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IN VITRO ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICAL ANALYSIS OF FIVE SELECTED PLANT MATERIALS USED FOR ORAL HEALTH AND HYGIENE AMONG PEOPLE OF DAKSHINA KANNADA

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ABSTRACT : A comprehensive study on the phytochemical contents and antioxidant activities of alcoholic extracts of five plant materials, such as mango leaves, fibrous pericarp of coconut and areca nut, tender twigs of *Jatropha curcus* and *Jatropha gossipifolia*, used for oral health and diseases by people of Dakshina Kannada district, Karnataka, India was conducted. The extract of different plant materials contained various levels of phenolics, flavonoids, saponins, tannins and so on. Various biochemical assays performed to assess the radical scavenging activity have shown good activity and was found to be concentration dependent. Of five selected materials, mango leaves, fibrous pericarp of coconut and areca nut showed more antioxidant activities than tender twigs of *J.curcus* and *J. gossipifolia*.

Key words: Phytochemicals, antioxidant activity, oral health, oral hygiene, Dakshina Kannada

INTRODUCTION

Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine. Plants produce hundreds to thousands of diverse chemical compounds with different biological activities (Hoareau and DaSilva, 1999). Thus, they have been used in the treatment of various human diseases for thousands of years all over the world. Traditional and folklore medicine bequeathed from generation to generation is rich in domestic recipes and communal practice and this valuable knowledge is facing the danger of being extinct as it is not being documented adequately. Recent and renewed interest in medicinal plants coupled to developments in technology has fuelled an explosion in the field of research focusing on screening programmes dealing with documentation of the traditional knowledge and systematic evaluation on the isolation of bioactive principles and in turn, the development of new drugs.

Since ancient times, traditional methods and techniques have been practiced in India, for the maintenance of oral health and prevention of oral diseases (Hebbar,et.al.,2004 and Harsha,et.al.,2002). Dakshina Kannada, a district in Karnataka of South India has a rich heritage of traditional medical practices. A survey conducted by the authors have identified 35 herbs and various traditional methods used by people of this region to maintain oral health and hygiene and as remedy for dental diseases.

Of various herbs, ones which were more frequently used were mango leaves, fibrous pericarp of coconut and areca nut, stem of *Jatropha curcus* and *Jatropha gossipifolia*. Tender stems of *J. curcus* and *J. gossipifolia* are used as chewing sticks to clean the teeth by people of rural villages, while the husks of areca nut and coconut are used like a brush. Mango leaves were rolled; one end of the roll was chewed to make it soft and fibrous and used for cleaning. Alternatively, whole leaves were chewed to make an infusion of the leaf extract and saliva. This mixture along with fibrous leaf material was rubbed against teeth and gum using finger for cleansing. Some people were also using fibrous pericarp of coconut and areca nut for cleaning teeth.

Research on identification of biologically active compounds of plant origin as an alternative remedy to various health issues is well advanced. But similar research related to oral health and diseases has not gained adequate popularity. In an attempt to identify the possible ways of the plant materials which are traditionally used for oral health and hygiene, a comprehensive study was conducted on the antioxidant properties of alcoholic plant extracts through several biochemical assays.

MATERIALS AND METHODS

Fresh leaves of *Mangifera indica,* fibrous pericarp of coconut and areca nut, tender stem of *J. curcus* and *J. gossipifolia* were collected from the wild. Plant materials collected were washed with distilled water to remove dirt and dried under shade for 21 days. The dried materials were powdered using household electric blender. 100 grams of the plant powder was soaked in 500 ml of ethanol. Extraction was done using Soxhlet apparatus. After complete extraction, the filtrate was concentrated using a rotary evaporator to obtain dry crude extract. The extracts were dissolved in dimethyl sulphoxide and used for various analyses.

Phytochemical screening

Basic qualitative phytochemical screening involving simple chemical tests was performed to detect presence of tannins, saponins, alkaloids, flavonoids, glycosides, carbohydrates, steroids and proteins in accordance with standard methods (Evans and Trease,2002).

Antioxidant activities and free radical scavenging properties:

Plant extracts were subjected to standard antioxidant assays. Different concentrations of all samples were analyzed in triplicate and the mean value was considered as value at that particular concentration. Following assays were performed:

DPPH radical scavenging assay (Furusawa,et.al.,2005)

The amount of extract per ml at which the absorbance at 517 nm decreased to half its initial value was used as the antioxidant value for the extract. 1.0 ml of 500µM DPPH (diphenylpicryl-hydrazyl) 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 presence of different concentrations of the extracts. Same procedure was also carried out with blank to determine the absorbance of DPPH before interacting with the extract. The amount of extract at which the absorbance was decreased at 517 nm, indicating DPPH used was taken as the antioxidant value of the extract.

