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RESEARCH ARTICLE

STUDIES ON THE EFFECT OF PHYTOCHEMICAL AND MINERAL ANALYSIS OF KALAKAI (*CARISSA CARANDAS* L.) FRUIT

[1]Thiyagarajan, G., [1]Rajasekaran, S. [2],*Balamurugan, S., and [1]Karthikeyan, S.

[1]Department of Botany, AnnamalaiUniversity,Annamalai Nagar Chidambaram 608 002.
[2]Department of Botany A.V.C. College (Autonomous), Mannampandal, Mayiladuthurai 609 305.

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ABSTRACT

Carissa carandas is a traditional and effective medicinal plant in India. It is used as various ailments to use in local peoples. The present research was finding to carry out the qualitative and quantitative phytochemical analysis, antioxidant potentials, macro and micronutrient contents of *Carissa carandas.* The qualitative phytochemical namely alkaloids, glycosides, flavonoids, terpenoids, carbohydrates, protein and aminoacids were identified. The quantitative phytochemicals namely alkaloids, saponins, flavonoids, phenols, terpenoids, glycosides, and carbohydrates were identified and observed the macro and micro elements such as nitrogen, phosphorous, calcium, magnesium, sodium, and chromium, cupper, ferrous, manganese, nickel, sodium, and zinc were identified and more high in this parameters. The maximum phytochemicals and minerals were observed in *Carissa carandas.*

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INTRODUCTION

Medicinal plants are a major source of biodynamic compounds of therapeutic values and are basis of many traditional medicines throughout the world for thousands of years. Medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries for primary healthcare not because they are inexpensive but also for better cultural acceptability, and compatibility with the human body and no side effects. However among the estimated 250,000-400,000 plants species, only 6% have been studied for biological activity and about15% have been investigatedphytochemicals. Owing to the global trend towards improved 'quality oflife', there is great demand for medicinal plants in the developing world for treating various ailments ofboth man and animals. Herbal medicine is an old practice as man himself. These plants are widely used by all sections of the society whether directly as folk remedies or indirectly as pharmaceutical preparation ofmodern medicine. In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential ofmedicinal plants used in various traditional systems (Ayurveda, Siddha and Unani).

Corresponding author: **Balamurugan, S.,**
Department of Botany A.V.C. College (Autonomous), Mannampandal, Mayiladuthurai 609 305.

Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. Therefore it seems necessary to evaluate the herbs properly. The fresh fruits are rich in ascorbic acid (Radhakrishnan *et al.,* 2001). The plant *Carissa carandas* (Apocynaceae) is an evergreen shrub or small crooked tree up to 3 m in tall with dichotomous branches armed with simple or forked, 2-4 cm long, paired axillary thorns. Bark is yellowish brown, peeling in square flakes. Leaves are short petiole, light green, leathery glabrous and shining on surfaces, obviate, elliptic or oblong, 3-7 cm long and 1.5-4 cm wide apex obtuse, base rounded faintly scented flowers in lax cymes. Fruits (berries) ellipsoid, up to 2 cm long, red turning dark purple when ripe, normally 8 seeded, flowers between March and February in central India. *Carissa carandas* traditionally has been used as stomachic, antidiarrheal and anthelmintic; stem used to strengthen tendons; fruits used in skin infections and leaves are remedy for fevers, earache and syphilitic pain. The seeds ofthe fruit are rich in potassium. The fresh fruit is used to prepare pickles, jams, and jelly etc., Alcoholic extract of root material was found to decrease blood pressure and aqueous extract of root has reported various pharmacological activities including histamine releasing, antihelmintic, sapsmolytic and cardiotonic. Fruits has also been studied it's analgesic, anti-inflammatory and lipase1 activity.Fruits of this plant were reported to contain a mixture of volatile principles like 2-phenyl ethanol, linalool, β- caryophyllene,

isoamyl alcohol and benzyl acetate and a novel (Carissol) triterpenic alcohol. Nowadays, herbal drugs are widely used as curative agent for different ailments.The present investigation carried to find out the qualitative and quantitative phytochemical analysis, antioxident properties, macro and micro nutrient contents of *Carissa carandas* (L.) fruits.

