RESEARCH ARTICLE

Phytochemical Evaluation and Anti-Oxidant activity of Extract of *Garcinia indica* and *Lannea coromandelica* plants

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ABSTRACT

Free radicals are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes including other micromolecules, resulting in cellular damage. Cell damage caused by free radicals appears to be a major contributor to aging and degenerative disease such as cancer, cardiovascular diseases, cataract, liver diseases, diabetes mellitus, inflammation, renal failure, etc. Naturally there is a dynamic equilibrium between the free radicals produced in the body and antioxidants that scavenge them to protect the body against deleterious effects. The amount of antioxidants present under normal physiological conditions may be insufficient to neutralize free radicals generated. Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases. Hence there has been an increased interest in the food industry and in preventive medicine in the development of "Natural antioxidant" from plant material. Considering the significance of antioxidant activity, crude extract from *Lannea coromandelica* (Hout.) Merill belonging to Family Anacardiaceae and *Garcinia Indica* was prepared in methanol and aqueous evaluated the pharmacognostical properties and for its radical scavenging activity using 1,1-diphenyl-2 picrylhydrazyl (DPPH) Radical Scavenging Assay, Nitric Oxide Radical Inhibition Assay, Reducing Power Assay and H O Radical 2 2 Scavenging Assay. The antioxidant activity of methanolic extract of *Lannea coromandelica* and aqueous extract of *Garcinia Indica* was studied in comparison with the standard ascorbic acid. The extract showed significant free radical scavenging activity as compared to ascorbic acid. The antioxidant activity observed in the present investigation might be due the presence of phenolics and flavonoids.

Keywords: DPPH Assay, H_2O_2 Assay, *Lannea coromandelica, Garcinia Indica,* Nitric Oxide Assay, Reducing Power Assay

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INTRODUCTION

Lannea coromandelica (Hout.) Merill belongs to Family Anacardiaceae. It is a deciduous tropical tree distributed throughout India, Bangladesh and few other tropical countries.[1] Traditionally boiled leaves are applied in sprains and bruises, local swellings, pains of body and in elephantiasis Five dihydroflavonols were isolated and identified from the stem barks of Lannea coromandelica.[2] The bark is useful in cuts, wounds, bruises, ulcers, opthalmia, gout, ulcerative stomatitis, odontalgia, sprains, diarrhea and dysentery. The fruit paste of Lannea coromandelica is therapeutically used for bone fractures by tribes in Eastern Ghats of Andhra Pradesh Various phytochemicals have been isolated from Lannea spp., including quercetin-3-O arabinoside and ellagic acid , 6,6-dimethyl-[2,3:7,6]- pyrano-8 (γ,γ-dimethylallyl) avanone rutin and quercetin, and lanceolatin-B and 7,2' dimethoxy-4',5 '- methylenedioxy avone from the leaves and flowers; phlobatannin and leucocyanidin , β-sitosterol, physcion and physcion anthranol B from the bark ; and a ferulic acid ester from the roots.[3] In the present study we investigated radical scavenging potential of methanolic extract of leaves of Lannea. The coromandelica. pharmacological properties of extract of Oxygen consumption inherent in cell growth Lannea coromandelica stem barks were screened for anti-inflammatory, hypotensive, and cytotoxicity effects. leads to the generation of a series of reactive oxygen species (ROS). They are continuously produced by the body's normal use of oxygen such as respiration and some cell-mediated immune functions.[4] *Garcinia indica* (dried rind known as 'kokum'), a tropical fruit, can be viewed as a wonder berry that has a pleasant, tangy-sweet taste and a myriad of health benefits. Garcinia is a rich source of active compounds including garcinol, xanthochymol, isoxanthochymol and Hydroxycitric acid.[5] These are flavonoids, benzophenones, xanthones, lactones and phenolic acids. The fruits contain citric acid, acetic acid, malic acid, ascorbic acid, hydroxycitric acid and garcinol. The major constituent of Kokum rind is garcinol, a polyisoprenylated benzophenones, isogarcinol and camboginol. Garcim-1, Garcim-2 and cambogin are the chief oxidative products of garcinol, along with isogarcinol, gambogic acid, mangostin, clusianone, macurin, oblongifolin (A, B, C), guttiferone (I, J, K, M, N). Kokum fruit is naturally very acidic with a pH between 1.5 to 2.0. The rind of ripe Kokum fruits consists of

