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EVALUATION OF DPPH RADICAL SCAVENGING ACTIVITY OF THE LEAF AND BARK EXTRACTS OF *SAMADERA INDICA* FROM SOUTH INDIA

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ABSTRACT

The DPPH radical scavenging activity of the petroleum ether, ethyl acetate, chloroform, methanol and aqueous extracts of the leaf and bark of *Samadera indica* was investigated. Among the leaf and bark extracts of *Samadera indica* studied, chloroform, ethyl acetate and methanol extracts of leaf and bark extracts showed potent scavenging activity on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. The remarkable antioxidant activity exhibited by the leaf and bark extract scan be attributed to the synergic effect of the active compounds present in it. Phytochemical screening of the leaf and bark extracts showed the presence of phenolic compounds, flavonoids, alkaloids,steroids and terpenoids in them and these compounds are reported to have antioxidant properties. The results showed that the leaf and bark extracts of *Samadera indica* can be considered as good source of natural antioxidants and can be incorporated into drug formulations.

KEYWORDS: *Samadera indica*, DPPH radical scavenging activity, Phytochemical screening, Antioxidants, Drug formulations.

1.1 INTRODUCTION

Samadera indica (Simaroubaceae) isa bitter plant widely distributed throughout India, and mostly found in evergreen forest of Western Ghats and along river shore.*Samadera indica* is a small tree up to 11 m in height with stout branches and pale yellow bark and leaves are

large, up to 25 cm long and 9 cm broad, elliptic-oblong, shortly acuminate, entire, shining and base rounded. Flowers are pinkish yellow in few or many flowered umbels, peduncles longer than the leaves, pedicels red. Fruits are large, flat, pear shaped, much compressed, smooth reticulate. The bark and wood are stomachic, emmenagogue, febrifuge , tonic and are useful in vitiated conditions of vata, dyspepsia, flatulence, colic, dysmenorrhoea and generaldebility. The leaves are used to cure puritis, leprosy, scabies, pruritus, skin diseases, constipation and bilious fever. The seed oil is astringent, acrid, thermogenic, depurative, emetic, purgative and febrifuge,[1,2] The plant is popularly known as Gucchakaranjah in Sanskrit, Lokhana Hindi Niepa bark tree in English and karingota in Malayalam.[3]Invitro antiplasmodial activity was reported in roots and leaves of *Samadera indica* .[4] Samaderin B and C isolated from the seed kernels of *Samdera indica* were shown to exhibit anti-feedant activity against *Spodopteralitura*.[5]

Samadera indica was evaluated for its physiochemical property and polyherbalointments with antiseptic activity was formulated using its extract.[6] Its leaves when bruised is applied for curing insect bites. *Samadera indica* is playing a significant role in its anti-inflammatory activity.[7] Flavone-o-glycoside, luteoline-7-o-β-D-glucopyranoside were isolated from the kernels of *Samadera indica*.[8] Antioxidant as well as antimicrobial potential of the alcoholic extract of crude plant were investigated.[9]

There is little knowledge about crop resistance to termite attack. However, in general indigenous crops are more resistant to termites than exotic crops. It may be advisable to establish small plantations in the field prior to larger scale plantations in order to discover if the crop or tree is resistant to local termites in local conditions. The leaves of *Samadera indica* (Gaertn) shows remarkable termite control properties.[10,11]

The plant was taken for investigation because of its abundance, the high number of folklores circulating about this plant and the carry out of Literature Survey emphasized the need for more study on this plant.Antioxidant has gained importance in the current scenario as it has an ability to trap free radicals which are produced during the degenerative diseases. Natural antioxidant is considered superior to synthetic as it is safe and produces a prominent action. The bitterness of *Samadera indica* is due to the presence of flavanoids like Quassinoids which is reported to as an antioxidant.[12,13]

The potential of plants as a medicine and for healing diseases were known to humans for a long time and we were bequeathed the knowledge from our ancestors. In developing countries, synthetic medicines are used, who's knowledge was derived from plant constituents; but synthetic medicines have huge side-effects. So, the present study highlights the need for identifiying phytoconstituents present in *Samadera indica* and to investigate the anti-oxidant potential of the extracts of leaf and bark of the plant, hoping in the future the study will formulate new drugs that can compete against dreadful diseases for the well fare of the human race.

1.2 MATERIALS AND METHODS

1.2.1 PlantMaterial

From the coastal regions of Thrissur District, Kerala (Perinjanam and Vellankallur), *Samadera indica* leaves and barks were separately collected in the month of December 2013 and authenticated by Dr. Kochuthressia M.V., HOD, Department of Botany, Vimala College, Thrissur, Kerala. Voucher specimen is deposited in the specially maintained herbarium, Department of Botany, Vimala College, Thrissur, Kerala.

