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Phytochemical investigation and pharmacological activity in the roots of *thottea siliquosa* lam.

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Abstract

The main aim of the research work is to investigate the *in vitro* antioxidant and cytotoxic activity in the root of medicinal plant *Thottea siliquosa* Lam. (Synonym: *Apama siliquosa* Lam.) the member of *Aristolochiaceae* family. The dried roots of *T. siliquosa* were powdered and extracted with methanol and the chemical composition of extract was further subjected to preliminary phyto-chemical screening, the plant extract proved the presence of alkaloids, tannins, phenols and other major chemical compounds. The major compounds of the plant extract was eluted and evaluated by TLC, HPLC, UV-VIS and IR methods. *In vitro* antioxidant and cytotoxic activity of the extract was analyzed using DPPH (1, 1-diphenyl-2-picrylhydrazyl) and human cancer cell line K562. The DPPH and cytotoxicity assay of methanolic root extract of *Thottea siliquosa* showed strong inhibitory activity. This finding demonstrated that, roots of *T. siliquosa* possess free radical and hydroxyl radical scavenging activity as well as antioxidant and anti cancer activity for *in vitro*. These results show that *T. siliquosa* is a promising source of natural products with potential anticancer and cytotoxic activity. The results will guide the selection for further pharmacological and phytochemical investigations.

INTRODUCTION

Plants are important for the continuation of life in the universe. They not only amalgamate food, necessary for the well being of human, but also synthesize different chemicals necessary for human health [1]. Medicinal plants have been used as major source of therapeutic agent. In India, indigenous systems of medicine namely Ayurveda, Siddha and Unani have been in existence for several centuries. These systems of medicine cater to the need of nearly 70% of our population residing in the village and are source of very potent plant drug. Poor easily afford these drugs and people preferred them because these are easily tolerated and less side effects [2].

Several medicinal plants were used against various types of tumours / cancers such as sarcoma, lymphoma, carcinoma and leukemia. Many of these medicinal plants have been found effective in experimental and clinical cases of cancers. And these facts spice up a growing interest in the ethnopharmacological evaluation of various plants used in Indian traditional system of medicine [3].

The species *A. siliquosa* Lam. or *Thottea siliquosa* (Lam.) is an erect rarely scandent slender shrubs or under-shrubs, the roots of *Tottea siliquosa* are used in the Indian systems of treatment, as curative for diarrhoea and dysentery, often administered along with lemon juice. [4] Have also recorded that a paste prepared from the plant with oil is effective against chronic sores and ulcers.

MATERIALS AND METHODS

Plant Material Collection

Fresh root of *Thottea siliquosa* were collected from Arya Vaidya Sala, Centre for Medicinal Plants, Mallapuram District, Kerala, India and authenticated from the Botany division of Centre for Medicinal Plants Research, Kottakkal, India. Voucher specimens of the plant material used in the study are obtained in the Herbarium of Centre of Medicinal Plant Research, Changuvetty, Malapuram, Kerala.

Preparation of methanolic extract in the roots of *Thottea siliquosa* Lam

The fresh plants were collected and the roots were separated. The roots were shade dried for five days and crushed to coarse powder. The coarse powder thus obtained was cold macerated with methanol (after selecting the solvent) and kept for 3 days at room temperature, with occasional stirring. The suspension was filtered through a fine muslin cloth and was evaporated to dryness at a low temperature (at 40 ºC) under reduced pressure in a rotary evaporator. The crystals were used for the further studies.

Preliminary phytochemical screening, Biochemical Estimations and Isolation of Secondary Metabolites in the Roots of *Thottea siliquosa*

The roots of *Thottea siliquosa* were tested for the phytochemical presence. The Antioxidant Activity was evaluated according to [5]. Thin Layer Chromatography was performed by [6]. The components were further identified by High Performance Liquid Chromatography using standards. Cytotoxic Activity in the Human Cell Line K562 was determined according to [7] by Trypan blue exclusion and MTT assay methods.

Statistical Analysis

The statistical analysis was performed by using Microsoft office excel 2003. In each of the tests minimum of three replicates were used and results were presented in the form of mean ± SD. The significance of the difference between the results was assessed using the student's *t*-test, and significance was accepted for p-values <0.01.