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Table1: Phytochemical Analysis of extract of *Lannea coromandelica* and *Garcinia indica*

Sr. No	Test	*Lannea coromandelica* Extract	*Garcinia indica* Extract
1.	Tannins	+	+
2.	Saponins	+	+
3.	Flavanoids	+	+
4.	Alkaloids	+	+
5.	Phenols	+	+
6.	Carbohydrates	+	+
7.	Proteins	-	+
8.	Glycosides	-	+
9.	Sterols	-	-
10.	Terpenoids	+	+

hydroxyacetic acid and hydroxycitric acid. It also contains 2.4% pigment as a mixture of two anthocyanins namely, cyanidin-3-sambubioside and cyanidin 3-glucoside in the ratio 4:1.[6]

ROS include free radicals such as superoxide anion radicals (O2•−), hydroxyl radicals (OH•) and non-free radical species such as hydrogen peroxide (H O) and 2 2 singlet oxygen (1O). ROS are 2 continuously produced during normal physiologic events and can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides.[7] ROS are also capable of damaging crucial biomolecules such as nucleic acids, lipids, proteins and carbohydrates and may cause DNA damage that can lead to mutations. If ROS are not effectively scavenged by cellular constituents, they lead to disease conditions. ROS have been implicated in more than 100 diseases. Antioxidants can protect the human body from free radicals and ROS effects.[8] They retard the progress of many chronic diseases as well as lipid peroxidation. Hence, a need for identifying alternative natural and safe sources of food antioxidants has been created, and the search for natural antioxidants, especially of plant origin, has notably increased in recent years.[9] Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods. At the present time, the most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate and tert-butyl hydroquinone.[10] However, BHA and BHT have been suspected of being responsible for liver damage and carcinogenesis. Therefore, there is a growing interest in natural and safer antioxidants.

MATERIAL AND METHODS

Bark of *Lannea coromandelica* and Fruits of *Garcinia Indica* collected and were authenticated from Botanical Survey of India, Jodhpur. Dried bark were ground to coarse powder, extracted with methanol, which was further evaporated to dryness to obtain alcoholic extract. For the aqueous extract of *Garcinia Indica* the dried fruits were grounded to coarse and macerated with distilled water in flask for 7days and filtered. It is then further evaporated till dryness to obtained extract.

The ELC and AGI was subjected to the following chemical tests separately for the presence of various phytoconstituents such as alkaloids, flavonoids, saponins, carbohydrates, sterols and terpenoids, anthraquinone glycosides, carotenoids, tannins and phenolic compounds. The antioxidant activity of the plant extracts and standard were assessed on the basis of the radical scavenging effect of the stable DPPH free radical. The ability of the extract to scavenge hydrogen peroxide was determined. Nitric oxide was generated from nitroprusside and measured by the Griess reaction.

RESULTS

The preliminary phytochemical components from the analysis showed that tannins, phenols, flavonoids, alkaloids, saponins, terpenoids and anthraquinones were present in the ethanolic extract *of Lannea Coromandelica* and *Garcinia Indica* Table 1 shows the results obtained from the qualitative analysis.

Free radical scavenging activity

The antioxidant activity of extracts has been studied by its ability to reduce DPPH. Interaction of antioxidant compounds with DPPH is based on the transfer of hydro- gen atom or electron to DPPH radical and converts it to 1, 1- diphenyl-2- picrylhydrazine. The result of reduction DPPH radicals causes discoloration from purple color to yellow pale color which demonstrates the scavenging activity. The antioxidant activity of the two solvents extracts and ascorbic acid against DPPH assay was tested with concentrations ranging from 50 to 300 µg/ ml as the results shown in Table 2.

As shown in Table 2, both the MeOH and Aq extracts were displayed a comparable and significant concentration-dependent free radical scavenging activ- ity from 91.54% to 93% and 91.04% to 93.39%, respec- tively, compared with that of the standard AA 94.57% to 95.8%). The IC50 values of DPPH assay for the H_2O, MeOH extracts were 94.92 µg/ml and 94.83 µg/ml, respectively. While the standard antioxidant had an IC50 of 127.737 µg/ml. Low IC_{50} values correspond to high antioxidant activity.