MATERIALS AND METHODS

Selection of plant species: The plant fruits of *Carissa carandas* were collected from the Thirunageshwaram, at nearby Kumbakonam, Thanjavore District, of Tamil Nadu. The plant materials were washed thoroughly 2-3 times with running tap water and once sterile with distilled water. Then the plant parts were shade dried and coarsely powdered separately and stored in well closed bottles for further analysis in laboratory.

Authentication of Plant Materials: The plant was authenticated at The Rapinat Herbarium, St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu and Botanical Survey of India (BSI), Southern Circle, Coimbatore, India. The specimen was labelled, numbered and annotated with the date of collection and locality.

Extraction of the Plant Materials: The fresh plant fruits were washed with running tap water and shade dried. These coarse powders (25g) were then subjected to successive extraction in 250ml of methanol solvent by using Soxhlet apparatus. The collected extracts were stored and then used for further analysis. The DMSO (Dimethyl sufloxide) is act as dissolved solvents for these extracts.

Qualitative Phytochemical Analysis: Preliminary phytochemical analysis was carried out for the extract as per standard methods described by Brain and Turner (1975) and Evans (1996).

Detection of Alkaloids: Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

Mayer s test. Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Wagner's test. Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Detection of Flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates that the presence of flavonoids.

H_2SO_4 test. Extracts were treated with few drops of H2SO4. Formation of orange colour indicates that the presence of flavonoids.

Detection of Steroids. Two ml of acetic anhydride was added to five mg of the extracts, each with two ml of H_2SO_4. The colour was changed from violet to blue or green in some samples indicate that the presence of steroids.

Detection of Terpenoids

Salkowski's Test: Five mg of the extract of the leaves, flowers and seeds was mixed with two ml of chloroform and concentrated H2SO4 (3ml) was carefully added to form a layer. An appearance of reddish brown colour in the inner face was indicates that the presence of terpenoids.

Detection of Anthroquinones

Borntrager's Test. About five mg of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl3 was added to the filtrate. Few drops of 10% NH3 were added to the mixture and heated. Formation of pink colour indicates that the presence anthroquinones.

Detection of Phenols

Ferric chloride test.10mg extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol.

Lead acetate test.10mg extracts was treated with few drops of lead acetate solution. Formation of yellow colour, precipitate indicates that the presence of phenol.

Detection of Saponins. About 0.5mg of the extract was shaken with five ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows that the presence of saponins.

Detection of Tannins. A small quantity of extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green color was formed. It indicates that the presence of tannins.

Detection of Carbohydrates. 0.5mg extracts were dissolved individually in five ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Detection of Protein and Amino acids

Biuret test. To 0.5 mg of extract equal volume of 40% NaoH solution and two drops of one percent copper sulphate solution was added. The appearance of violet colour indicates that the presence of protein.

Ninhydrin test. About 0.5 mg of extract was taken and two drops of freshly prepared 0.2% Ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates that the presence of proteins, peptides or amino acids.

Detection of Oils and Resins. Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates that the presence of oils and resins.

Antioxidant activity

Ferric-Reducing/Antioxidant Power (FRAP) assay by Pulido*et al.* (2000). The absorbance of the reaction mixture was read at 593 nm. The values are expressed as mmol Fe (II)/g extract.

Antioxidant activity by the ABTS + assay (Re *et al.* 1999) Radical scavenging activity of extracts was assessed spectrophotometricallyby [2,20-azino-bis(3-ethylbenzothiazoline-6- sulfonic acid)] ABTS+ cationdecolorization assay and the absorbance was taken at 734 nm (Re *et al.*, 1999). The unit of total antioxidant activity is defined as the concentration of Trolox having equivalent antioxidant activity expressed as l mol/g extracts.

Metal chelating activity by Dinis *et al.* (1994): The chelating activity of ferrous ions by different extracts of *Carissa carandas*fruitswas estimated by the method described by Dinis*et al.* (1994). Absorbance of the solution was measured spectrophotometricallyat 562 nm. The results were expressed asmg ethylene di amine tetraacetic acid (EDTA) equivalent/gextract.

Phosphomolybdenum assay (Prieto *et al.* 1999): The antioxidant activity of extracts was evaluated by the green phosphomolybdenum complex formation according to the previously described method of Prieto*et al.*, (1999). The absorbance of the mixture was measured at 695 nm. The results reported are mean values expressed as grams of ascorbic acid equivalents (AAE) per 100 g extract.

