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Research Article

In vitro Antioxidant Activity and HPTLC Fingerprinting of Seeds of *Spermacoce hispida* Linn.

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ABSTRACT
Spermacoce hispida Linn., has been extensively used in Siddha system of medicine for curing various diseased conditions. The plant has been widely studied for its phytochemical composition and a large number of active ingredients. The present study reveals the *invitro* antioxidant activity of hydroalcoholic extract of seeds of *Spermacoce hispida* by DPPH, ABTS, Hydrogen peroxide and Superoxide dismutase method. The result indicate that the plant possess considerable antioxidant activity. Fingerprinting profile of *Spermacoce hispida* was established by using high performance thin layer chromatography (HPTLC) technique. It was carried out with CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS-4 software. It can be concluded that HPTLC fingerprint analysis of seed extract of *Spermacoce hispida* can be used as a diagnostic tool for the correct identification of the plant and it is useful as a phytochemical marker.

Key words: *Spermacoce hispida*, antioxidant, HPTLC, fingerprint

INTRODUCTION

Damage to cells caused by free radicals is believed to play a central role in the ageing process and in disease progression. The need for antioxidants becomes even more critical with increased exposure to free radicals because they possess defence against free radicals. Pollution, cigarette smoke, drugs, illness, stress, and even exercise can increase free radical. Reactive oxygen species (ROS) include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. Other than dietary antioxidants, plant derivatives known as "phytochemicals" are becoming popular for their antioxidant activity. In plants Phenolic compounds such as flavonoids are ubiquitous and have therapeutic values. Approximately 3,000 flavonoid substances have been described[1].

One of such plants with medicinal and a food value is *Spermacoce hispida* Linn. Popularly known as 'Nattaiccuri' in Tamil and 'Shaggy button weed' in English and belongs to the family of Rubiaceae. The plant is widely distributed in the Western Ghats of Kerala and Maruthamalai forest of TamilNadu[2]. The whole plant is used for various medicinal properties. Seeds of *Spermacoce hispida* are crushed into paste and taken orally to treat stomach problems. The seed extract has been used as a remedy for curing internal injuries of nerves and kidney[3]. The plant is rich in flavonoids[4]. The plant exhibits various pharmacological activities like anti-inflammatory, analgesic, hypolipidemic, antidiabetic, antihypertensive, antifungal, anticancer and hepatoprotective activity[5].

Standardization of plant materials is becoming essential now a day. Describing only the physicochemical parameters of the plant material is not enough for their standardization. Hence the methods describing the identification and quantification of active constituents in the plants may be useful for proper standardization. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time[6].

In this present study *invitro* antioxidant activity of seeds of *Spermacoce hispida* has been done to identify the free radical scavenging property and HPTLC fingerprinting of *Spermacoce hispida* seed extract has been performed which may be used as markers for quality evaluation and standardization of the drug.

MATERIALS AND METHODS

Collection of plant material

The seeds of *Spermacoce hispida* were collected from Kollimalai, TamilNadu. The dried seeds were made into fine powder and were kept separately in an airtight container until use.

In-vitro free radical scavenging activity of Spermacoce hispida

DPPH radical scavenging activity was carried out by the method of Molyneux, (2004)[7]. ABTS radical scavenging activity was performed as described by Re *et al.*, (1999)[8]. The hydrogen peroxide radical scavenging activity of the test sample was estimated by following the method of Ruch *et al.*, (1989)[9]. The superoxide radical scavenging activity of the test sample was studied using the method of

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Table 1: ABTS radical scavenging activity of HAE and Vitamin c at various concentrations

	Vitamin C		HAE	
S.no	Conc (µg/ml)	% Inhibition	Conc (µg/ml)	% Inhibition
1	0.2	24.7 ± 0.1	25	10.5 ± 4.9
2	0.4	43.1 ± 0.4	50	39.8 ± 1.5
3	0.6	58.1 ± 0.4	100	56.3 ± 0.4
4	0.8	77.5 ± 0.3	150	69.6 ± 0.5
5	1.0	88.3 ± 0.1	200	92.3 ± 0.3
IC_{50}		0.53 ± 0.05		70.1 ± 3.9

Values are Mean ± SD of triplicate

Table 2. DPPH radical scavenging activities of HAE and vitamin c at various concentrations