Scavenging of hydrogen peroxide

The scavenging effect of different extracts on hydrogen peroxide was concentration-depend- ent (25–300 µg/mL) as shown in Table 3. The methanol extract displayed strong H2O2 scavenging activity (IC50 141.6 µg/mL), whereas water extract exhibited IC50 value 180.6 µg/mL. All solvent extracts of tested plant exhibited concen- tration-dependent free radical scavenging activity. The MeOH extract showed good scavenging ability (70.38%). The IC50 values of MeOH, H2O extracts and standard AA extract were 141.6, 156, and 112 µg/ml, respectively. The MeOH extract had higher antioxidant activity while the H2O extract had the lowest antioxidant activity.

DISCUSSION

DPPH is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples.[11] The assay is based on the reduction of DPPH radical in methanol which causes an absorbance drop at

Table 2 DPPH scavenging activity of extracts

Conc. µg/ml	H_2O extract GC			MeOH extract LC			AA		
	A	%	IC50	A	%	IC50	A	%	IC_{50}
50	0.151	91.54	94.92	0.160	91.04	94.83	0.097	94.57	127.737
100	0.149	91.65		0.153	91.43		0.092	94.85	
150	0.143	91.99		0.142	92.04		0.087	95.13	
200	0.138	92.27		0.129	92.77		0.084	95.29	
250	0.131	92.66		0.126	92.94		0.079	95.57	
300	0.125	93		0.118	93.39		0.075	95.8	

Table 3 DPPH scavenging activity of extracts

Conc. µg/ml	H_2O extract GC			MeOH extract LC			AA		
	A	%	IC50	A	%	IC50	A	%	IC_{50}
50	0.322	48.73	141.6	0.399	36.46	156	0.231	63.22	112
100	0.287	54.3		0.308	50.96		0.199	68.31	
150	0.241	61.62		0.298	52.55		0.152	75.8	
200	0.205	67.36		0.244	61.15		0.117	81.37	
250	0.198	68.47		0.207	67.04		0.079	87.42	
300	0.186	70.38		0.198	68.47		0.047	92.52	

517 nm. DPPH is known to abstract labile hydrogen.[12] DPPH being a stable free radical can accept an electron or hydrogen radical to become stable diamagnetic molecule. Due to its odd electron, the methanolic solution of DPPH shows a strong absorption band at 517 nm; reduction of the DPPH radicals can be observed by the decrease in absorbance at 517 nm. DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses color stoichometrically with the number of electron taken up.[13-15] Such reactivity has been widely used to test the ability of compounds/ plant extracts to act as a free radical scavengers. Antioxidant molecules can quench DPPH free radicals and convert them to a colorless/bleached product (i.e. 2,2 diphenyl-1-hydarzine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at the 517 nm band.[16] Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of extract in terms of hydrogen atom-donating capacity. The dose-response curve of DPPH radical scavenging activity of the MELC, compared with ascorbic acid, as standard. The MELC extract scavenged 42.50% of DPPH radical at concentration of 25 µg and ascorbic acid scavenged 55.4% at same concentration i.e. 25 µg. The EC50 for MELC extract was found to be 29.41 µg and 22.56 µg for ascorbic acid respectively.

Hydrogen peroxide itself is not very reactive, but can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells.[17-18] Scavenging of H O by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water.[19] The MELC was capable of scavenging hydrogen peroxide in a concentration dependent manner. Figure 2 shows that MELC shows less scavenging activity (H_2O_2) than that of Ascorbic acid. The EC50 value for scavenging of for MELC was 28.06 µg while EC50 value for ascorbic acid was 25.73µg.

The crude extract contains phenolic and tannin compounds which are potential sources of antioxidants.[20] These bioactive compounds possess many bio- logical properties including antioxidants such as anti- fungi, antibacterial, anti-inflammatory, antioxidant, anticancer, antiviral.[21-23] The antioxidant scavenging activities of all solvent extrac-tion in DPPH assay were found to be the highest as compared to the others assays at 300 µg/mL.

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that methanolic extract of *Lannea coromandelica* and Aqueous extract of *Garcinia Indica*, exhibits high antioxidant and free radical scavenging activities. It also chelates iron and has reducing power. These in vitro assays indicate that this plants extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

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