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COMPARATIVE EVALUATION OF ANTIOXIDANT ACTIVITY OF 50% HYDROALCOHOLIC EXTRACT OF *NALPAMARAM*

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ABSTRACT

Nalpamaram is an important group of trees in Ayurveda system of medicine, which constitutes the four lactiferous trees ie, *Ficus benghalensis, Ficus religiosa, Ficus racemosa* and *Ficus microcarpa* of the Family *Moraceae*.Traditionally these plants are used separately or in combination for treatment of various diseases . Free radicals play important roles in ageing and in the pathogenesis of cancer, hypertension, atherogenesis, Alzheimer's disease, and Parkinson's disease. In the present study, an attempt was made to carry out the comparative evaluation of antioxidant activity of the barks of the above 4 trees. In this study, we carried out separate and combined extraction of four barks using 50% hydroalcoholic mixture. Preliminary phytochemical studies showed that extracts contained flavonoids, phenolics, tannins, carbohydrates, sterols and terpenoids. The Folin ciocalteau method and Aluminium chloride colorimetry studies revealed that *F.religiosa* contained more phenolics and flavonoids than other extracts. Combined Nalpamaram extract and its individual components showed good Nitric oxide scavenging activity {combined Nalpamaram extract(IC_{50} =102.1μg/ml)>*F.religiosa*>*F.microcarpa* >*F.racemosa*>*F.bengalensis*.}and Hydrogen peroxide scavenging activity {*F.religiosa* (IC_{50}=139.21μg/ml)> Combined Nalpamaram extract> *F.microcarpa*> *F.racemosa*> *F.bengalensis*}.

Key Words:- Antioxidant, Moraceae, Nalpamaram, Nitric oxide, Hydrogen peroxide.

INTRODUCTION

Cell damage caused by free radicals appears to be a major contributor to ageing and to degenerative diseases of ageing such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases.

NO is an important bioregulatory molecule, which has a number of physiological effects. During infections and inflammations, formation of NO is elevated and may bring about some undesired deleterious effects. There is now increasing evidence to suggest that NO and its derivatives produced by the activated phagocytes may have a genotoxic effect and may contribute in the multistage carcinogenesis process. The plant products may have the property to counteract the effect of NO formation and in turn preventing the ill effects of excessive NO generation *in vivo.* (Ganesh Chandra J *et al*., 2004)

When oxygen is supplied in excess or its reduction is insufficient, reactive oxygen species or free radicals such as superoxide anions, hydroxyl radicals and hydrogen peroxide are generated. Hydrogen peroxide may not be highly toxic in itself; it is easily reduced further to hydroxyl radical, which is extremely damaging. Thus the scavenging of H_2O_2 in cells is critical to avoid oxidative damage (Michalak A, 2006).

Antioxidants are capable of stabilizing or deactivating, free radicals before they attack cells. However, antioxidant supply is limited as one antioxidant molecule can react only with a single free radical.

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Therefore, there is a constant need to replenish antioxidant resources, either endogenously or through supplementation as they render beneficial effects to mankind. For these reasons, antioxidants are of interest for the treatment of many kinds of cellular degeneration.

Phenolic compounds are powerful antioxidants and exhibit a wide range of physiological properties, such as anti-allergenic, antiatherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio protective and vasodilatory effects. The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity. The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations (Ali Aberoumand *et al*., 2008).

In the traditional Indian system of medicine, Ayurveda , mixtures of plants are used rather than one species.*Nalpamaram* is in an important group of trees in Ayurveda which constitutes four milky latex secreting trees namely; *Ficus benghalensis ,F.religiosa ,F.racemosa* and *F.microcarpa* of the Family *Moraceae*. The barks of these species are used for various diseases like skin diseases, ulcers, inflammation, dysentery, diarrhoea, diabetes.