	Vitamin C		HAE	
S.no	Conc (µg/ml)	% Inhibition	Conc (µg/ml)	% Inhibition
1	0.2	7.1 ± 0.4	25	17.4 ± 1.3
2	0.4	19.1 ± 0.3	50	34.6 ± 1.8
3	0.6	45.0 ± 0.5	75	51.3 ± 0.9
4	0.8	59.2 ± 0.2	100	64.3 ± 1.2
5	1.0	87.5 ± 0.6	200	84.9 ± 1.8
IC_{50}		0.79 ± 0.07		55.1 ± 2.4

Values are Mean ± SD of triplicate

Table 3: Superoxide radical scavenging activities of hae and vitamin c at various concentrations

	Vitamin C		HAE	
S.no	Conc (µg/ml)	% Inhibition	Conc (µg/ml)	% Inhibition
1	10	9.1 ± 0.3	200	16.1 ± 0.3
2	20	26.3 ± 0.8	400	31.0 ± 0.4
3	30	50.1 ± 0.3	600	52.2 ± 0.7
4	40	66.0 ± 0.5	800	56.2 ± 0.4
5	50	88.3 ± 0.5	1000	59.1 ± 0.3
IC_{50}		32.41 ± 5.3		371.3 ± 12.4

Values are Mean ± SD of triplicate

Table 4: Hydrogen peroxide scavenging activities of HAE and vitamin c at various concentrations

	Vitamin C		HAE	
S.no	Conc (µg/ml)	% Inhibition	Conc (µg/ml)	% Inhibition
1	200	42.2 ± 0.7	200	46.7 ± 1.6
2	400	54.2 ± 0.4	400	49.1 ± 1.3
3	600	71.1 ± 0.3	600	76.2 ± 0.7
4	800	79.9 ± 0.4	800	92.4 ± 2.1
5	1000	82.5 ± 0.8	1000	96.8 ± 1.1
IC_{50}		180.5 ± 4.4		175.8 ± 5.5

Liu *et al.,* (1997)[10]. Different concentration of ascorbic acid was used as reference compound. Percentage of inhibition was calculated from the equation [(Absorbance of control - Absorbance of test)/ Absorbance of control)] X 100. IC_{50} value was calculated using Graph pad prism 5.0.

HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of (Harborne, 1998 and Wagner 1996)[11,12]. Application of bands of extract was carried out using spray technique. Sample were applied in duplicate on pre-coated silica gel 60F254 aluminium sheets (5 x 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software. After the application of sample, the chromatogram was developed in Twin trough glass chamber

RESULT AND DISCUSSION

Free radicals are the main factor responsible for causing various diseases including cardio vascular disease. Based on different reaction mechanisms, several *In-vitro* methods have been developed to measure the free radical scavenging activity of bioactive drug. Some of them are adopted in the present investigation to evaluate the same. The percentage inhibitions are tabulated in the Table. The ABTS and DPPH radical decolorisation assay are widely accepted methods for evaluating the free radical scavenging activity[8]. Addition of antioxidant to the radicals like ABTS and DPPH is always associated with a degree of decolorisation[13]. Quenching of ABTS and DPPH radical of HAE is observed in a dose dependent manner from 25 to 200 µg/ml (Table 1 & 2). The preliminary phytochemical investigation confirms the presence of saponins, Tannins, phenolics, Steroids, Essential oils, Flavonoids and Terpenoids[14]. Phenolic compound of HAE may attract the electron or hydrogen from ABTS and DPPH thereby, causing decolorisation and shows its free radical scavenging property.

The superoxide radicals are precursors of many other toxic Reactive Oxygen Species (ROS). These radicals can easily initiate the peroxidation of membrane lipids leading to the accumulation of lipid peroxides and damaging a wide range of essential molecules. In the reaction mixture, superoxide radicals are generated from the reaction of Phenazine methosulphate and NADH. These superoxide radicals, unless scavenged by the test samples, would readily reduce the electrophilic nitro blue tetrazolium to blue formazan that can be detected at 560 nm[15]. It is to be noted that Vitamin C is a very good superoxide radical scavenger as observed by its scavenging ability at lower concentration itself (Table 3). The presence of phenolic compounds and other superoxide radical scavengers present in HAE extract might be responsible for the observed activity. Hydrogen peroxide is not very reactive by itself but it can form hydroxyl radicals in the cells[16]. Thus, removing H_2O_2 is very important. The H_2O_2 scavenging ability of HAE in comparison with that of Vitamin C as standard is shown (Table 4). The reducing power of a compound is related to its electron transfer ability and may therefore; serve as an indicator of its potential antioxidant activity[17]. The results of the present study reveal that HAE is more potent in free radical scavenging activity. Moreover, the activity of HAE is comparable with that of standard Vitamin C.