1.2.2 Preparation of Extracts

Two kilograms of the powered leaves and two and a half kilograms of bark of the plant material were separately extracted successively with three lires of petroleum ether, chloroform, ethyl acetate, methanol and water.

1.2.3 Preliminary Phytochemical analysis

The sample extracts were analysed for the presence of various phytoconstituents like of flavonoids, alkaloids, glycosides, steroids, phenols, saponins and tannins according to standard methods.[14,15]

1.2.4 DPPH free radical scavenging assay

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants.[16] The hydrogen or electron donation abilities of the compounds were measured from the bleaching of the purple-coloured ethanol solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). This spectro-photometer assay uses the stable radical DPPH as a reagent. The sample solution of material (50 µl) at four concentrations (1.0, 0.5, 0.25 and 0.125 mg/ml) was mixed with freshly prepared methanolic solution of DPPH (634 μM) and allowed to stand for 30 min at room temperature. The

absorbance was then measured at 515nm using a spectrophotometer and the inhibition of free radical DPPH in percent (%) was calculated using the formula below:

The percent of inhibition of DPPH reduction (decolourization)

$$\% \text{ of inhibition} = \frac{A_0 - A_{sample}}{A_0} \times 100$$

The percent of inhibition of DPPH reduction (decolourization) where (A_0) is the absorbance of the control (blank) and (A sample) is the absorbance of the test compound. The compound concentration demonstrating 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against sample concentration. Tests were carried out in triplicate. Samples and DPPH were dissolved in methanol. L-ascorbic acid was used as positive control.[17]

1.3 RESULS AND DISCUSSION

1.3.1 Phytochemical screening: The phytochemical screening of the leaf extracts of *Samadera indica* revealed that alkaloids,flavanoids, glycosides,steroids and terpenoids are present large amount in the chloform and ethyl acetate extracts. (Table1) The phytochemical screening of the bark extracts gives significant results for alkaloids, flavanoids, glycosides, steroids and terpenoids. These phytochemicals confer antibacterial, antioxidant, anticancer activity on the root extracts. (Table2)Phytochemical studies revealed the presence of various secondary metabolites in the leaf and bark extracts of *Samadera indica.* The study shows the presence of alkaloids, steroids, glycosides, terpenoids, phenols and flavonoids in significant amount. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences andconfer antibacterial, antioxidant, anticancer activity on the leaf and bark extracts of the plant.The results of this study support the use of this plant for human diseases and reinforce the ethnobotanical importance of plant as a potential source of bioactive substances.[18]

1.3.2 Antioxidant activity: The antioxidant activity of*Samadera indica* leaf and bark extracts in solvents of varying polarity were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH. The method is based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant. DPPH solutions show a strong absorption band at 515 nm appearing as a deep violet color. The absorption vanishes and the resulting decolourization is stoichiometric with respect to degree of reduction. The remaining DPPH, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant.

The results of the free radical scavenging activity of the leaf and bark extracts of *Samadera indica* assessed by DPPH assay were summarized in Tables 3,4. *Samadera indica* chloroform extract of leaf and bark , ethyl acetate extract of bark and leaf and methanol extractof leaf and petroleum ether extract of bark possess potent free radical-scavenging activity. The amount of the sample needed for 50% inhibition of free radical activity is expressed by IC_{50}. Lower IC_{50} value indicates higher antioxidant activity. IC_{50} values of leaf and bark extractsand the authentic antioxidant L-ascorbic acid are also evaluated.

Antioxidants are resistance against the oxidative stress by scavenging free radicals and by other mechanism and prevent body from oxidative diseases. Plants are identified as the source of natural antioxidants that can protect body from oxidative decay and has reported less side effect.[19,20]

