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Indigenous Claim Supports i*n vitro* Antioxidant and Cytotoxic Screening of certain South Indian Medicinal Plants

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Abstract: *Plant drugs enjoy much acclaim and wide acceptability even in the midst of amazing advancements in modern medicine. Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological and molecular techniques. Evaluation of certain selected south Indian medicinal plants popularly used in Indian system of medicine has been taken up for their antioxidant and cytotoxic activities. The plants selected were Heliotropium indicum, Schleichera oleosa, Shorea robusta, Symplocos cochinchinensis and Wrightia tinctoria. Antioxidant activities involved DPPH, total antioxidant, iron chelating and nitric oxide assays. Cytotoxicity assay was carried out by MTT using SKMEL-28 and HCT-15 cell lines. Results obtained could very well support the therapeutic claims made for the investigated plants.*

Keywords: *antioxidant, cytotoxic, DPPH, iron chelating, MTT*

I. Introduction

Heliotropium indicum is used in the local application for ulcers, wounds, sores, gum boils and skin infection (1). *Schleichera oleosa* bark aqueous extract is astringent in nature. It is mixed with oil and applied externally in skin eruptions. Seed oil is used for massage in rheumatism and applied in alopecia, itch and acne. It is claimed to stimulates hair growth (2). *Shorea robusta* resin is popularly known as ' sal resin' is an antioxidant, astringent, detergent, antidiarrhoeal, antidysenteric and has antiseptic action, hence used in skin diseases (3). *Wrightia tinctoria* bark preparation finds use in the control of dysentery, piles and skin diseases (4). Bark and seeds are prescribed in flatulence and bilious affections (5). *Symplocos cochinchinensis* is traditionally used for the treatment of diarrhoea, dysentery, eye diseases, hemorrhagic gingivitis, uterine disorders, menorrhagia, bowel complaints, ulcers, snake bites, malaria and enteritis (6). Uncontrolled production of reactive oxygen species cause damage to living organisms which can be prevented by the use of free radical scavengers or antioxidants (7). Also, assessing the potential to inhibit the viability of cells in two human cancer cell lines namely skin and colon carcinomas by MTT assay would be worthwhile to explore the scope of these much used medicinal plants.

II. Materials and methods

S. robusta and *W. tinctoria* barks were collected from Idukki District, Kerala and *Heliotropium indicum*, *Schleichera oleosa* and *Symplocos cochinchinensis* were collected from Thengamom, Pathanamthitta District, Kerala during September 2010. These were authenticated by Mr. Rogimon. P. Thomas, Assistant Professor Department of Botany, C.M.S College, Kottayam, Kerala.

2.1 Preparation of the extract

Shade dried plant material was soaked in ethanol (95%) overnight and then refluxed for three hour; the clear extract was decanted off; it was repeated thrice. The extracts were pooled and concentrated by distillation under reduced pressure till a syrupy consistency was achieved. Solvent was evaporated to dryness on a water bath. The dry extract was used for the antioxidant assays and MTT assay (8).

Table: 1 Plants and plant parts used

Plant	*H.indicum* (HI)	*S. oleosa* (SO)	*S. Robusta* (SR)	*S.cochinchinensis* (SC)	*W.* tinctoria (WT)
Parts used	Whole part	bark	Oleoresin	Bark	Bark

2.2 Antioxidant activity

Antioxidant activity studies of the TEE of five plants were carried out using different models namely total antioxidant activity by phosphomolybdenum method, DPPH, iron chelating assay by orthophenanthroline and nitric oxide scavenging assay. Active oxygen species and free radicals are involved in a variety of pathological events. Total antioxidant activity was estimated by phosphomolybdenum assay and were expressed as the number of equivalents of ascorbic acid (9). The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid(10). The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen-donating antioxidant due to the formation of the nonradical form DPPH-H(11). In addition to ROS, nitric oxide is also implicated in inflammation, cancer and other pathological conditions(12).The ferric reduction capacity of the extract was measured as a mark of antioxidant capacity. The reducing powers of all the plant extracts were increasing with concentration dependent manner.

2.3 *In vitro* anticancer activity

2.3.1 MTT Assay

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells(13).