Hydroxyl radical scavenging activity (Hazra, et.al.,2008).

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 1ml of reaction buffer (100 µM $FeCl_3$, 104 µM EDTA, 1.5mM H_2O_2, 2.5mM deoxyribose and 100 µM L-Ascorbic acid; pH 7.4) and incubated for 1 hour at 37^0C. 1 µl of 0.5% 2- thiobarbituric acid in 0.025M NaOH and 1ml 2.8% TCA was added to the mixture and heated for 30 minutes at 80^0C. The color developed was measured at 532nm against a blank containing the phosphate buffer using spectrophotometer. The inhibitory effect on the activity of hydroxyl radical was calculated as:

Inhibitory effect = (Absorbance of Control- Absorbance of Sample) / Absorbance of Control X100

Nitric oxide radical (NO) scavenging assay (Balakrishnan,et.al.,2009**)**

Sodium nitroprusside (10mM) in phosphate buffered saline (PBS) was mixed with different concentrations of extract (100-1000µg/ml) dissolved in ethanol and water and incubated at 25^0C 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.

NO scavenged (%) = Absorbance of Control- Absorbance of Sample / Absorbance of Control X100

Superoxide radical scavenging assay (Hazra,et.al.,2008**)**

This activity was measured by the reduction of NBT according to method reported by Fontana et al. The nonenzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce nitro blue tetrazolium (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 (0–20µg/ml) of sample solution. After incubation for 5 minutes at 25^0 C temperatures, the absorbance at 562 nm was measured against a blank to determine the quantity of formazan generated.

Superoxide scavenging activity (%) = Absorbance of Control- Absorbance of Sample) / Absorbance of Control X100

Reducing power (Oyaizu M, 1986)

The Fe^{3+}-reducing power of the extract was determined by the method of Oyaizu with a slight modification. Different concentrations of the extract (0.5 ml) 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 min. After incubation, 0.5 ml of TCA (10%) is added to terminate the reaction. The upper portion of the solution (1 ml) is mixed with 1 ml distilled water, and 0.1 ml $FeCl_3$ solution (0.01%) is added. The reaction mixture is 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.

RESULTS AND DISCUSSION

In our study, we have observed various phytochemical constituents such as tannins, saponins, alkaloids, flavonoids, glycosides, carbohydrates, steroids and proteins in various concentrations in ethanolic extracts of leaves of *M. indica,* fibrous pericarp of coconut and areca nut, *tender* stem of *J. curcus* and *J. gossipifolia.* Saponins were observed only in mango leaves and twigs of *J. gossipifolia* (Table 1).

Table 1: Phytochemical analysis of selected plant materials

Compound	Mango leaves	Husk of coconut	Husk of Arecanut	Twigs of *J. curcas*	Twigs of *J. gossipifolia*
Alkaloids	++	+	+	+	+
Flavonoids	++	+	+	+	+
Saponins	+	-	-	-	+
Tannins	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Proteins	-	+	-	+	+
Steroids	+	+	-	+	+
Glycosides	++	-	-	+	+

+ = Present; - = Absent

Antioxidant activity of various concentrations of ethanolic extracts of leaves of *M. indica,* fibrous pericarp of coconut and areca nut, *tender* stem of *J. curcus* and *J. gossipifolia was* measured by different biochemical assays. All the selected plant parts have shown good free radical scavenging activities and therefore antioxidant properties. Our analyses have revealed that mango leaves, coconut husks and areca nut husks are more potent in terms of their free radical scavenging activity compared to twigs of *J. curcus and J. gossipifolia*.

One of the assays that were carried out to study the radical scavenging effects of ethanol extracts was DPPH assay in which DPPH, a stable free radical with a characteristic absorption at 517nm, was used. The decrease in absorption is taken as a measure of the extent of radical scavenging and the radical-scavenging activity values were expressed as the ratio percentage of sample absorbance decrease and the absorbance of $DPPH^{\cdot}$ solution in the absence of extract at 517 nm. From the analysis (Figure 1), it can be concluded that the scavenging effects of all extracts on DPPH radicals increased with the concentration increase and were excellent, especially in the case of coconut, areca nut husk and mango leaves while the values were fair for *J. curcus* and *J.gossipifolia*. Similarly all extracts revealed a good scavenging activity on hydroxyl radicals, superoxide radical and NO radical and in all assays the scavenging activity was found to be increasing with increasing concentration of extracts (Figure 2-4).