The DPPH free radical scavenging activity of the leaf and bark extracts of *Samadera indica* are sorted in descending order: leaf chloroform extract > leaf ethyl acetate extract > leaf methanol extract > leaf aqueous extract> leaf petroleum ether extract, bark chloroform extract > bark ethyl acetate extract > bark petroleum ether extract > bark aqueous extract > bark methanol extract. Out of the samples tested, *Samadera indica* bark chloroform extract showed the highest scavenging activity (% inhibition 100, 100, 98.75 and 90 at 1.0, 0.5, 0.25 and 0.125 mg/ml respectively), followed by *Samadera indica* leaf chloroform extract(% inhibition 100, 98.75, 96.25 and 94.25 at 1.0, 0.5, 0.25 and 0.125 mg/ml respectively). Leaf petroleum ether extract exhibited least DPPH radical scavenging ability with % inhibition 70, 33.75, 22.5 and 7.50 at 1.0, 0.5, 0.25 and 0.125 mg/ml respectively. By comparing the IC_{50} value of the leaf and bark extracts of *Samadera indica* with that of the authentic antioxidant L-ascorbic acid, it was found that the antioxidant activity of *Samadera indica* bark chloroform (IC_{50}: 44.61 µg/ml) and leaf chloroform (IC_{50}: 47.7 µg/ml) extracts was lower than IC_{50}value of standard antioxidant L-ascorbic acid (IC_{50}: 53.15 µg/ml). IC_{50} value of *Samadera indica* bark ethyl acetate extract (IC_{50}: 55.7 µg/ml), bark petroleum ether extract (IC_{50}: 61.09 µg/ml) and ethyl acetate extract of leaf (IC_{50}: 62.36 µg/ml) and leaf methanol extract (IC_{50}: 66.36 µg/ml) are quite comparable to $IC_{50}$of L-ascorbic acid (IC_{50}: 53.15 µg/ml). The lower IC_{50} values of these extracts attributes to the higher antioxidant potential of these extracts of the plant.

Table 1 :Phytochemical analysis of leaf extracts of *Samadera indica*

Phyto constituents	Petroleum ether	Ethyl acetate	Chloroform	Methanol	Water
Phenolic component	+	++	+++	++	_
Flavanoids	+	+++	+++	+++	++
Glycosides	+++	+++	+++	++	-
Steroids	+++	+++	+++	+++	+++
Terpenoids	+++	+++	+++	+++	++
Saponines	+++	+++	+++	++	++
Alkaloids	+++	+++	+++	+++	++

+ present ++ moderately present +++ appreciable amount

Table 2: Phytochemical analysis of bark extracts of *Samadera indica*

Phyto constituents	Petroleum ether	Ethyl acetate	Chloroform	Methanol	Water
Phenolic component	+++	+++	++	+++	+++
Flavanoids	+++	+++	++	+++	+++
Glycosides	+++	++	+++	+++	+++
Steroids	+++	+++	+++	+++	++
Terpenoids	+++	+++	+++	+++	++
Saponines	_	+	+	++	+
Alkaloids	+++	+++	+++	+++	++

+ Present ++ moderately present +++ appreciable amount

Table 3: DPPH scavenging activityof leaf extracts of *Samadera indica*

***Samadera indica* leaf extracts**	**Concentration of sample in mg/ml**				**IC_{50} in µg/ml**
	1mg/ml	**0.5mg/ml**	**0.25mg/ml**	**0.125mg/ml**	
	% of Inhibition of sample				
Petroleum ether	70	33.75	22.5	7.50	710.66
Ethyl acetate	95	91.25	87.5	75	62.36
Chloroform	100	98.75	96.25	94.25	47.7
Methanol	92.5	91.25	82.5	70	66.36
Water	95	82.5	70	45	219.5
L Ascorbic acid	97.5	96.25	95	93.75	53.19

Table 4: DPPH scavenging activity of bark extracts of *Samadera indica*

Concenration of sample in mg/ml					
***Samadera indica* bark extracts**	**1mg/ml**	**0.5mg/ml**	**0.25mg/ml**	**0.125mg/ml**	**IC_{50} in mg/ml**
	% of Inhibition of sample				
Petroleum ether	100	93.75	87.5	81.25	61.09
Ethyl acetate	100	100	96.25	85	55.7
Chloroform	100	100	98.75	90	44.61
Methanol	96.25	93.75	90	66.25	94.7
Water	88.75	80	72.5	62.5	86.02
L Ascorbic acid	97.5	96.25	95	93.75	53.19

1.4 CONCLUSIONS

Phytochemical screening of plant extracts of leaf and bark shows that phytochemical constiuents such as alkaloids, terpenoids, phenolic components, flavonoids, glycosides and steroids are present significantly high inbark methanol extract and leaf chloroform extract of *Samadera indica* and these phytochemicals are reported to have antioxidant activities.[20]

Among the leaf and barkextracts of *Samadera indica* studied, the IC_{50} value of chloroform extract of bark, chloroform extract of leaf, ethyl acetate extract of bark, petroleum ether extract of bark, ethyl acetate extract of leaf and methanol extract of leaf is very low.The lower IC_{50} value shows their higher free radical scavenging activity. Higher scavenging activity on DPPH free radical of chloroform extract of leaf and bark, ethyl acetate extract of leaf and bark, petroleum ether extract of bark and methanol extract of leaf can be attributed to the presence of antioxidants in them and can be incorporated into the drug formulation.

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