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Research Paper

Free radical scavenging activity screening of medicinal plants from Tripura, Northeast India

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Abstract

Antioxidative effects of 123 extracts (Direct methanolic and sequential per ether, dichloromethane, ethyl acetate, methanol) prepared from 32 plants species (59 plant samples) collected from Tripura, Northeast India have been studied. Their ability of scavenging free radicals was measured by DPPH reduction spectrophotometric assay. Sixteen extracts showed strong antioxidant capabilities, which were, subjected for their dose dependent activity at different concentrations to calculate IC_{50} values.

Keywords: Antioxidant, DPPH, Free radical scavenging activity, Medicinal plants, Northeast India, Tripura.

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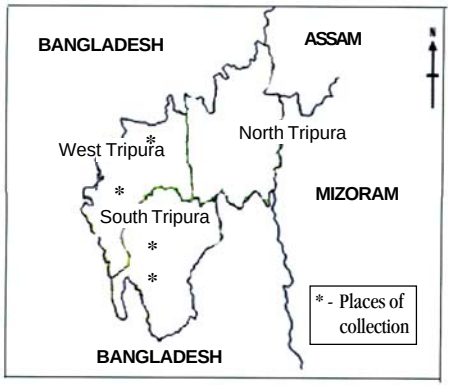
Introduction

Antioxidants, which scavenge active oxygen species (free radicals), are found in a variety of foodstuffs and are commonly referred to as scavengers. Many antioxidants are plant based and play an important role in protecting plants that are exposed to strong sunlight and live under severe oxygen stress. Antioxidants also play an important role in human health because the biologic defense mechanisms cannot operate under severe oxygen stress. According to recent research, activated oxygen is thought to be a major factor in ageing, hardening of the arteries, diabetes, cancer and tissue injury of skin. Indeed approximately 90% of age-related diseases are linked to activated oxygen. When human skin is exposed to ultraviolet rays active oxygen (free radical) is generated, which is scavenged by excess melanin. Pigmentation from excess melanin can cause the appearance of spots and freckles on the skin[1]. Plants are rich source of antioxidants, which is evinced through many reports on medicinal plants with antioxidant potential[2-5]. Almost all the sources for such phytochemical studies are from published or unpublished ethnobotanical knowledge[6-9].

Study area

Tripura is endowed with vast natural resources. The State has a total area of 10,169 sq. km out of which 6,168 sq. km is occupied by forest (exclusive of tea gardens). Geographically, it lies in a strategic zone as it falls in between the Indo-Malayan and Indo-Chinese biological realms. Thus, Tripura stands at the gateway to floral and faunal confluence. Tripura also lies in bio-geographical zone of nine big North East hills. Tripura occupies 0.32 per cent area of India and accounts for 12.78 per cent of the plant resources species found in the country. Tripura has been listed as one of the 26 endemic centres in India. Scientific studies have revealed that it possesses 1545 plant species with 28 varieties, 379 tree species, 320 shrubs, 581 herbs, 165 climbers, 34 ferns, 45 epiphytes, four parasites and 16 climbing shrubs out of which seven are endangered, seven are endemic and 18 rare species. The State also has 24 species of orchids and 266 species of medicinal plants. Scientific study has also shown that the Maximum Plant Diversity Index lies at 5.23, one of the highest in India. Rare species like ***Angiopteris erecta* Desv.**, a tree fern and ***Gnetum montanum* Markgraf**, a climbing gymnosperm occupy a key position. At present, most of the wild species have been confined to the sanctuaries due to habitat destruction. Tripura has 603.65 sq. km of forests within the four sanctuaries—*Sipahijala* (18.4 sq. km), *Gumti* (389.54 sq. km), *Trishna* (194.71 sq. km) and *Roa* (0.86 sq. km). The forest density of Tripura in terms of percentage is higher than the national figure. It is 17.35 per cent, the national figure being 11.73 per cent. The



Map of Tripura showing places of collection

Aquillaria malaccensis

Pterospermum semisagittatum

Litsea glutinosa

Grewia sapida

Alpinia officinarum

Syzygium cerasoides

percentage wise figure of open forests lies at 35.4 per cent, which is greater than the national figure of 7.61 per cent. Overall, 52.79 per cent of the total land is covered by forest whereas the national figure stands at 19.47 per cent. The forests of Tripura have been classified as evergreen and deciduous. The evergreen type has been diminishing[10].