Leucopelargonodin-3-O-α-L rhamnoside ,leuco cynidin 3-O- α-D galactosyl cellobioside, Glucoside, 20 tetratriaconthene-2-one,6- heptatriacontene-10-one, pentatriacontan-5-one,β-sitosterol-alpha-Dglucose and meso-inositol have been isolated from the bark of the *Ficus bengalensis* (Baby Joseph *et al.,* 2010) *F.religiosa* has been reported to contain β-sitosteryl-d-glucoside, vitamin K, n-octacosanol, methyl oleanolate, lanosterol, stigmasterol, lupen-3-one, bergapten and bergaptol (Bhanumathy M *et al*., 2010). The stem bark of *F.racemosa* showed the presence of leucocyanidin -3-o-β-glucopyranoside, leucopelarogonidin -3-o-α-L-rhamnopyranoside , β-sitosterol, ceryl behenate,lupeol, lupeol acetate, α-amyrin acetate (Padmaa M Paarakh, 2009). Lupenyl acetate, friedelin, glutinol, epifriedelinol, *β*-amyrin acetate, β-amyrin, protocatechuic acid, marmesin, trans-catechin, 4, 5-dihydroblumenol and isoflavones have been isolated from stem barks of *F.microcarpa* (Changwei A, 2009)

The stem barks of *F.bengalensis, F.religiosa, F.racemosa* and *F.microcarpa* are used separately or in combination in many Ayurvedic formulations to treat various disorders. Their additional property to scavenge NO may add to its therapeutic effects. Various antioxidant studies are reported in these plants. The objective of the present study was to carry out comparative evaluation of nitric oxide scavenging activity and hydrogen peroxide scavenging activity of 50% hydroalcoholic extract of these barks and the combined extract of these barks.

MATERIALS AND METHODS

The stem barks of *F.bengalensis*(Fb), *F.religiosa*(Fr), *F.racemosa* (Fra) and *F.microcarpa* (Fm) were collected from different districts of Kerala.All the barks were identified and authenticated by Mr. Joby Paul, Botanist, Environmental Sciences; M. G .University, Kottayam and voucher specimens were deposited in the University herbarium. (424,425,428,429)

Extraction

All the stem barks were cut into small pieces, dried in shade for 2 weeks and coarsely powdered. All the powdered barks were separately extracted in soxhlet apparatus using 50% v/v hydroalcoholic mixture as solvent. The residues were collected after evaporating the solvent and the yield of each extract was calculated.

Equal amount of all the four powdered barks were mixed together and extracted using 50% v/v hydroalcoholic mixture, evaporated the solvent to collect the residue and termed as Combined *Nalpamaram* extract. (Ne)

Preliminary phytochemical screening

The hydroalcoholic extracts of four barks were subjected to qualitative tests for the identification of various phytoconstituents.

Total phenolic content

Total phenolic estimation was carried out by using the standard Folin-Ciocalteau method. The extracts and the standard, gallic acid, were dissolved in methanol separately for the total phenolic estimation. One milliliter of each extract was mixed with 5 ml of Folin-Ciocalteau reagent, and 4 ml of sodium carbonate solution added after 3 min and kept at room temperature for two hours. Absorbance values were measured at 750 nm and a standard curve was prepared using gallic acid. The total phenolic content was expressed as gallic acid equivalents in milligrams per gram dry material (Akhila S *et al*., 2009)

Total flavonoid content

Estimation of flavonoids was carried out by the aluminium chloride colorimetric method. The extracts and the standard, rutin, were dissolved in methanol separately for the total flavonoid estimation. Each extract (1 ml) were mixed with 4 ml of water and 0.3 ml of sodium nitrate. After 5 min, 0.3 ml of 10% aluminium chloride solution was added and at the 6th minute, 2 ml of 1 M sodium hydroxide were added. After proper mixing, absorbance was measured at 510 nm and a standard curve was

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prepared using rutin. The total flavonoid content was expressed as milligram equivalents of rutin. (Akhila S *et al*., 2009)

Nitric oxide scavenging assay

The standard quercetin and the extracts were prepared in phosphate-buffered saline at 50, 100, 150 and 200 µg/ml concentrations. The assay reaction mixture (3 ml) was prepared by mixing 2 ml of 10 mM sodium nitroprusside solution, 0.5 ml of phosphate-buffered saline, and 0.5 ml of the sample or standard solution. These were then incubated at 25°C for 2.5 hours. After incubation, 0.5 ml of the reaction mixture was pipetted out and mixed with 1 ml of sulphanilic acid reagent (final concentration of 0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for complete diazotization. Then, 1 ml of 1-naphthylamine solution was added, mixed, and allowed to stand for 30 min to form pink chromophores. The absorbance was then measured at 540 nm against the corresponding blank solution. All samples were prepared and assayed in triplicate and averaged. The antioxidant activity was measured using the formulae:

$$\%\,\text{Inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

Where, A_c - is the absorbance of the control; A_s - is the absorbance of sample/standard