The hydroalcoholic extract (HAE) of *Spermacoce hispida* was applied to the HPTLC plate in microgram quantities, and chromatogram was then, developed using

Table:5 Peak Table of HPTLC Profile at 254nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.01	69.4	0.02	191.2	22.40	0.04	1.7	3538.3	8.08
2	0.06	0.2	0.08	78.3	9.18	0.11	1.0	1651.4	3.77
3	0.15	3.2	0.18	43.5	5.10	0.21	0.7	1031.8	2.36
4	0.38	0.6	0.39	12.2	1.43	0.40	8.5	94.2	0.22
5	0.40	8.7	0.41	14.1	1.66	0.44	2.0	256.0	0.58
6	0.44	2.7	0.45	13.3	1.56	0.46	7.4	98.8	0.23
7	0.46	8.4	0.46	17.5	2.05	0.48	7.5	186.4	0.43
8	0.49	5.0	0.49	16.5	1.93	0.50	10.5	140.7	0.32
9	0.50	10.8	0.52	18.4	2016	0.54	1.3	281.7	0.64
10	0.56	1.8	0.57	12.7	1.48	0.58	3.8	103.9	0.24
11	0.58	3.8	0.61	37.6	4.41	0.62	7.9	462.6	1.06
12	0.67	13.5	0.69	24.8	2.90	0.70	19.0	483.5	1.10
13	0.72	18.9	0.76	40.8	4.79	0.78	33.9	1328.5	3.03
14	0.78	32.4	0.83	332.4	38.95	0.99	5.8	34117.0	77.94

Table:6 Peak Table of HPTLC Profile at 366nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.00	7.6	0.03	150.2	11.04	0.05	0.2	3171.4	4.85
2	0.06	0.6	0.08	57.6	4.23	0.11	0.2	1196.2	1.83
3	0.12	0.0	0.17	76.5	5.62	0.20	0.6	1959.5	3.00
4	0.25	6.3	0.28	17.4	1.28	0.29	16.0	360.4	0.55
5	0.38	14.7	0.41	28.0	2.06	0.44	17.4	1005.8	1.54
6	0.45	18.5	0.46	49.9	3.67	0.48	30.8	827.9	1.27
7	0.48	31.4	0.48	46.9	3.45	0.50	28.4	529.4	0.81
8	0.50	29.6	0.51	43.3	3.18	0.52	24.6	633.1	0.97
9	0.55	26.9	0.56	46.1	3.39	0.57	33.2	800.0	1.22
10	0.58	34.3	0.59	56.5	4.16	0.60	40.6	685.3	1.05
11	0.60	40.9	0.61	46.8	3.44	0.62	35.0	451.6	0.69
12	0.63	39.0	0.66	63.1	4.64	0.67	47.5	1427.8	2.18
13	0.68	50.6	0.72	85.1	6.25	0.73	77.4	1981.0	3.03
14	0.73	78.3	0.75	96.6	7.10	0.75	90.1	1678.3	2.57
15	0.76	99.5	0.84	496.3	36.49	0.99	2.9	48693.9	74.45

Toluene:ethylacetate:formic acid (50:35:5) as mobile phase, which separated the components of the sample under investigation. The chromatogram thus, developed was scanned using a computer controlled viewing system. The report has brought out the TLC picture along with the areas of each peak (Table 5 and 6; Fig 1 and 2). The unique spectra of any peak may be used to confirm the identity of any particular compound. Moreover, the results have shown the presence of 14 peaks at 254 nm and 15 peaks at 366 nm exhibiting many phytoconstituents and probably bioactive also. The highest concentration of the phytoconstituents was found to be 38.95% and its corresponding Rf value was found to be 0.83 at 254nm. At 366nm, the highest concentration of the phytoconstituents was found to be 36.49% and its corresponding Rf value was found to be 0.84.

CONCLUSION

Spermacoce hispida is a one of the vital plant which has most significant medicinal value. It is an important source of many pharmacologically and medicinally important phytochemicals. From the HPTLC studies it has been found that seeds of *Spermacoce hispida* is a rich source of various phytoconstituents. Various pharmacological actions of the plant may be due to the presence of these phytoconstituents. *Invitro* antioxidant activity of *Spermacoce hispida* also confirms the above.