Free radical scavenging activity on DPPH by Blios (1958). The DPPHradical scavenging activity ofdifferent extracts of *B. vahlii*leaves was measured according to the method of Blios (1958). IC50 values ofthe extract i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50%was calculated.

Hydroxyl radical scavenging activity by Klein *et al.*, (1991).The scavenging activity of different extracts of *B. vahlii*leaves (20, 40, 60 and 80 lg) on hydroxyl radical activity was measured. The intensity ofthe color formed was measured spectroscopically at 412 nm against reagent blank. The hydroxyl radical scavenging activity of the sample extracts was evaluated as % ofantioxidant activity.

The b-carotene/linoleic acid antioxidant activity (Taga*et al.*, 1984). The b-carotene/linoleic acid antioxidant activity of the antioxidant (fruit extracts, or a-tocopherol (a T), 100 lL) solution was measured according to the previously described method at 470 nm. And the antioxidant activity of the bark extracts and standard was evaluated as % of antioxidant activity.

Quantitative Phytochemical analysis

Estimation of Alkaloidsby Harborne (1973): One gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and its covered and allowed to stand for 4 h. It was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated NH4OH was added by drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH4OH and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Flavonoidsby (Krishnaiah *et al.*, 2009*):* One grams of plant sample was repeatedly extracted with 100ml of80% aqueous methanol at room temperature. The mixture was filtered through a Whatman No1 filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed.

Estimation of Total Phenols: The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. Five ml of the extract was pipetted out into a 50 ml flask, then 10 ml of distilled water was added. Two ml of NH_4OH solution and 5 ml ofconcentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was read at 505nm.

Estimation of Carbohydrate by (Krishnaveni*et al.*, 1984): 100 mg of sample was hydrolysed in a boiling tube with 5 ml of2.5 N HCl in a boiling water bath for a period of3 hours. It was cooled at room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml by using distilled water. From this 0.2 ml of sample was pipetted out and made up the volume to one ml with distilled water. Then one ml of phenol reagent was added and followed by 5.0 ml ofsulphuric acid. The tubes were kept at 25-30 C for 20 min. The absorbance was read at 490 nm.

Estimation of micro and macro nutrient contents. Nitrogen and phosphorous (Yoshida *et al.*,1972), potassium (Williams and Twine, 1960), calcium, magnesium, and sodium(Yoshida *et al.*,1972).

RESULTS AND DISCUSSION

Medicinal plants were of great importance to the health of individuals and communities(Pascaline, *et.al* ,,2011). *Carissa carandas* is a species of flowering shrubs in the dogbane family, Apocynaceae. It produces berry-sized fruits that are commonly used as a condiment in Indian pickles and spices. The fruit is a rich source of iron, so it sometimes used in treatment of anemia. It contains a fair amount of vitamin C and therefore is an antiscorbutic. Mature fruit contains pectin and accordingly is a useful ingredient in jelly, jam, syrup and chutney.

Qualitative phytochemical analysis of *Carissa carandas* fruit with methanol solvent: Qualitative phytochemical analysis of *Carissa carandas*revealed that the presence and absence of alkaloids, glycosides, flavonoids, steroids, terpenoids, anthroquinone, phenols, saponins, tannins, carbohydrates, protein, aminoacids, oil, and resin were analysed in *Carissa carandas* fruits. (Table.1) The qualitative phytochemicals such as alkaloids, flavonoids, glycosides, steroids, terpenoids, phenols, saponins, tannins, carbohydrates, proteins, aminoacids, oils and resins were tested maximum phytochemicals were positively resulted in *Carissa* plant fruits. Similar reports were absorbed in the screening of phytochemicals in *C. carandas*. revealed the presence of tannins, alkaloids, saponins, sterols and flavonoids. Tannins such as tannic acid and propylgallate inhibit growth of food-borne bacteria (Sharma,*et.al,* 2011). Tannins have the ability to inactivate microbial adhesions, enzymes, cell envelope, transport proteins and polysaccharides (Akiyama,*et al.,* 2001).Dixit,(1994) observed that the flavonoids isolated from the leaves of *Ocimum sanctum* have shown to exhibit antibacterial activity against *S. aureus*, *Staphylococuscohni*, *E. coli*, *Proteus* and *Klebsialla pneumonia.*

Table 1. Qualitative Phytochemical Analysis of *Carissa carandas* fruit extracted with methanol solvent