III. Results And Discussion

Phytochemical analysis indicated the presence of secondary metabolites like polyphenols, tannins, flavonoids and alkaloids(14). From the *Symplocos* species betulinic, oleanolic, acetyl oleanolic and ellagic acids reported having cytotoxic and antioxidant presence are reported from the plant. Glycosides, isolated from the ethanolic extract of the stem bark are highly astringent, and are reported to be responsible for the medicinal properties of the bark (15). Sterols schleicherastins (1-7) and two related sterols such as 8 & 9 designated as schleicheolos 1 & 2 are isolated from the *Schleichera oleosa*(16). Aerial parts of *H.indicum* contain pyrrolizidine alkaloids, indicine (principal), echinitine, supinine, heleurine, heliotrine, lasiocarpine, its N-oxide, acetyl indicine, indicinine and antitumor alkaloid, indicine-N-oxide. The plant also contains rapanone, lupeol and an ester of retronecine. Roots contain high amount of estradiol (17).

3.1 Antioxidant activity

TEE of all the five plant extracts showed very potent total antioxidant capacity. On the basis of results of the four assays each plant extract contributes in one way or other towards the antioxidant activity, with a clear gradation becoming difficult(18)(17). Nitric oxide scavenging assay of all the five TEE of the plants were evaluated at a concentration of 125 to 2000 µg/mL and the results were compared with the standard ascorbic acid. (19). The scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Large amounts of NO may lead to tissue damage (20).

Table: 2 IC_{50} values of antioxidant screening of the plants by iron chelating, DPPH, nitric oxide and total antioxidant activity

Sl.no:	Plant extracts	Iron chelating	DPPH	Nitric Oxide	Total antioxidant activity (Ascorbic acid Equivalents /100gextract)
		IC_{50} (µg/mL)			
1	**WT**	86.64	166.77	864.20	55.8
2	**SR**	*	*	**522.74**	*
3	**HI**	**71.05**	259.45	1295.37	74.8
4	**SO**	147.517	**62.29**	1224.24	**131.4**
5	**SC**	362.406	174.55	1313.47	119
6	**STD**	37.59	17.25	157.10	-

*reference(3)

In each case for iron chelating assay, *H.indicum* showed 71.05, DPPH assay, *Schleichera oleosa* given 62.29 and for nitric acid assay, *Shorea robusta* showed 522.74 lower IC_{50} values and higher activity when compared with that of the standard ascorbic acid 37.59, 17 25 and 157.10 IC_{50} values respectively. Total antioxidant assay *Schleichera oleosa* given good quantity (131.4) of ascorbic equivalents when compared with other extracts. The observed results demonstrate a marked capacity of the extract for iron binding, suggesting that their action as a peroxidation protector may be related to its iron binding capacity (21).The results revealed *S.robusta* and *W.tinctoria* showed good scavenging property. The IC_{50} values are tabulated in the table:2

3.2 *In vitro* anticancer activity

3.2.1 MTT ASSAY

An *in vitro* cytotoxicity study had been carried out by MTT assay for the TEE of all the plants using SKMEL-28 and HCT-15 cell lines. IC_{50} values of *H.indicum, S.cochinchinensis, S. oleosa, S.robusta* and *W. tinctoria* in SKMEL-28 cells were found to be 276.90, 278.71, 81.57, 22.17 and 62.22 µg/mL respectively (22)

Table: 3. IC_{50} values of plant extracts by MTT assay

Sl.no:	Plant extract	SKMEL-28	HCT-15
		IC_{50} (µg/mL)	
1	WT	62.22	91.97
2	SR	**22.17**	**69.57**
3	HI	276.90	106.98
4	SO	278.71	77.55
5	SC	81.57	81.71

The cytotoxic potential investigated indicate that among the five TEE *S.robusta* imparted the highest growth inhibitory activity in selected human cancer cell line followed by *W. tinctoria, S.cochinchinensis, H.indicum* and, *S. oleosa* (table:3). The anticancer activities of TEE of all plants were evaluated by MTT assay using HCT-15 cell line (table-3) at a concentration of 10, 25, 50, 75 and 100 µg/mL for 24 h and the activity varied in a concentration dependent manner. IC_{50} values of *H.indicum, S.cochinchinensis, S. oleosa, S.robusta* and *W. tinctoria* in HCT-15 cell lines were found to be 106.98, 77.55, 69.57, 81.71 and 91.97µg/mL respectively.