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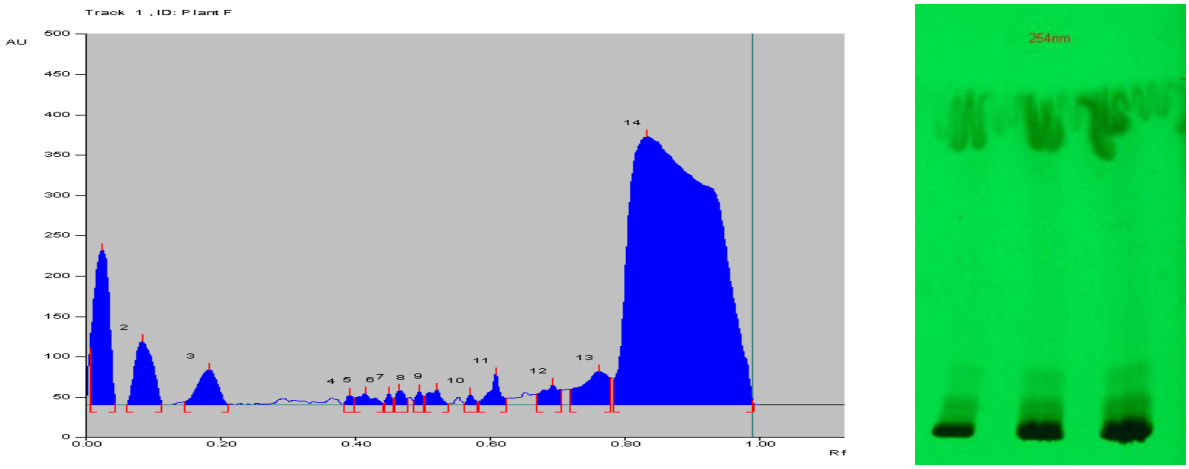

Fig 1: HPTLC finger printing of *Spermacoce hispida* seed extract at 254nm

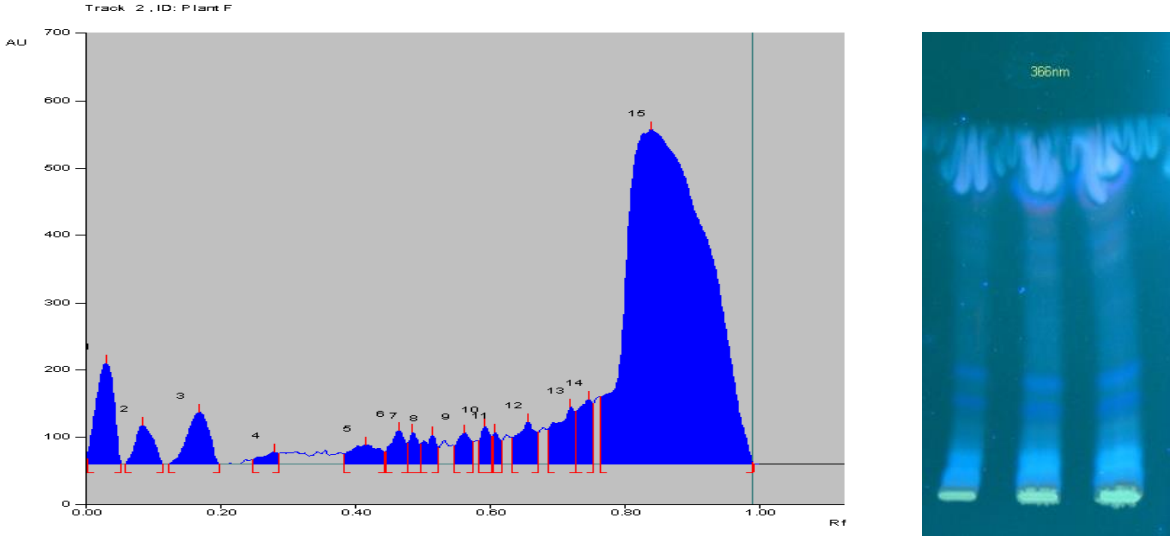

Fig 2: HPTLC finger printing of *Spermacoce hispida* seed extract at 366nm

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