S.No	Phytochemical Test	Inference	
1.	Alkaloids	Mayer 's test	+
		Wagner 's test	+
2.	Glycosides		+
3.	Flavonoids	Lead acetate test	+
		H_2SO_4 test	-
4.	Steroids: Liebermann Burchardtest		-
5.	Terpenoids:Salkowski test		+
6.	Arthroquinone:Borntrager's test		-
7.	Phenols	Ferric hloride test	-
		Lead acetate test	-
8.	Saponins		-
9.	Tanins		-
10.	Carbohydrates		+
11.	Protein and aminoacids	Biuret test	+
		Ninhydrin test	+
12.	Oils and Resins		-

(+ Positive –Negative)

Table 2. Antioxidant property of *Carissa carandas* fruit *by* various methods

S.No	Antioxidant properties (%)	Fruit (mg/g)
1.	FRAP	734.4± 19.40
2.	ABTS	8569.7±994.50
3.	Metal chelating property	0.9 ±0.10
4.	Phosphomolybdenum	25.4± 0.80
5.	Hydroxyl radical scavenging activity	189.5±10.40
6.	DPPH	131.0±4.80
7.	b-carotene/linoleic acid	110±4.70

± Standard deviation

Table 3. Quantitative Phytochemical analysis of the *Carissa carandas* fruit extract with methanol solvent

S.No	Phytochemicals test	Methanolic extract (fruit)
1	Alkaloids	02.15±0.06
2.	Saponins	5.8±0.174
3.	Flavonoids	10.08±0.30
4.	Phenols	07.49±0.22
5.	Terpenoids	7.8±0.234
6.	Glycosides	6.8±0.204
7.	Carbohydrates	05.89±0.17

± Standard deviation

Table 4. Macro element levels (mg/kg) in *Carissa carandas* fruits

S.no	Macro elements	Quandity(Mg/g)
1.	Nitrogen	19.74±0.592
2.	Phosphorous	2845.1±154.60
3.	Potassium	2625.2±143.50
4.	Calcium	11.3±0.06
5.	Megnesium	260.3±37.20
6.	Sodium	827.6±50.60

± Standard deviation

Table 5. Micro element levels of *Carissacarandas* fruits

S.no	Micro elements	Quandity(Mg/g)
1	Cromium	0.462±0.14
2	Cupper	3.31±0.60
3	Ferrous	3.65±0.69
4	Mangamese	0.929±0.22
5	Nickel	0.338±0.02
6	Seledium	1.827±0.47
7	Zinc	17.46±3.08

± Standard deviation

The hydroxyl group of flavonoids attributes to antioxidant and chelating action to enhance antimicrobial activity (Cowan, 1999, Heim, 2002).

Saponin, the natural detergent, has the ability of forming stable foam in water (Abid, *et.al* ., 2012). The present study showed *C. carandas* methanolic extract containing more

1. *Carissa carandas* tree with fruits

Figure 2. *Carissa carandas* fruits

saponin than other extracts, hence, linked with exhibited antibacterial activity. Antimicrobial activity of saponin is associated with the ability of forming pore in the cell membrane and hence giving the toxic material free access to the cell (Abid, *et al*., 2012). Alkaloids have been credited as good source of many drugs and destruct the microbe by intercalating the DNA. However, results of the present study showed alkaloids were absent in *C. carandas* extracts. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowora, 1993). Analysis of the plant extracts revealed the presence of phytochemicals, such as proteins, charbohydrates, phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids. Several studies have described the antioxidant properties of different parts of various medicinal plants which are rich in phenolic compounds (Brown, Rice-Evans, 1998, Berger, 2001). The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation(Garcia, *et al.,* 1997).

This methanolic extract has great free radical scavenging property and also contains liberal amount of flavonoid components. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. The carbohydrates proteins and amino acids were positively resulted in the *Carissa* plants. Similar findings were recorded in *Terminaliaarjuna* bark consists of many useful compounds, such as flavonoids, tannins, phenols, phytosterols, saponins and alkaloids. Its antioxidant activity is largely due to flavonoids.