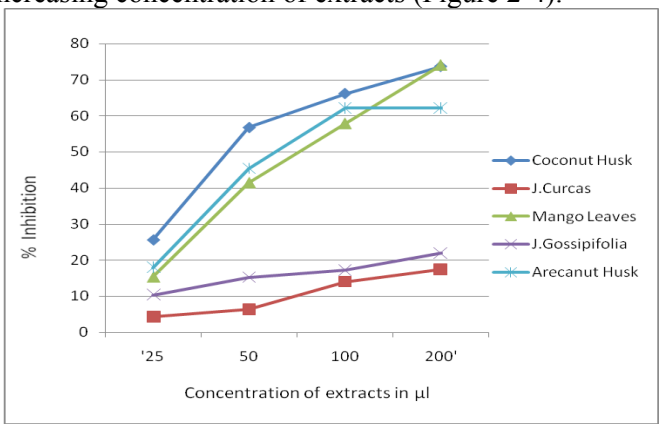

Figure 1: DPPH radical Scavenging activity of different plant extracts

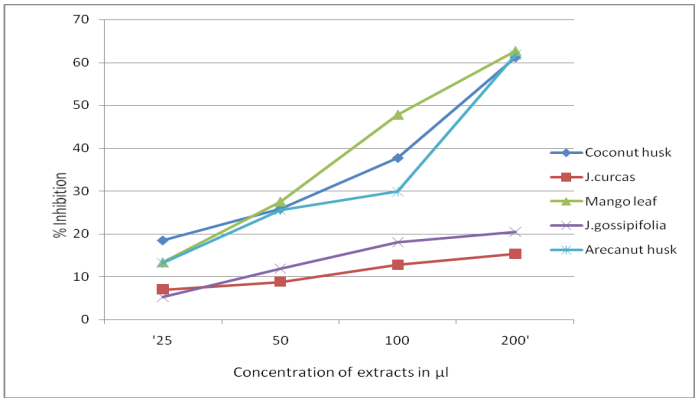

Figure 2: Nitric Oxide scavenging activity of different plant extracts

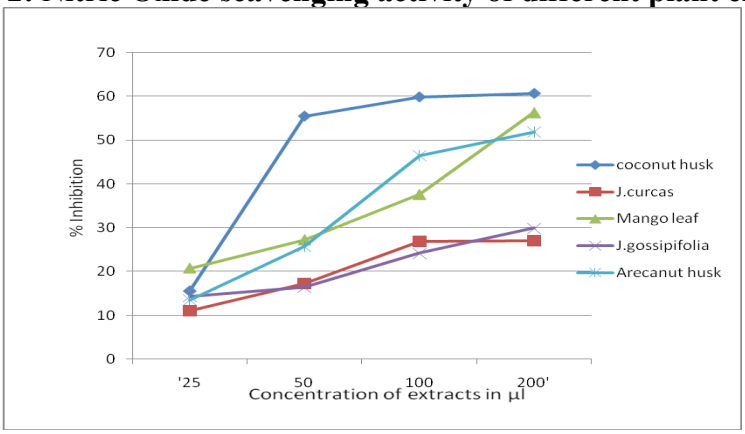

Figure 3: Hydroxyl radical scavenging activity of different plant extracts

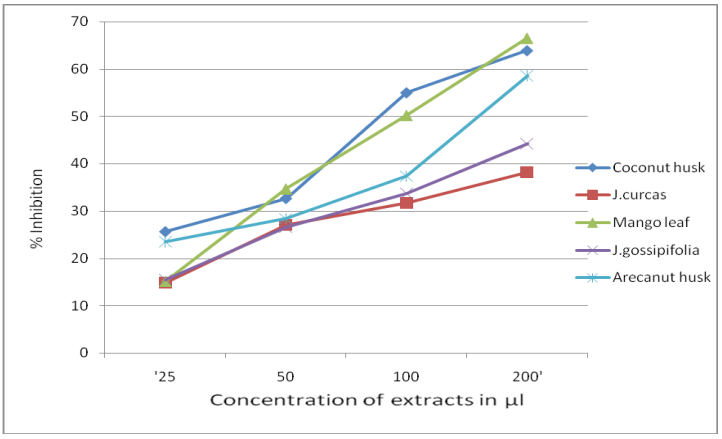

Figure 4: Superoxide radical scavenging activity of different plant extracts

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Higher absorbance indicated a higher ferric reducing power. Figure 5 shows the reducing powers of the ethanol extracts as a function of their concentration. The reducing power also increased with concentration, and the values obtained for all the extracts were good. As in other assays mango leaves, coconut and areca nut husks were found to have higher reducing power when compared to other plant materials.

