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"STUDIES ON ANTIOXIDANT PROPERTIES AND INHIBITION OF MONOAMINE OXIDASE ABILITIES USING COCONUT WATER (*COCOS NUCIFERA* LINN.)

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ABSTRACT

Coconut water (*Cocos nucifera* L.) is a refreshing drink. It has been widely used for mood stabilizer, anti-aging and anti-carcinogenic properties. The present study assessed antioxidant activity and Monoamine oxidase inhibition ability of Coconut water. Antioxidant potential has been demonstrated by using Phenollic content,2,2-diphenyl-1Picrylhydrazyl(DPPH),Superoxide Radical(SOR),Hydroxyl radical(OH⁻),and reducing power scavenging activity. The enzyme activity study has been carried out to understand MAO inhibition. The DPPH, SOR⁻ ,OH⁻ and reducing power activities of *C.nucifera* showed IC50 as (0.95) at 10%,(0.054) at 10%,(0.044) at 10% and (0.044) at 10%. It also showed high Phenol content as 25 μg /ml and ability to inhibit Monoamine Oxidase. This study reveals that Coconut water (*C. nucifera*) potentially used as a natural antioxidant and anti-depressant.

KEY WORD- Coconut water, Anti-aging, Anti-carcinogenic, Antioxidant, Monoamine Oxidase.

INTRODUCTION

Depression is the most severe neurological disorder .Everyone experiences sadness from time to time but depression lasts longer, interferes with daily life, and cause physical pain. Depression caused due to combination of genetic, chemical, biological, psychological, social and environmental factors (Dar and Khatoon , 1998). The prevalence of depression in general population is estimated to be around 5%. At present 121 million people are estimated to suffer from depression. An estimated 5.8% of men & 9.5% of women experience depression in their lifetime with suicide being one of the most common outcomes of depression. If left untreated; it may disrupt work, family, personal life. Although currently used antidepressants provide some improvement in patients, it is at the cost of having adverse effects (Ibrahim et.al. 2007) Symptoms of depression include a depressed mood, feeling of loneliness, sadness and unhappiness. Along with classical theory of decrease in neurotransmitter levels in brain leading to pathogenesis of depression, recent study has shown involvement of oxidative stress (George Boeree, 2007). Ayurveda mentions a number of drug formulations of plant origin used in treatment of psychiatric disorders(Tripathi ,2008 and Sembulingam ,1997).Neurotransmitters are the chemicals which allow the transmission of signals from one neuron to the next across synapses (Graeme et.al.,2004).Some of the most significant neurotransmitters are Acetylcholine, Norepinephrine, Dopamine, Serotonin, Endorphin.
Monoamine oxidase is an enzyme that oxidizes variety of monoamines, several of which are neurotransmitters (serotonin, norepinephrine dopamine).these compounds act as neurotransmitters in CNS and also agents that prepare body for physical activity. There are two types of monoamine oxidase as-Monoamine Oxidase A and Monoamine Oxidase B.Both are found in neurons and outside the CNS in brain. Monoamine oxidases catalyses oxidative deamination of monoamines. Oxygen is used to remove an amino group from a molecule resulting in aldehyde and ammonia (Tipton et. al., 2009). Because of the vital role that MAOs play in the inactivation of neurotransmitters, MAO dysfunction (too much or too little MAO activity) is thought to be responsible for a number of psychiatric and neurological disorders. There are many treatment options for depression such as use of antidepressants, mood stabilizers, counselling, yoga, relaxation, etc.(Bodkin et.al.,1995).But there are certain side effects of MAO inhibitors such as Daytime sleepiness, Dizziness or light headedness ,Low blood pressure, Diarrhea , Dry mouth , Altered sense of taste, Nervousness, Muscle aches, Insomnia, Weight gain.
To overcome these side effects, use of herbal drugs is needed. From ancient times, herbal drugs are used as therapeutic agents. Medicinal herbs are reputed for management of various diseases including depression.

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Free radical is any atom or molecule that has a single unpaired electron in an outer shell and can be formed when oxygen interacts with certain molecules. Once formed, these highly reactive oxygen species can start a chain reaction. When they react with important cellular components such as DNA, RNA etc, cells may die also. To prevent free radical damage, body has defense system of antioxidants.