Materials and Methods

Plant material

Botanical species or allied species of reported medicinal plants were collected from West and South Tripura districts of Tripura State (see Map), Northeast India during May 2005. While collecting samples the herbarium voucher specimens were also collected for future reference. Mounted herbarium specimens with proper labels and accession numbers were authenticated by using Flora of Tripura, Manipur, Mizoram[11-13] and deposited at herbarium of Reliance Pharmaceuticals Pvt. Ltd, Dhirubhai Ambani Life Sciences Center, Navi Mumbai. Collected samples were chopped in small pieces, shade dried and pulverized to coarse powder (10 mm sieve size)[14]. The plant species with family, local names, accession number, plant part/s studied and percentage antioxidant activity in direct methanol extract is detailed in Table 1.

Table 1: Antioxidant (AO) activity for direct methanol extracts of plant samples

S. No.	Name of the species/Family/ Acc. No.	Local name	Plant part/s	AO activity (%)
1.	***Alocasia fornicata* (Roxb.) Schott**/ Araceae/ RLS-12&13	*Baibing*	Rhizome Aerial part	41.06 21.63
2.	***Alpinia malaccensis* Rosc.** Zingiberaceae/ RLS-5	*Aiphal*	Aerial part	5.69
3.	***Alpinia officinarum* Hance** Zingiberaceae/ RLS-3&4	*Aichal*	Rhizome Aerial part	94.02 74.97
4.	***Aquilaria malaccensis* Lam.** Thymelaeaceae/ RLS-44&45	*Agar*	Stem Aerial part	43.30 92.03
5.	***Artocarpus chaplasha* Roxb.** Moraceae/ RLS-30	*Harikothong*	Stem Leaf	47.70 76.94
6.	***Callicarpa arborea* Roxb.** Verbenaceae/ RLS-22	*Hnahkiah*	Stem Leaf	53.65 47.20
7.	***Cassia nodosa* Buch.-Ham. ex Roxb.** Caesalpiniaceae/ RLS-31	*Tisibi*	Stem Twig	78.96 71.44
8.	***Cassia renigera* Wall. ex Benth.** Caesalpiniaceae/ RLS-52	*Radhachura*	Stem Twig	36.11 17.65
9.	***Clerodendrum indicum* (Linn.) Kuntze** Verbenaceae/ RLS-8&9	*Kuthap*	Aerial part Root	47.07 1.0
10.	***Dalbergia volubilis* Roxb.** Fabaceae/RLS-36&37	*Dadbari*	Stem Twig	86.44 88.93
11.	***Diplazium polypodioides* Blume** Athyriaceae/ RLS-24	*Chakawkei -chii*	Aerial part	5.87