RESULTS AND DISCUSSION

The extract was subjected to preliminary phytochemical screening to detect the presence of different phytochemical compounds. The table-1 shows the presence of the chemical compounds like phenols, tannins anthra-quinones, amino acids, and carbohydrates.

The total phenolic content in the methanolic root extracts of *T.*

silquosa was evaluated and found to be 17.15%. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in the absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [8]. The Phenolic compounds are also thought to be capable of regenerating endogenous α-tocopherol, in the phospho-lipid bi-layer of lipoprotein particles, back to its active antioxidant form; and also known to inhibit various types of oxidizing enzymes [9]. The presence of flavonoid content was evaluated and the levels were found to be 0.92%. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [10, 11]. Phenolic compounds are a class of antioxidant compounds which act as free radical terminators [12].

The TLC fingerprint showed a total of 12 bands, in which yellow, violet, green, dark green, light violet, grey, dark blue, and orange color were observed with the help Ansaldehyde reagent. The solvent front of the TLC was 8.1 cm (Figure-1) and *Rf values* was depicted in the table-2. The TLC finger print conform the presence β-sitosterol in methonolic extracts of *T. siliquosa* by comparing to a standard. The five new unknown bands were isolated from TLC chromatogram and were subjected characterization by UV-Vis spectrum.

The isolated compounds from TLC plates were named as compound 1, 2, 3, 4, and 5. The spectrogram of the each compounds were depicted in figure-2 to 6. UV-Visible spectroscopy helps in structure elucidation of presence or absence of organic molecules like un-saturated or saturated hetero-atoms like S, O, N, or halogens. Usually molecules with steroidal nucleus in a saturated system give a λ*max* in the region of 210 to 230nm.

The absorption maximum (λ*max*) of eluted compounds were λ = 3.358, 3.381, 3.021, 2.437, and 2.788 Abs respectively at 260, 248, 260, 220, and 225 nm. From the above observation the compounds 4 and 5 could be steroids and the absorption λ*max* was 2.437 and 2,788 at 220 and 225nm.

The HPLC analysis of the root methanolic extracts of *T. siliquosa* showed similarity in their composition (Figure-7) evident that presence of β-sitosterol in the extract. The investigated compound was identified by comparison of their retention time (t_R) with the standard. The identity of HPLC peaks was definitely assessed by co-chromatography after spiking the samples with reference compounds.

The peaks showed maximum similarity at following t_R = 2.94, 2.94, 3.19, 3.97, 4.38, 5.42, 6.44, 6.95, 7.63, and 10.02 minutes. And it showed 2.21, 0.66, 7.44, 0.65, 32.92, 3.14, 1.25, 5.5, and 2.28 % of peak area with standard respectively. The maximum 32.93% of peak area was observed at t_R = 5.42min, and minimum 0.65% of peak area was observed at t_R = 4.38min among the similar area. The t_R like 5.88, 6.1, 7.32, and 8.68 min shows prominent peaks with 13.15, 3.96, 2.02, and 2.2 percentage of peak area. These results indicated compounds with t_R = 5.88, 6.1, 7.32, and 8.68 minutes needs more standard or/and spectroscopic studies for characterization of the peaks.

The antioxidant activity of methanolic extract of the *T. siliquosa* exhibited IC_{50} 10.2µg/ml, when compared to BHA, a control, which is presented in table-3. The antioxidant effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes [13]. [12] Have suggested that

Table 1: Preliminary phytochemical screening of methanolic root extract of Thottea siliquosa

S. No.	TESTED FOR	METHANOLIC EXTRACT
01	**Alkaloids**	
	Draggendorf's test	+
	Hager's test	+
	Wagner's test	+
02	**Fixed oils and fats**	-
03	**Anthraquinones**	
	Bornrager's test	+
04	**Amino acids**	
	Ninhydrin test	+
05	**Carbohydrates**	
	Molish's test	+
	Fehling's test	+
06	**Flavanoids**	
	Shinoda test	+
	Lead acetate test	+
07	**Saponin's**	
	Foam test	-
08	**Glycosides**	
	Legal's test	-
09	**Steroids / Terpenoids**	
	Liebermann – Burchardt test	-
10	**Tannins**	
	Braemer's test	+
11	**Phenols**	
	Phosphomolybdic acid test	+

