent extracts. With respect to the positive control Quercitin (94.78%), acetone extracts of *C. nucifera*(70%) exhibited considerable scavenging activity when compared to other extracts.

Table 2D, refers to Reducing power assay. The reducing efficiency of the plant extract to convert the oxidized form of iron (Fe+3) in ferric chloride to ferrous (Fe+2) is measured. BHT, the positive control showed 83.84% of scavenging activity which is followed by methanol and acetone extract of *M. indica* showing 68.26% and 55.5% activities respectively.

Table 3, explains the antihemolytic activity of various extracts of M. indica and C. nucifera. The lysis of erythrocytes in the presence of H_2O_2, $FeCl_3$ in the absence of plant extract was considered as 100% hemolytic activity. Among all the extracts used for the study, methanol extract of *C. nucifera* flower showed considerable antihemolytic activity, by showing partial lysis of the RBC's and. Antihemolytic activity of methanol extract showed maximum activity than the positive control ascorbic acid used. The MDA content was significantly lesser in the tube containing methanol extract of *C. nucifera*.

DISCUSSION

For several years, many researchers have been searching for powerful but non-toxic antioxidants from natural sources, especially edible or medicinal plants. Such natural antioxidants could prevent the formation of the reactive species-related disorders in human beings without the use of synthetic compounds, which may be carcinogenic and harmful to the lungs and liver[18]. Also, antioxidants play an important role in nutritional by lengthening the shelf life of food and reducing nutritional losses and formation of harmful substances. Thus, attention is now increasingly paid to the development and utilization of more effective and non-toxic antioxidants of natural origin. A great number of natural medicinal plants have been tested for their antioxidant activities and results have shown that the raw extracts or isolated pure compounds from them were more effective antioxidants *in vitro* than BHT or vitamin E[19]. Hence medicinal plants can be a potential source of natural antioxidants[20].

Polyphenols are ubiquitously distributed group of plant secondary metabolites ranging from simple molecules like phenolic acids, phenyl propanoids, and flavonoids to highly polymerized compounds namely lignins, melanins and tannins. Flavanoids are the most common and widely distributed subgroups[21].

In the present study proximate analysis showed higher concentrations of phenols, flavonoids and tannins in the methanol as well as acetone extract of M. indica and C. nucifera flowers. The significant antioxidant activities namely DPPH, Hydroxyl, reducing power and Superoxide radical scavenging activities of methanol and acetone extracts of M. indica and C. nucifera may be endorsed for the presence of higher amounts of the above mentioned plant secondary metabolites.

Many studies revealed that polyphenols exhibit a wide range of pluripharmacological effects including antimicrobial, anti-inflammatory, antiallergic, hepatoprotective and anticarcinogenic actions. Many of these biological functions have been attributed to their antioxidant activity in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides[22].
The effective antihemolytic activity of C.nucifera methanol extract is because of the ability of phenolic compounds including flavonoids in neutralizing the free radicals generated by $H_2O_2$and thereby protecting the erythrocytes membrane from destruction and lysis. Insignificant hemolysis was observed when erythrocytes were treated only with plant extracts, indicating the nontoxic nature of plant extracts. Thus the extract can be justified as harmless for the cells.

Membrane lipid peroxidation is regarded as a key factor for cell lysis. Malondialdehyde (MDA), a low molecular weight end product of lipid hydroperoxide decomposition is the most often measured parameter of membrane destruction. Lesser the cell lysis lesser will be the MDA content. Earlier reports suggested the potent antihemolytic activity of bioactive components namely Flavanoids and phenols from plant extracts[23]. Thus the plant secondary metabolites could be regarded as efficient scavengers of reactive oxygen species offering the cell protective function.

CONCLUSION

Presence of substantial amounts of secondary metabolities like Phenols, Flavanoids as well as tannins in the methanolic extract of plant stem may be responsible for exhibiting significant antioxidant and antihemolytic activity thereby serving for significant cytoprotective function.

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