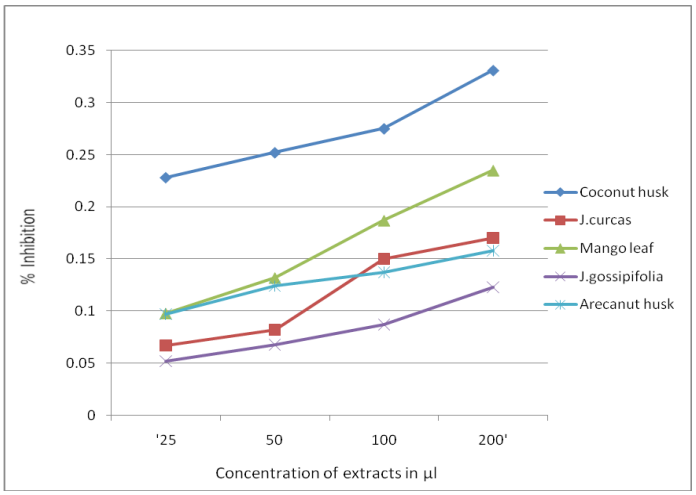

Figure 5: Reducing power of different plant extracts

Traditional methods of dental and oral care are still practiced by some people residing in rural villages. As the modern methods of oral hygiene measures gained importance, traditional knowledge is slowly vanishing away and now they are not part of everyday life. Use of leaves and fibers of some plants have been found to be highly effective for prevention and heal diseases of gum, oral mucosal diseases and tooth decay(Deepa,et.al.,2011). However, all these beneficial effects have to be scientifically validated and proved.

J.curcus and J. gossipifolia belong to the family "Euphorbiaceae". These are shrubs with smooth gray bark, which exudes whitish colored, watery, latex when cut. The root, stems, leaves, seeds and fruits of the plant have been widely used in traditional folk medicine for various ailments, in many parts of West Africa as well as other parts of world. The young stem of these plants are used as toothbrush as well as to clean the tongue in the treatment of thrush. The latex of *Jatropha* contains an alkaloid known as "jatrophine" which is believed to have anti-cancerous properties (M. Reyadh, Ogundare, 2007 and Oduola, 2007).

The coconut (*Cocos nucifera* Linn. belongs to Arecaceae family) is native to coastal areas of Southeast Asia. The Husk covering the fruit is part of its pericarp. Recent studies have revealed various medicinal properties of husk extracts such as antibacterial and antiviral (Esquenazi,et,al.,2002), antitumoral (Kirszberg, et.al.,2003 and Koschek,et.al.,2007) and antileishmanial properties (Mendonca-Filho,et.al.,2004). This extract also exhibited *in vivo* and *in vitro* analgesic and free radical-scavenging properties (Alviano,et.al., 2004).
Betel nut (*Areca catechu*) is a slender, single-trunked palm that can grow to 30 meters. Betel nut palm yields diverse products that are used throughout its range. In addition to the well known stimulant properties, the seed is used medicinally in numerous internal and external preparations. The husks, shoots, buds, leaves and roots also have local medicinal uses (George and Robert 2006).
Oral cavity being the first body part confronted by external materials that is taken in to the body as a part of food, drinks or inhaled volatile ingredients, has developed its own antioxidant defense mechanism in the form of salivary antioxidant system (Liskmann,et.al.,2007). However additional protection against the damaging free radicals generated as a part of biological function or due to exposure to tobacco chemicals can contribute to better oral health and protection from various oral diseases. Various studies have revealed the role of oxidative stress in periodontal diseases and oral cancer.

A study has identified that periodontal disease is associated with reduced salivary antioxidant status and increased oxidative damage within the oral cavity (Sculley and Evans,2003). Similarly it is also identified that in addition to cessation of tobacco use, supplementation of antioxidants are also significant in prevention of oral cancer (Garewal, 1995). From this, it can be presumed that local increase in antioxidants in oral cavity can contribute significantly to oral health.