MATERIALS AND METHODS-

Chemicals-

Ascorbic acid, potassium chloride, sodium hydroxide, Tris Hcl, ferrous sulphate, DPPH, ethanol, oxalic acid, potassium ferric cyanide, catechol, monobasic and dibasic salts of sodium phenanthroline, H_2O_2 ,SNP, benzylamine, semicarbazide DNPH, EDTA, mannitol, sucrose. The chemicals were obtained from S.D.Fine Chemicals Ltd. Mumbai. All chemicals used were of AR grade and were purchased from commercial sources.

Reagents-

Follin – Ciocalteu's reagent

Collection of plant sample -

The plant, *Cocos nucifera* L., was collected from Local market of Nanded in the month of January 2012.The plant was identified and authenticated in the Department of Botany, School of Life Science, and the voucher specimen was deposited in the herbarium of the host institute. The plant part of *Cocos nucifera* L, fruits (Water) used for further study.

Etimation of Vitamines C-

A ascorbic acid or vitamin 'c' is an anti-ascorbic. It is present in gooseberry, bitter guard in high amount generally. Pipette out 5ml of working std. solution into a 100ml conical flask add 10ml or 4% oxalic a acid against the dye (10ml) end point is appearance of pink colour which pink colour persist for few min. the ant of dye consumed is equivalent to amount of ascorbic acid extract the sample (0.5-5gm) depending on sample in 4% oxalic acid and make up known volume (100ml) and centrifuge pipette out 5ml of this supernatant acid 10ml of 4%oxalic acid & titrate against the dye ($v_2$ml) .

Estimation of Phenol-

The total phenol content was determined spectrophotometrically with folin-ciocalteu reagent using the modified method of wolfe et at (2003). An aliquot of the crude extract (0.5ml) was mead with 1ml Folin Ciocalteu's reagent and 2ml of incubation in 1ml after add in 2ml of na2co3 (75% w/v). The resulting mixture was vortexes for 55 and incubated at 40c for 30 min for colour development. The absorbance of the samples was censured at percentage & total phenol content was calculated form calibration curve of catechol and was expressed as percent equivalent to catechol.

Determination of DPPH Radical Scavenging Activity-

The DPPH radical scavenging assay was carried out according to reported method [26].the reaction mixture contains different concentrations of plant sample (100-500 µg/ml, in absolute ethanol) and DPPH radical (10^{-4} M in absolute ethanol) solution. The contents of the reaction mixture were observed spectrophotometrically at 517 nm for 20 min. Ascorbic Acid (1 mM) was used as a reference compound. The DPPH radical scavenging activity (%) was calculated by using following formula, DPPH radical scavenging activity (%) =1-T/C*100, Where, T=absorbance of test sample, C=absorbance of control at 517 nm.

Superoxide Radical (SOR) Scavenging Activity-

The superoxide anion scavenging assay was performed by the reported method (Liu. et al., 1997). Superoxide anion radicals were generated in a non-enzymatic Phenanzine methosulphate - Nicotinamide Adenine Dinucleotide (PMS-NADH) system through the reaction of PMS, NADH and Oxygen. It was assayed by the reduction of Nitroblue tetrazolium (NBT). In this experiment superoxide anion was generated in 3ml of Tris HCL buffer (100mM, pH 7.4) containing 0.75ml of NBT (300µM), 0.75ml of NADH (936 µM), and 0.3ml of plant sample (1mg/ml). The reaction was initiated by adding 0.75ml of PMS (120 µM) to the mixture. After 5min. of incubation at room

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temperature the absorbance at 560 nm was measured in spectrophotometer. Ascorbic acid (1mM) was used as reference compound.

Hydroxyl Radical (OH) Scavenging Activity -

The OH radicals scavenging activity was demonstrated with Fenton reaction (Rollet-Labelle *et al.,* 1998). The reaction mixture contained, 60µl of $FeCl_2$ (1mM), 90µl of 1-10 phenanthroline (1mM), 2.4 ml of phosphate buffer (0.2M, pH 7.8), 150µl of H_2O_2 (0.17M) and 1.5 ml of individual plant extract (1mg/ml). The reaction was started by adding H_2O_2. After 5 min. incubation at room temperature, the absorbance was recorded at 560 nm. Ascorbic acid (1mM) was used as reference compound

Determination of Reducing Activity-

The principle of reduction assay is that the reducing agents reduce the $Fe3^{+}$ to Fe^{2+}. Higher absorbance (as compared to control) of the reaction mixture indicates greater reducing power. The reducing power of coconut water was determined by method of Oyaizu (Bartolome et.al., 2004). In brief, at various concentrations coconut water (10%-50%)was mixed with 0.75 ml of phosphate buffer (0.2 M, pH 6.6)and 0.75 ml of potassium hex cyanoferrate ($K_3Fe(CN)_6$) (1%w/v) followed by followed by incubating at 50c in water bath for 20 min. The reaction was terminated by adding 0.75ml of tri-chloro acetic acid solution (10%) and then centrifuged at 800*g for 10 min .1.5ml of supernatant was mixed with 1.5ml of distilled water and 0.1ml of ferric chloride (fecl3) solution (0.1%w/v) for 10 min. The absorbance at 700nm was measured as the reducing power. Ascorbic acid (1mM, 155.7%) was used as a reference compound. The values of absorbance obtained were multiplied by a factor of 100 for the calculation of % reducing power.