Table 2: TLC finger print of *T. siliquosa* root

Extract	Visible		UV-254		UV-365	
	Color	*Rf*	*Color*	*Rf*	*Color*	*Rf*
Methanol	Yellow	0.14			Orange	0.14
	Dark Blue	0.16	Dark	0.16		
	Dark Green	0.19	Dark	0.19		
	Light Violet	0.26			Pink	0.26
	Grey	0.31				
	Green	0.48	Dark	0.48	Blue	0.48
	Light Violet	0.52				
	Violet	0.65	Dark	0.65	Dark	0.65
	Light Violet	0.70			Pink	0.70
	Orange	0.74				
	Light Violet	0.80			Pink	0.80
	Dark Violet	0.99	Dark	0.99		

Table 3: Antioxidant activity of methanolic root extract of *T. siliquosa*

Sample tested	Concentration µg/mL	% of inhibition	EC_{50} µg/mL
Butylated Hydroxy Anisole (BHA)	100	20.34	12.97
	200	38.24	
	300	65.44	
	400	84.32	
	500	89.22	
	600	87.87	
T. siliquosa methanolic extract	100	31.69	14.51
	200	37.95	
	300	59.49	
	400	66.41	
	500	68.41	
	600	80.62	

Table 4: Cytotoxic activity of methanolic root extract of *T. siliquosa*

	Control	DMSO	0.5 mg	1 mg
48hrs	100	97.28	16.59	20.45
72hrs	100	92.45	7.9	7.67

Figure 1: Thin Layer Chromatographic profile of methanolic extract of *T. siliquosa*