Plants synthesize a number of antioxidant compounds as secondary products, mainly phenolics, serving in plant defense mechanisms to counteract deleterious action of reactive oxygen species (Wollgast and Anklam, 2000). Flavonoids and some other phenolic compounds of plant origin have been reported to be good scavengers of free radicals (Rechner,et.al.,2002).

With the help of various antioxidant and radical scavenging assays, the present study demonstrated efficient antioxidant activity in plant extracts. We have also observed that all the plant materials that have been selected have phenolic compounds that contribute to antioxidant property. Results of our study are consistent with previous reports. Researchers in their investigations on different parts of *C. nucifera* demonstrated *in vitro* antioxidant activities of coconut husk (Naskar, et.al., 2011and Alviano,et.al., 2004). Only one study was found in literature on areca nut husk (Hai-De Zhang et al. 2009). As observed in the present study, the authors have reported good antioxidant activity of areca nut husks. Similarly as noted by us antioxidant properties of mango leaves (Doughari and S.Manzara, 2008 and Olabinri, 2010) and *J. curcus* (Diwani,et.al.,2009) and *J. gossipifolia* (Shahwar,et.al.,2010) have been reported earlier.

CONCLUSION

Oxidative stress induced by free radicals is identified to play a definite role in various oral diseases such as periodontal diseases and tobacco induced lesions including oral cancer. Antioxidants scavenge the free radicals and protect the oral environment from their damaging effects. The present investigation confirmed that the plant materials that have been used traditionally for oral health and hygiene have good antioxidant activity, reducing power and free radical scavenging activity. Hence we can conclude that the traditional oral hygiene measures contribute to oral health and hygiene through the efficient antioxidant activity. Further work is required to analyze other possible beneficial effects such as antimicrobial and antiproliferative activities. The plant materials selected in this study are relatively less investigated for their antioxidant capacity. Therefore more studies on these are recommended which can lead to identification of natural antioxidants, without undesirable side effects that may be incorporated in to modern oral care systems.

REFERENCES

A.R. Rechner, G.Kuhnle, P.Bremner, G. P.Hubbard, K.P.Moore, and C.A. Rice-Evans (2002).The metabolic fate of Dietary phenolics.Free Radical Biology and Medicine:Vol. 33, 220–235.

A.O. Ogundare(2007). Antimicrobial Effect of *Tithonia diversifolia* and *Jatropha gossypifolia* Leaf Extracts. Trends in Applied Sciences Research: Vol.2, 145-150.

B.Hazra, S.Biswas and N.Mandal(2008). Antioxidant and free radical scavenging activity of *Spondias pinnata.* BMC Complementary and Alternative Medicine:Vol. 8,63 doi:10.1186/1472-6882-8-63.

B.M. Olabinri,M.T.Olaleye, O.O.Bello, L.O.Ehingie and P.F. Olabinri(2010). In vitro comparative antioxidative potentials of mango and pawpaw leaves. International journal of tropical medicine:Vol. 5(2):40-45.

C.Kirszberg, D.Esquenazi, C.S. Alviano and V.M. Rumjanek(2003). The effect of a catechin-rich extract of *Cocos nucifera* on lymphocytes proliferation. Phytother Res:Vol. 17, 1054-1058.

D.Esquenazi, M.D.Wigg, M.M.Miranda, H.M.Rodrigues, J.B.Tostes and S.Rozental(2002) .Antimicrobial and antiviral activities of polyphenolics from *Cocos nucifera* Linn. (Palmae) husk fiber extract. Res Microbiol: Vol.15, 647-652.

D.S.Alviano, K.F.Rodrigues, S.G.Leitao, M.L.Rodrigues, M.E.Matheus and P.D Fernandes(2004). Antinociceptive and free radical scavenging activities of *Cocos nucifera* L. (Palmae) husk fiber aqueous extract.J Ethnopharmacol: Vol. 92, 269-273.

D.Shahwar,S. Rehman, N. Ahmad, S.Ullah, M.A. Raza(2010). Antioxidant activities of the selected plants from the family Euphorbiaceae, Lauraceae, Malvaceae and Balsaminaceae. African Journal of Biotechnology: Vol. 9(7), 1086-1096.

D.V.Sculley and S.C. Evans (2003). Periodontal disease is associated with lower antioxidant capacity in whole saliva and evidence of increased protein oxidation. Clinical Science: Vol. 105, 167–172.