Hydrogen peroxide scavenging assay

The standard, α-tocopherol, and all the extracts were prepared in phosphate buffer, pH 7.4. Sample and standard solutions (0.5 ml) were taken in different test tubes and to each test tube; 0.6 ml hydrogen peroxide solution (2 mM hydrogen peroxide in phosphate buffer, pH 7.4) was added. A control was prepared by replacing the sample/standard with phosphate buffer. These solutions were then kept at room temperature for ten minutes. The absorbance was measured at 230 nm against the blank solution containing phosphate buffer without hydrogen peroxide. All samples were prepared and assayed in triplicate and averaged. The antioxidant activity was measured using the same formulae as that in NO scavenging assay (Akhila S *et al.,* 2009)

RESULTS

All the extracts were found to contain flavonoids, tannins, phenolics saponins, sterols, carbohydrates and triterpenoids by preliminary phytochemical screening.

The 50% hydroalcoholic extract of stem bark of *F.religiosa* had highest concentration of total phenolics and flavonoids than the other extracts (table 1.).

All the extracts exhibited good NO scavenging activity *in vitro* and the scavenging of NO by the extracts was increased in dose dependent manner.

F.bengalensis and *F.religiosa* showed maximum activity of 61.74 % and 69.09% respectively at 200 µg/ml. *F.racemosa* and *F.microcarpa* showed maximum activity of 63 % and 64.83% respectively at 200 µg/ml .The combined Nalpamaram extract showed maximum activity of 68.49% where as the standard quercetin showed maximum activity of 74.54 % at 200 µg/ml.

The combined Nalpamaram extract and its individual components demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner. *F.bengalensis* and *F.religiosa* showed maximum activity of 64.96% and 68.30 % respectively at 200 µg/ml. *F.racemosa* and *F.microcarpa* showed maximum activity of 66.33 % and 67.57 % respectively at 200 µg/ml. The combined Nalpamaram extract showed maximum activity of 67.89% where as the standard alpha-tocopherol showed maximum activity of 87.43 % at 200 µg/ml [Table 2].

Table1. Estimation of Total Phenolics and Flavonoids

Extract	Absorbance at 750 nm		Conc.obtained from graph µg/ml	
	Total Phenolics	Total Flavonoids	Total Phenolics	Total flavonoids
F.bengalensis	0.760	0.075	74.1	61.9
F.religiosa	0.895	0.089	85.6	72.6
F.racemosa	0.852	0.081	81	66.7
F.microcarpa	0.787	0.077	75.3	63.7

Table 2. Antioxidant activities of extracts

Extracts	IC_{50} values of extracts µg/ ml	
	NO scavenging assay	H_2O_2 scavenging assay
F.bengalensis	141.8	154.6
F.religiosa	108.2	139.2
F.racemosa	120.0	150
F.micrcarpa	113.4	146

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Nalpamaram	102.1	143.2
Quercetin	85.9	106.9

DISCUSSION

The hydroalcoholic extract of stem bark of *F.bengalensis, F.religiosa, F.racemosa* and *F.microcarpa* are rich source of phenolics and flavonoids. The antioxidant activity of the 50% hydroalcoholic extract of these barks may be due to the presence of phenolics and flavonoids.The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations.

Nitric oxide has been found to be involved in a number of regulatory functions in inflammation. Although nitric oxide and superoxide radicals are involved in host defense, over production of these two radicals contributes to the pathogenesis of some inflammatory diseases. Moreover in the pathological conditions, nitric oxide reacts with superoxide anion and form potentially cytotoxic molecules, peroxynitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspect of inflammation and tissue damage seen in inflammatory diseases (Hazeena Begum H, 2007) . In this study all the four extracts decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro.* Their efficacy in the NO scavenging may partly be responsible for their clinical activity, as excess NO is known to damage the immune system and deteriorate health. Further, the high scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health.

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. Thus the scavenging of H_2O_2 in cells is critical to avoid oxidative damage.

CONCLUSION

The hydroalcoholic extracts of stem barks of *F.bengalensis, F.religiosa*, *F.racemosa* and *F.microcarpa* have good free radical scavenging activity due to the presence of phenolics and flavonoids. The broad therapeutic effects of the Ayurvedic formulations of these barks can be largely attributed to their antioxidant properties. These Ficus species may be useful in the management of free radical mediated diseases

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Nil

CONFLICT OF INTEREST

No interest

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