Antioxidant property of *Carissa carandas* fruit *by* various methods: The antioxidant properties of *Carissa carandas* fruits were analysed in milligram per gram in fresh weight basis. The antioxidant properties such as ferriic reducing of assay of plasma is 734.4, AzinoBis 3- ethyl enzoThazoline 6-Sulphonic acid(ABTS) is 8569.7, metal chelating property 0.9, phosphomolybdenum 25.4, hydroxyl radical scavenging activity is 189.5, 2,2-Di Phenyl 1-Picrylhydrozyl (DPPH) is 131.0 and b-carotene/linolic acid is 110.(Table.2).The antioxidant properties such as Ferric Reduction of plasma (FRAP), Azino-Bis (3-Ethyl enzothazoline 6-Sulphonic acid

(ABTS), metal chelating property, phosphomolybdenum ,hydroxyl radical scavenging, 2, 2-Diphenyl 1-Picryll Hydrazyl (DPPH), and b-carotene were identified. *Carissa carandas* is used in traditional medicinal system for its various diseases curing property. Extraction of dried fruits of *Carissa carandas* were carried out with petroleum ether and methanol. The methanol extract and petroleum ether extract were selected for performing antioxidant activity. Here we have performed various in-vitro antioxidant assays including DPPH, metal chelating, H2O2, super oxide, anti- lipid peroxidation of petroleum ether and methanol extracts from the selected fruit. The methanol extract have showed strong antioxidant activities when compared with petroleum ether extract, which were correlated with its high level of phenolic and flavonoid. The extract possesses more phenolic and flavonoid content that causes the antioxidant activity.

Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. They are used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals. Antioxidants are involved in the defence mechanism of the organism against the pathologies associated to the attack of free radicals. Endogenous antioxidants are enzymes, like superoxide dismutase, catalase, glutathione peroxidase or nonenzymatic compounds, such as uric acid, bilirubin, and albumin. There is an increasing interest in antioxidants, particularly in thoseintended to prevent the presumed deleterious effects of free radicalsin the human body, as well as the deterioration of fats and otherconstituents of foodstuffs (Molyneux2004)).

Quantitative Phytochemical analysis of the *Carissa carandas* fruit extract with methanol solvent: The present study revealed the quantity of different phytochemicals present in *Carissa carandas* fruit extract. In *Carissa carandas* fruits contain alkaloids (02.15±0.06), saponins (5.8±0.174), flavonoids (6.4±1.3), phenols (11.54±0.7), terpenoids (7.8±0.234) and glycosides (6.8±0.204)and carbohydrates 05.89±0.17) mg/g were recorded in photochemical compounds.(Table.3). Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignin, tannins, flavonoids, quinones, coumarins, alkaloids, amines, and other metabolites, which are rich in antioxidant activity (Zheng , Wang , 2001, Cai ,*et.al*., 2003) and in recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables (Kitts ,*et.al.,* 2000, Wang ,*et.al*., 2000).

Macro elements of *Carissa carandas* Friuts: The result on the effect of application of macro elements of *carissacarandas* on nitrogen, phosphorous, and potassium, calcium, magnesium, and sodium, content of *carissacarandas* is shown in table 4.The mineral content such as nitrogen, phosphorous, potassium, calcium, magnesium, and sodium, content of of *carissacarandas* viz.,(19.74±0.592, 2845.1±154.60, 11.3±0.06, 2625.2±143.50, 260.3±37.20, and 827.6±50.60,) were recorded in *carissacarandas*.

Micro elements of *Carissa carandas* fruits: The result on the effect of application of macro elements of *carissacarandas* on micro nutrient content such as chromium, cupper, ferrous, manganese, nickel, selidium, sulphor, and zinc content of*carissacarandas* is shown in table 5.The mineral content such as chromium, cupper, ferrous, manganese, nickel, selidium, sulphor, and zinc content of *carissacarandas* viz.,(0.462±0.14, 3.31±0.60, 3.65±0.69, 0.929±0.22, 0.338±0.02, 1.827±0.47, and 17.46±3.08) were recorded in *carissacarandas*.

Conclusion

The analysis of phytochemical test such as alkaloids, glycosides, flavonoids, steroids, terpenoids, arthoquinone, phenols, saponin, tannin, carbohydrates, proteins, aminoacids, oil, resins qualitative, quantitative phytochemicals such as alkaloids, saponins, flavonoids, phenols, terpenoids, glycosides and carbohydrates, micro element levels such as chromium, cupper, ferrous, manganese, nickel, selidium, sulphor, and zinc were measured in *Carissa crandas* fruits.