Preparation of Rat Brain Mitochondrial MAO-

The isolation of rat brain mitochondria was carried out as per the method reported by Satav and Katyare (Paniappan et.al., 2012). In brief, the brain tissue was homogenized in buffer containing 0.3 M Mannitol,0.1 mM EDTA, and pH 7.4. Homogenate was centrifuged at 600*g for 1 min at 4 0c. The supernatant was collected and followed by centrifugation at 10*g for 1 min at 4 c to obtain brain mitochondria. The mitochondrial pellets thus obtained were washed 3 times with 0.25 M sucrose buffer containing 0.1 mM EDTA, pH 7.4, resuspended in 0.25 M sucrose buffer, pH 7.4 and stoared at 4 c for further studies.

Determination of protein and assay of Monoamine Oxidase-

The protein concentration was determined by using a method of (Bartolome et.al.,2004) the assay of MAO with different concentrations (100-500 µg/ml) of plant extract and coconut water (10%-50%) was carried out as per the methods (Oyaizu et.al. 1986 and Satav et,al., 1988)with slight modification. In brief, the reaction mixture contains 0.025 M phosphate buffer of pH (7.0). 0.125 M semicarbazide, 10 mM benzylamine (pH-7),and enzyme and ethanolic extract of selected plant sample in a total reaction volume of 2 ml. after 30 min,1 ml acetic acid was added and boiled for 3 min in boiling water bath followed by centrifugation. The resultant supernatant (1 ml) was mixed with equal volume of 0.05% of 2, 4 DNPH and2.5 ml of benzene was added. After 10 min incubation at room temp, and after separating benzene layer, it was mixed with equal volume of 0.1 N NAOH. Alkaline layer was decanted and heated at 80 c for 10 min. The orange yellow colour developed was measured at 450 nm. One unit of enzyme activity was defined as amount of enzyme which caused an increase in absorbance of 0.001min -1 at 450 nm at 25 c and pH 7 which corresponds to formation of 0.01 µm of product.

OBSERVATION TABLES -

1. Estimation of Vitamin C (Ascorbic Acid)-

Observation Table 1 – Estimation of Vitamin C (Ascorbic Acid)

Sr. no.	Plant sample	Concentration Ascorbic Acid (in mg/gm of sample)
1.	*Cocos nucifera*	223.21

Hence, the amount of Vitamin C (Ascorbic Acid) =223.21 mg/ gm of Coconut water.

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Observation Table 2 – Estimation of Phenol

Sr. no.	Plant sample	Concentration of Phenol (in µg/ml of sample)
1.	*Cocos nucifera*	25

Hence, the concentration of Phenol for Coconut water = 25 µg/ml.

Observation Table 3- Antioxidant potential of *Cocos nucifera* of DPPH, SOR, OH radicals, and Reducing activity.

Conc. in %	DPPH	SOR	OH	Reducing Activity
10	0.95	0.054	0.044	0.044
30%	1.10	0.056	0.051	0.051
60%	1.54	0.059	0.066	0.061
90%	1.62	0.060	0.075	0.078
Ascorbic acid	0.34	0.057	0.068	0.068
$IC_{50\ (v/v)}$	10%v/v	10%v/v	10% v/v	10% v/v

Observation Table 4 –Estimation of Protein by Follin Lawry method -

Sr. no.	Conc. of protein (µg/ml)	Amt of protein (ml)	D/W (ml)	Retag. C (ml)		Follin reagent (ml)		O.D.at 650 nm
1	100	0.1	3.9	5.5	Incubation for 10 Min At R.T.	0.5	Incubation for 30 Min At R.T.	0.45
2	200	0.2	3.8	5.5		0.5		0.84
3	300	0.3	3.7	5.5		0.5		1.02
4	400	0.4	3.6	5.5		0.5		1.21
5	500	0.5	3.5	5.5		0.5		1.34
6	600	0.6	3.4	5.5		0.5		1.41
7	700	0.7	3.3	5.5		0.5		1.48
8	800	0.8	3.2	5.5		0.5		1.50
9	900	0.9	3.1	5.5		0.5		1.51
10	1000	0.10	3.0	5.5		0.5		1.51
Blank			3 ml	5.5		0.5		
Unknown	3 ml			5.5		0.5		0.95

Hence, the amount of Protein in Monoamine oxidase isolated from rat brain is 390 µg/ml.