S. No.	Name of the species/Family/ Acc. No.	Local name	Plant part/s	AO activity (%)
12.	***Dipterocarpus turbinatus* Gaertn. f.** Dipterocarpaceae/ RLS-1&2	*Garjan*	Stem Leaf Fruit	75.86 10.76 97.26
13.	***Garuga pinnata* Roxb.** Burseraceae/ RLS-21	*Kanjikara*	Stem Leaf	87.56 65.01
14.	***Grewia nervosa* (Lour.) Panigrahi** Tiliaceae/ RLS-9&10	*Yongkomla*	Stem Twig	67.09 89.51
15.	***Grewia sapida* Roxb. ex DC.** Tiliaceae/ RLS-38&39	*Yongkomla*	Stem Twig	95.46 97.01
16.	***Hydnocarpus kurzii* (King) Warb.** Flacourtiaceae/ RLS-50&51	*Khavitur*	Stem Twig Fruit	45.96 53.27 47.95
17.	***Litsea glutinosa* (Lour.) C.B. Robinson** Lauraceae/ RLS-32&33	*Kalimendi*	Stem Twig	90.57 41.53
18.	***Mallotus tetracoccus* (Roxb.) Kurz** Euphorbiaceae/ RLS-16&17	*Thingkhei*	Stem Leaf	23.02 31.42
19.	***Melastoma malabathricum* Linn.** Melastomataceae/ RLS-40	*Builukham*	Aerial part	36.58
20.	***Mitragyna rotundifolia* (Roxb.) O. Kuntze** Rubiaceae/ RLS-15	*Viteaval*	Stem Twig	91.51 93.58
21.	***Murraya koenigii* (Linn.) Spreng.** Rutaceae/ RLS-79	*Karipatta*	Aerial part	41.75
22.	***Persicaria hydropiper* (Linn.) Opiz** syn. *Polygonum hydropiper* Linn. Polygonaceae/ RLS-19	*Leipung*	Whole plant	46.68
23.	***Phlogacanthus thyrsiflorus* Nees** Acanthaceae/ RLS-48&49	*Nongmang -kha*	Stem Twig	33.70 14.43
24.	***Psidium guineense* Sw.** Myrtaceae/ RLS-06	*Sibiki*	Aerial part	67.34
25.	***Pterospermum semisagittatum* Buch.-Ham. ex Roxb.**/Sterculiaceae/ RLS-46&47	*Bandarhola*	Stem Twig	96.99 50.25
26.	***Saraca asoca* (Roxb.) de Wilde** Caesalpiniaceae/ RLS-26&27	*Malhawih*	Stem Leaf	95.52 79.50
27.	***Schima wallichii* (DC.) Korth.** Theaceae/ RLS-29	*Kanak*	Stem Leaf	96.46 96.72
28.	***Spilanthes paniculata* Wall. ex DC.** Asteraceae/ RLS-25	*Athlo*	Whole plant	23.37
29.	***Sterculia villosa* Roxb.** Sterculiaceae/ RLS-28	*Udal Kanak*	Stem Leaf	42.70 24.62
30.	***Syzygium cerasoides* (Roxb.) Raiz.** Myrtaceae/ RLS-34&35	*Botijam*	Stem Leaf	93.60 94.65
31.	***Vitex peduncularis* Wall. ex Schauer.** Verbenaceae/ RLS-13&14	*Awal*	Stem Twig	24.47 24.96
32.	***Wendlandia wallichii* Wight & Arn.** Rubiaceae/ RLS-42&43	*Lengpat*	Stem Twig	96.99 95.71
	Standard-Curcumin			99.48
	Standard-Catechin			99.47
	Standard-Trolox			100

Chemicals

Pet ether, dichloro methane, ethyl acetate, methanol were purchased from Thomas baker, DPPH, catechin, curcumin, trolox from Sigma Aldrich Inc. and DMSO from s. d. fine-chem Ltd.

Extraction and evaluation of extracts

Powdered samples were subjected to cold extraction by using methanol. The samples were charged in percolators and solvent is added to it in 1:6 proportions at room temperature with intermittent agitation and filtered. The process repeated for thrice and accumulated solution from methanol was concentrated to dryness under reduced pressure and controlled temperature (42-45°C) using rotary evaporator. Then methanol extracts were evaluated in DPPH assay for antioxidative property as has been evinced in Table 1.

Antioxidant screening

One mg of sample was dissolved in 100 ml of DMSO and methanol was added to it to make concentration of 1 mg/ml. 50 µl of sample solution was taken in a micro titer plate and 200 µl of DPPH solution (10 mM concentration of 2, 2-diphenyl-1-picryl-hydrazyl prepared in methanol AR grade) was added to it. The plate was incubated in dark for half an hour. Methanol blank (250µl) and DPPH blank

(200 µl DPPH + 50 µl methanol) was used for calculating the percentage antioxidant activity. Absorbance measured at 540 on Eliza plate reader. Antioxidant activity in percentage was calculated by the formula: 1-(Absorbance of sample/ Absorbance of DPPH) × 100 [Ref. 15]. Dose dependent study was carried out by same protocol but with following concentrations; 1, 0.5, 0.25, 0.12 and 0.062 mg/ml.