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REFERENCES

Abid M.M, Khan A, Naqvi ST, Nagvi SM. 2012. Identification of phytosaponins as novel biodynamic agents.*Asian J Biol Sci.*3(3):459-467.

Akiyama H, Fujii K, Yamasaki O, Oono T, Lwatsuki K. 2001. Antibacterial action of several tannins against *Staphylococcus aureus*.*J Antimicrob Chemother*.;48(4):487-491.

Blios, M.S., 1958. Antioxidant determinations by the use of a stable free radical.*Nature;*26, 1199–1200.

Brain, K.R., and T.D. Turner, 1975. The practical evaluation of phytopharmaceuticals, Wrightscience technical, 1st Ed ,*Bristol Britain*., 144.

Brown J.E, Rice-Evans CA. 1998.Luteolin rich artichoke extract protects low density lipoprotein from oxidation in vitro. *FreeRadic Res*; 29: 247-255.

Cowan, M.M. 1999.Plant products as antimicrobial agents. ClinMicrobiol Rev..12:564– 582.

Dinis, T.C.P., Madeira, V.M.C., Almeida, L.M., 1994. Action of phenolic derivatives (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers.*Arch. Biochem. Biophys*. 315, 161–169.

Dixit, S.P., 1994. Effect of paddy straw management of soil fertility build up and yield of cowpea in wheat paddy cropping system in North-West Himalayan soil.*In:* Proceedings of Symposium on Sustainability of Rice-Wheat System in India, Karnal, Haryana.

Evans, W.C., 1966. *Trease Evans Pharmacognosy.*, 14th Ed, London:WB Saunders Ltd, 119-159.

Garcia, B., and Castillo, J. 2008. Update on Uses and Properties of Citrus Flavonoids: New Findings in Anticancer, Cardiovascular, and Anti-inflammatory Activity. *J. Agric. Food Chem;*56: 6185–6205.

Harbone, J.B., 1973. Phytochemical methods. London. *Chapman andHall, Ltd.*, 49-188.

Heim KE, Tagliaferro AR, Bobilya D.J. 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nut Biochem.*;13(10):572-584.

Kitts D.D., Yuan Y.V., Wijewickreme A.N., Hu C.2000. Antioxidant properties of a North American gingseng extract. *Mol Cell Biochem;*.20(3):1-10.

Krishnaiah, D., T. Devi, A. Bono and R. Sarbatly, 2009.Studies on phytochemical constituents of six Malaysian medicinal plants. *J. Med. Plants*. Res; 3(2): 67-72.

Krishnaveni, S., B. Theymoli., S. Sadasivam, 1984.Phenol Sulphuric acid method.*J.Pl.Sci*3 21-29.

Molyneux P. 2004.The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin *J Sci Technol* 26:

Pulido, R., Bravo, L., Saura-Calixto, F., 2000.Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing antioxidant power assay.*J. Agric. Food Chem*; 48, 3396–3402.

Radhakrishnan R, Zakaria MNM, Islam MW.2001. Analgesic and Anti- Inflammatory Activities of Teucriumstocksianum. Pharm. Bio.; 39 (6): 455- 459.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice- Evans, C., 1999.Antioxidant activity applying an improved ABTS radical cationdecolorization assay. *Free Radic. Biol. Med*; 26, 1231–1237.

Sharma C, Aneja KR, Kasera R. 2011.Screening of *Berberisaristata* DC. For antimicrobialpotential against the pathogens causing ear infections. *Int J Pharmacol*.;7(4):536-541.

Sofowora A. 1993.Medicinal plants and traditional medicine in Africa. *Int. J. of Pharma. Res*. 2(4) 1766-1788.

Taga, M.S., Miller, E.E., Pratt, D.E., 1984. Chia seeds as a source of natural lipid antioxidants. *J. Am. Oil Chem. Soc.*61, 928–931.

Wang S.Y., Jiao H. Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. *J Agric Food Chem;* 2000.48*:* 5672-5676.

Williams, C.H. and V. Twine, 1960.*In:* Peach, K. and M.V. Tracey (eds.), *Modern Methods of Plant Analysis*, Vol. 5, Springer Verlag, Berlin, pp. 3-5.

Yoshida, S., D. Fordo, J. Cork and K. Gomez, 1972. Laboratory manual for physiological studies of rice 3rdedn., *The International Rice Research Institute, Philippines*, pp.11-23.

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