G.E.I. Diwani, S.E.Rafie and S.Hawash(2009). Antioxidant activity of extracts obtained from residues of nodes leaves stem and root of Egyptian *Jatropha curcas* African Journal of Pharmacy and Pharmacology: Vol. 3(11), 521-530.

H. Garewal(1995). Antioxidants in oral cancer prevention.Am J Clin Nutr:Vol.62 (Suppl), 1410s-6s.

Hai-De Zhang, W.M.Zhang, B.Li, L. Han, (2009). Antioxidant activities of extracts from areca (*areca catectu* l.) flower, husk and seed. Electronic journal of environment, agriculture and food chemistry:Vol. 8 (9), 740-748.

J. H Doughari and S.Manzara(2008). *In vitro* antibacterial activity of crude leaf extracts of *Mangifera indica* Linn.African Journal of Microbiology Research: Vol.(2), 67-72.

K.Deepa, M.Jose and V.Prabhu(2011). Ethnomedicinal practices for oral health and hygiene of tribal population of Wayanad, Kerala. International Journal of Research in Ayurveda and Pharmacy: Vol. 2(4), 1246-1250.

L.Hoareau and E.J. DaSilva. (1999).Medicinal plants: a re-emerging health aid. Journal of Biotechnology: Vol.2 (2), 56-70.

M. Reyadh. The cultivation of *Jatropha curcas* in Egypt. www.fao.org/docrep/ x5402E/X5402e11.htm

M.Furusawa, T.Tanaka,, T.Ito, A.Nishikawa, N.Yamazaki and K.Nakaya(2005). Antioxidant activity of hydrohyflavonoids.Journal of Health Science: 51(3), 376–378.

N.Balakrishnan, A.B.Panda, N.R.Raj, A.Shrivastava and Prathani R(2009*)*. The evaluation of nitric oxide scavenging activity of *Acalypha indica* linn Root. Asian J. Research Chem: Vol. 2(2),148-150.

Oyaizu M (1986). Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. Jap J Nutr, Vol.44, 307-315.

P.R. Koschek1, D.S. Alviano,C.S. Alviano and C.R. Gattass(2007). The husk fiber of *Cocos nucifera* L. (Palmae) is a source of anti-neoplastic activity. Brazilian Journal of Medical and Biological Research:Vol. 40,1339-1343.

R.R. Mendonca-Filho, I.A.Rodrigues, D.S.Alviano, A.L.Santos, R.M.Soares and C.S. Alviano (2004). Leishmanicidal activity of polyphenolic-rich extract from husk fiber of *Cocos nucifera* Linn. (Palmae). Res Microbiol: Vol.155, 136-143.

S.Liskmann, T.Vihalemm, O.Salm, K.Zilmer,K.Fischer and M.Zilmer(2007). Characterization of antioxidant profile of saliva in peri-implant health and disease. Clin Oral Impl. Res: Vol.18, 27-33.

S.Naskar, U.K.Mazumder, G.Pramanik, A.Bala, P.K.Haldar, A. Islam and M. Gupta(2011). Comparative *in vitro* antioxidant activity of different parts of *Cocos nucifera* (linn.) on reactive oxygen and nitrogen species. Int J Pharm Pharm Sci: Vol 3(3), 104-107.

S.S.Hebbar, V.H.Harsha, V.Shripathi and G.R.Hegde(2004). Ethnomedicine of Dharwad district in Karnataka India. Plants used in oral health care. Journal of Ethnopharmacology:Vol. 94, 261-266.

T. Oduola, G. B. Popoola1, O. G. Avwioro, T. A. Oduola, A.A. Ademosun1 and M. O. Lawal(2007). Use of *Jatropha gossypifolia* stem latex as a haemostatic agent: how safe is it? Journal of Medicinal Plants research: Vol.1 (1), 14 – 17.

V.H.Harsha, S.S.Hebbar, G.R. Hegde and V.Sripathi(2002).Ethnomedical knowledge of plants used by Kunabi Tribe of Karnataka in India. *Fitoterapia*:Vol. 73, 281-287.

W.Evans and G.Trease(2002). Pharmacognosy. Churchill Livingstone Harcourt Health services, London. 15th edition; p. 2, 26, 127- 147.

W.S.George and F.B.Robert (2006).Species Profiles for Pacific Island Agroforestry www.traditionaltree.org. August,ver. 1.3

Wollgast, and E. Anklam(2000). Review on polyphenols in *Theobroma cacao*: changes in composition during the manufacture of chocolate and methodology for identification and quantification. Food Research International: Vol. 33, 423–447.