Results

The antioxidant activity of various plants species in direct methanol extract is given in Table 1.

Only those crude samples, which had shown more than 90% antioxidant activity in direct methanol extract, were sequentially extracted. Solvents used for sequential extraction were, petroleum ether (60-80), dichloro methane, ethyl acetate and methanol. The process of extraction followed for all the extracts preparation is same as that of direct methanolic extraction. The name of the species, its respective plant part, type of extract studied and percentage antioxidant activity has been enumerated in Table 2.

The extracts that showed potential activity (>90%) were further studied for their IC_{50} values, which are enumerated in Table 3.

Discussion

In the present study, out of 123 extracts prepared from 59 plant samples, 59 methanolic extracts and 54 successively prepared extracts were tested for antioxidant activity using standard *in vitro* model. Interestingly, successively prepared two ethyl acetate and 16 methanol extracts showed strong antioxidant nature in DPPH *in vitro* assay. Out of 59 methanolic extract samples, which were screened in DPPH assay, 16 samples showed more than 90% antioxidant activity. Those 16 crude samples were then freshly subjected for successive extraction by using pet ether (60-80), dichloro methane, ethyl acetate

Table 2 : Antioxidant activity (AO) profile of potential sequential extracts

S. No.	Name of the species with plant part	Percentage AO in PE	Percentage AO in DCM	Percentage AO in EA	Percentage AO in Me
1.	***Alpinia officinarum***-Rhizome	15.03	21.15	72.67	95.36
2.	***Aquilaria malaccensis*** - Aerial Part	2.96	7.66	10.37	92.62
3.	***Grewia sapida*** - Twig	45.94	54.26	56.24	82.38
4.	***Grewia sapida*** - Stem	34.65	9.50	79.41	95.05
5.	***Grewia nervosa*** - Twig	4.16	31.68	33.27	98.40
6.	***Litsea glutinosa*** - Stem	28.31	56.97	92.89	91.39
7.	***Mitragyna rotundifolia*** - Twig	2.55	7.52	39.05	97.34
8.	***Mitragyna rotundifolia*** - Stem	6.39	17.65	50.25	93.03
9.	***Pterospermum semisagittatum*** - Stem	32.67	85.15	72.56	94.65
10.	***Saraca asoca*** - Stem	23.37	28.32	44.55	93.07
11.	***Schima wallichii*** - Leaf	23.56	34.85	78.81	96.63
12.	***Schima wallichii*** - Stem	32.29	51.49	96.37	97.84
13.	***Syzygium cerasoides*** - Leaf	1.37	12.44	48.30	95.39
14.	***Syzygium cerasoides*** - Stem	6.45	7.97	11.58	93.13
15.	***Wendlandia wallichii*** - Twig	20.20	14.65	28.32	95.07
16.	***Wendlandia wallichii*** - Stem	17.96	23.56	81.58	93.47
	Standard - Curcumin				99.48
	Standard - Catechin				99.47
	Standard - Trolox				100

PE-pet ether extract, DCM-dichloro methane extract, EA-ethyl acetate extract, Me-methanol extract

Table 3: Promising extracts with their IC_{50} values

S. No.	Name of the species with plant part	Type of extract	IC_{50} value in mg
1.	***Alpinia officinarum*** - Rhizome	Methanol	0.42
2.	***Aquilaria malaccensis*** - Aerial Part	Methanol	0.05
3.	***Grewia nervosa*** - Twig	Methanol	0.044
4.	***Grewia sapida*** - Twig	Methanol	0.04
5.	***Grewia sapida*** - Stem	Methanol	0.045
6.	***Litsea glutinosa*** - Stem	Methanol	0.10
7.	***Mitragyna rotundifolia*** - Twig	Methanol	0.034
8.	***Mitragyna rotundifolia*** - Stem	Methanol	0.11
9.	***Pterospermum semisagittatum*** - Stem	Methanol	0.044
10.	***Saraca asoka*** - Stem	Methanol	0.06
11.	***Schima wallichii*** - Stem	Ethyl acetate	0.062
12.	***Schima wallichii*** - Leaf	Methanol	0.039
13.	***Syzygium cerasoides*** - Stem	Methanol	0.13
14.	***Syzygium cerasoides*** - Leaf	Methanol	0.041
15.	***Wendlandia wallichii*** - Twig	Methanol	0.05
16.	***Wendlandia wallichii*** - Stem	Methanol	0.12
	Standard - Curcumin		0.048
	Standard – Catechin		0.033
	Standard - Trolox		0.031