Observation Table 5 - The effect of treatment of coconut water on activity of Monoamine Oxidase (MAO)

Concentration (%)	Monoamine Oxidase (MAO) activity
Control	0.192
10%	0.117
20%	0.108
30%	0.061

RESULTS AND DISCUSSION

Antioxidant Activities of Cocus nucifera

The results of the DPPH, SOR, OH, Reducing radical scavenging activities of coconut water are summarized in Table 3.The obtained results clearly indicates concentration dependent activities of the selected plants towards the DPPH, SOR, OH and Reducing radical scavenging activities. DPPH, SOR, OH and Reducing radical scavenging activities of coconut water shows IC_{50} as (0.95) at 10%, (0.054) at 10%, (0.044) at 10%,and(0.044) at 10% respectively. The antioxidant capacity is widely used as a parameter for medicinal bioactive components. Polyphenols are major plant compounds with high level of antioxidant activity. This activity could be due to their ability to absorb, neutralise and to quench free radicals (Duh et al., 1999).Their ability as a free radical scavenger could be also

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attributed to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Rice-Evans et al., 1995).
In the present study, it was found that coconut water contains high phenol content as 25µg/ml that might account for strong activity observed against free radicals. On the other hand, the activity depicted in DPPH and superoxide anion may be as a result of content of flavonoid which has been reported to possess high antioxidant activity.

Monoamine Oxidase (MAO) Inhibitory Activity-
Inhibitory activities of the coconut water is summarized in Observation Table-5, Coconut water shows (68%) activity at 50% v/v respectively. There are several categories of MAO inhibitors which belong to a variety of chemical classes such as isoquinolines, tetrahydroisoquinolines , oxadiazoles , and natural xanthones .In addition, oxygen-containing phytochemicals such as coumarin, xanthone, thioxanthone have been reported to inhibit MAO activities. Flavonoids, a naturally occurring group of plant phenolics, have been reported to possess MAO inhibitory activities. Some of the notable flavonoids investigated for MAO inhibition include leuteolin, quercetin, apigenin, chrysin, genistein and daidzein. Equally the plant derived coumarins such as aesculetin, aesculetin-7-methyl ether and scopoletin have been attributed with MAO inhibition. Of the reported flavonoids apigenin has been shown to be the highly effective inhibitor of mouse brain MAO (Thull and Kneubuhler,1995). Moreover variety of other plant derived compounds such as isoquinoline (Nunez et.al.,2004) xanthones (Han et.al.,1995) stilbenoids (Huong et.al.,1990) and coumarin derivatives(Haraguchi et.al.,2004) have been identified as MAO inhibitors. The presence of abovementioned ethanol soluble phytochemicals in the coconut water may be the possible cause for the inactivation of rat brain MAO.In recent years it has been critically investigated that the catecholamines manifest a crucial role in the generation of reactive oxygen species. The reaction of MAO has been reported to yield H2O2, for e.g. R-CH_2-NH_2, O_2 - H_2O. R-CHO-NH_3, H2O2. The H_2O_2 radicals thus generated are further implicated in the generation of hydroxyl (OH) radicals, the most powerful and hyper reactive free radicals involved in insulting the cellular functions (NEJM, 1994). Selected plant Coconut water has good phenolic content as 25µg/ml. Therefore Coconut water can be used as potential antidepressant of herbal origin.

CONCLUSION-
The present study demonstrated that coconut water inhibit rat brain Monoamine oxidase. Moreover, sample possesses significant antioxidant potential. It has been shown that phenolic compounds show good inhibitory activity on rat brain Monoamine Oxidase (MAO). This result shows that *C. nucifera* can be considered as a possible source of Monoamine Oxidase (MAO) inhibitor used in treatment of depression and other neurological disorders. However, further pharmacological studies such as kinetics of Monoamine Oxidase and *In Vivo* Monoamine Oxidase (MAO) inhibition of coconut water needed for categorizing it as an effective antidepressant herbal ingredient.

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