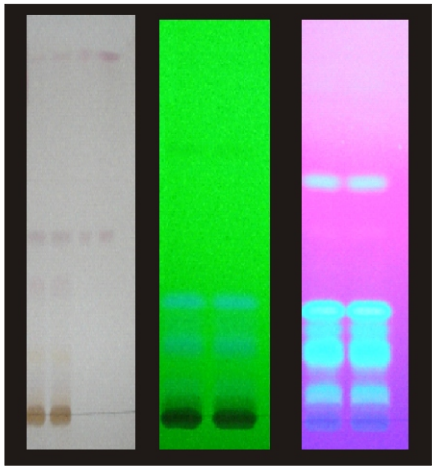


Figure 2: UV-VIS spectrogram of methanolic extract of *T. siliquosa* compound 1

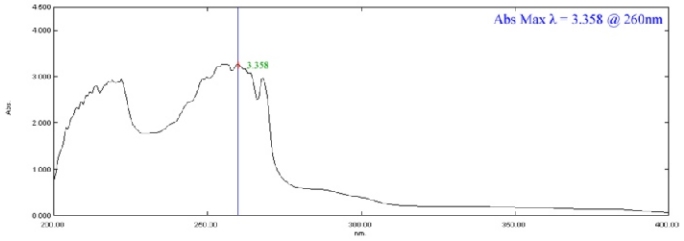


Figure 3: UV-VIS spectrogram of methanolic extract of *T. siliquosa* compound 2

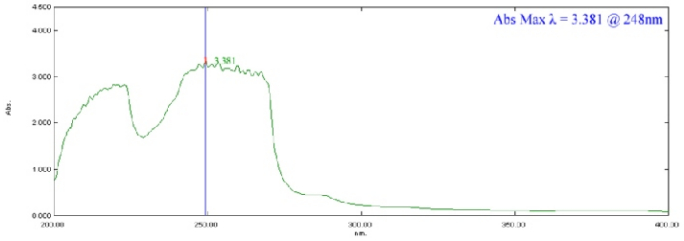


Figure 4: UV-VIS spectrogram of methanolic extract of *T. siliquosa* compound 3

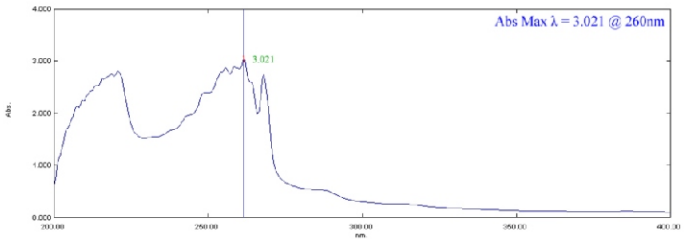


Figure 5: UV-VIS spectrogram of methanolic extract of *T. siliquosa* compound 4

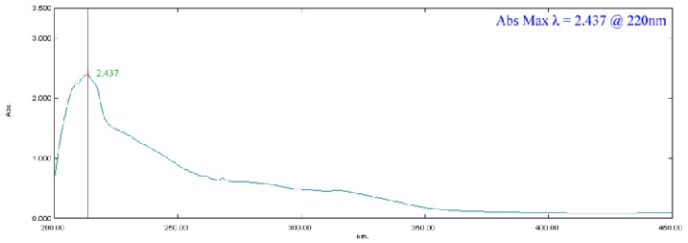


Figure 6: UV-VIS spectrogram of methanolic extract of *T. siliquosa* compound 5

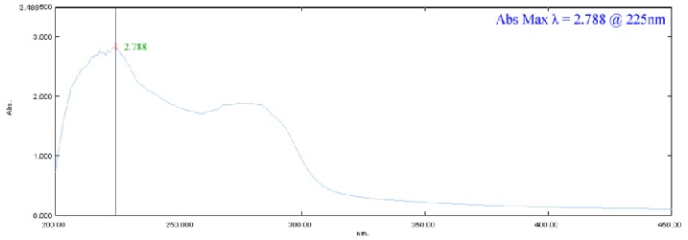
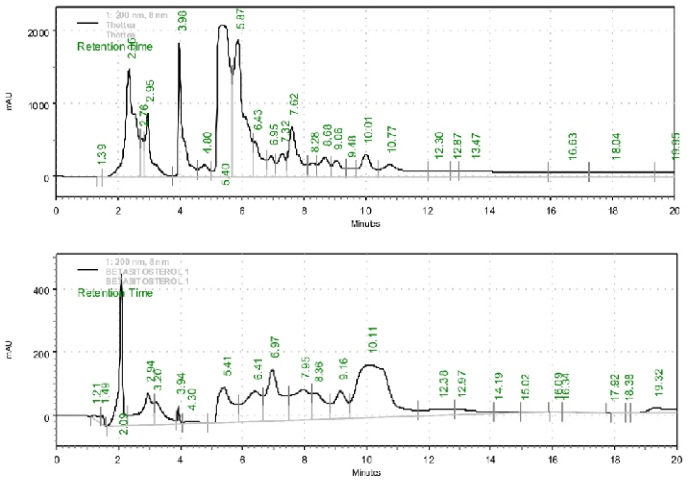


Figure 7: HPLC chromatogram of methanolic extract of *T. siliquosa* and β-sitosterol



there may be relationship between phenolic compounds and reducing powers. Presence of phenolic compounds might be the reason for reducing power. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [8].

Similar studies like *in vivo* were supported by the work of [14] in *Teucrium* species; the inhibitory effect of the extracts was greater than that of reference [11]. The crude extract of *T. siliquosa* appeared to be as potent as BHA with a maximum inhibition at the same concentration (0.5 mg/ml and 10 mg/ml). These results indicate that the reducing power of the extract might be mostly related to their concentration of hydroxyl hydrogen. Phenolic compounds could easily donate hydroxyl hydrogen due to resonance stabilization. Reducing power of a compound is related to the electron transfer ability of a compound. Therefore, the reducing capacity of a compound may serve a significant indicator of its potential antioxidant activity from this result.

The crude extract inhibits the cell proliferation significantly in a time dependent manner (Table- 4). IC_{50} values were calculated after 72 hrs of exposure of these extract. *Thottea siliquosa* methanolic extract showed excellent inhibitory activity against the cell line tested at tested concentrations. Among *Thottea siliquosa* root methanolic extract exhibit good anti-proliferative activity.

This study suggests that the roots of the medicinal plant *T. siliquosa* possess antioxidant activities which can neutralize the human cancer cell line (K562). The crude extract of *T. siliquosa* appeared to be as potent as BHA with a maximum inhibition at the same concentration (0.5 mg/ml and 10 mg/ml). And this result indicated that the reducing power of the extract might be mostly related to their concentration of hydroxyl hydrogen. Phenolic compounds could easily donate hydroxyl hydrogen due to resonance stabilization. Reducing power of a compound is related to electron transfer ability of a compound. Therefore, the reducing capacity of a compound may serve a significant indicator of its potential antioxidant activity from this result [15].

It is concluded that *Thottea siliquosa* found to have potent antioxidant and anti-cancer activity further investigations were required to isolate and identify the secondary metabolite responsible for the pharmacological action.

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