and methanol. Out of all successively prepared extracts only 12 % ethyl extracts showed almost same antioxidant activity as that of alcoholic extracts. Therefore, it can be said that the efficiency of each species differs depending on the particular assay methodology, reflecting complexity of the mechanisms involved in total antioxidant activity. The remarkable example of such diversity is the extracts of ***Litsea glutinosa*** (stem) and ***Schima wallichii*** (stem). Potent antioxidant extracts and compounds are known to increase the levels of catalase and SOD and decrease the level of TBARs in blood and tissues when compared with CCl_4 treatment[16].

There is antioxidant serum in the market by the name '*Juice Beauty*' which is a polyherbal preparation and one of the phytoconstituents is ***Litsea cubeba* Pers.**[17]. Leaves of ***L. glutinosa*** are used in nausea, vomiting and possess antidiarrhoeal activity. Decoction of bark may be applied to the sores and scabies and also aches and pains. Juice of crushed leaves may be applied to relieve the sore eyes[18, 19]. This corroborates the antioxidant credentials of the ***L. glutinosa*** reported in this paper.

The butanol fraction of ***Schima wallichii*** leaves have been shown to have antimutagenic and antioxidant activities [20]. In the present study, we have found that leaf has antioxidant activity in methanol fraction and the stem has both in ethyl acetate and methanol fractions.

Water extract of ***Syzygium gratum* (Wt.) S.N. Mitra** (leaf)[21] and ethanolic extract of ***S. cuminii* (Linn.) Skeels** (Stem bark)[22] are reported to have antioxidant and anti-inflammatory properties. In the present study we found ***S. cerasoides*** stem is having antioxidant property in methanolic fraction.

Antioxidative compounds were isolated from the methanol extract of fresh rhizome of ***Alpinia officinarum***[23]. In the present study it is noticed that dry rhizomes of same species from Tripura also have potent antioxidant property. Likewise the ethyl acetate extract of heartwood of ***Aquilaria agallocha*** also possess antioxidant activity[24] and the activity in methanol extract of aerial parts is also noticed by us.

Conclusion

All the selected extracts, subjected to dose dependent studies to calculate IC_{50} values are from successive methanolic extraction. Therefore, it could be concluded that only direct methanol extract is not sufficient for investigations in antioxidant property, but every sample has to be successively extracted with different solvents with increasing polarities as is evident in case of two plants, viz. ***Litsea glutinosa*** (stem) and ***Schima wallichii*** (stem) in which successive ethyl acetate extracts have shown equal antioxidant activity as that of successive methanol.

Mitragyna rotundifolia (twig), ***Schima wallichii*** (leaf), ***Syzygium cerasoides*** (stem) have good activity in dose dependent study i.e. 50% reduction of DPPH is achieved at the dose of 0.03 mg/ml that is comparable to the standards (which are pure molecules), viz. Catechin and Trolox. Moreover, their activities are better than that of Curcumin for which 50% reduction is a chieved by

0.04 mg/ml. ***Grewia nervosa*** (twig), ***G. sapida*** (twig, stem), ***Pterospermum semisagittatum*** (stem) and ***Syzygium cerasoides*** (leaf) have same activity profile as that of Curcumin. Therefore, further isolation of antioxidant constituents and *in vivo* studies are warranted for above samples. Further phytochemical work on isolation of bioactive molecules is going on.

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