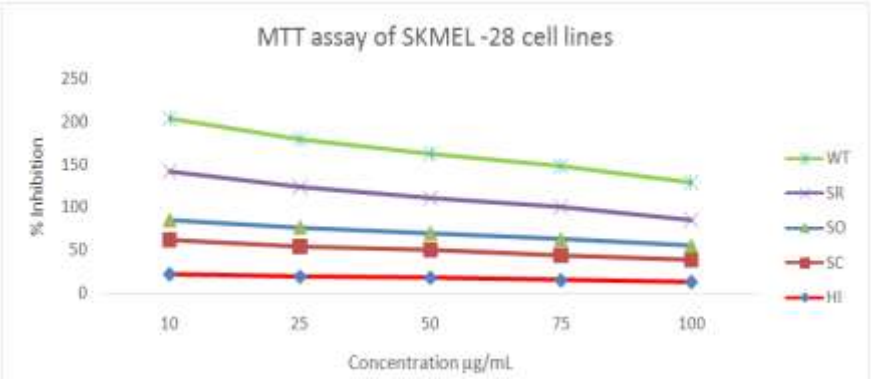

Fig: 1 Comparative evaluation of IC_{50} values of various extract using SKMEL-28 cell lines by MTT assay.

The cytotoxic potential results indicated that among the five plants TEE of *S.robusta* imparted the highest growth inhibitory activity in selected human cancer cell line followed by *S. oleosa, S. cochinchinensis, W. tinctoria,* and *H.indicum*. *In vitro* cytotoxicity study of all the five plant parts by MTT assay carried out using SKMEL-28 and HCT-15 cell lines. All the extracts gave good results. Among the plants *H.indicum, S.cochinchinensis, S. oleosa, S.robusta* and *W. tinctoria, S.robusta* showed best activity with lowest IC_{50} values of 22.17 and 69.57µg/mL.

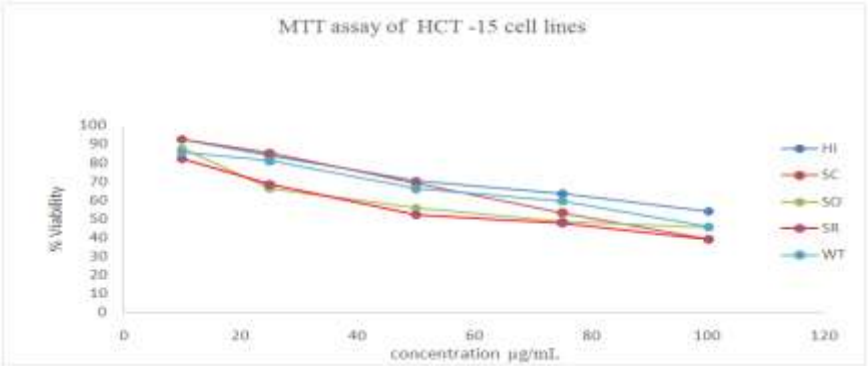

Fig: 2 Comparative evaluation of IC_{50} values of various extract using HCT-15 cell lines by MTT assay.

The better activity of the plant may be due to the presence secondary metabolites present. All the extracts gave good results, among the plants *S.robusta* showed best activity with lowest IC_{50} values of 22.17 and 69.57µg/mL in SKMEL-28 and HCT-15 cell lines respectively. The cell lines used for the study were colon

cancer cell lines and skin cancer cell lines. The results revealed the very good activity of the plant *S. robusta* and *W. tinctoria* as good candidates and confirmed its traditional use.

IV. Conclusion

The literature review shows the presence of gallic acid, lignan and triterpenoids in the plant (23). Crude extracts of all the five plant parts were prepared and evaluated for *in vitro* cytotoxic and antioxidant activity. All the extracts did not show hemolytic activity suggesting their biocompatibility. *Shorea robusta* and *Wrightia tinctoria* was found to be significantly antioxidant and cytotoxic on the different methods carried out on antioxidant activity and on cancer cell lines in dose dependent manner implying potential antitumor activity of both the plants and scope for further studies. The observed activity of the plant was shown by the whole extract. The activity showed only can be confirmed by the isolation of the particular compound present.

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