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THE USE OF SIDA PLANT IN THE PREPARATION OF NAYAPAYAM KASHAYAM

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ABSTRACT
The plants coming under the genus Sida are used in various Ayurvedic and ethnic medicines. Different species of Sida are found to be used at different places for preparation of medicines. Examination of the chemical profiles (literature and HPTLC comparison) of five common species of Sida found to have similarities with some significant differences. Nayopayam Kashayam prepared using these species show considerable differences in their antioxidant activity. Naturally pharmacological activities should also differ. Selection of species for medicines demands high caution.

Key words: Sida species, HPTLC, Nayopayam Kashayam, antioxidant studies.

INTRODUCTION

The medicinal plant named as 'Bala' in Sanskrit belonging to the genus Sida of the family Malvaceae finds application in many Ayurvedic preparations and ethnic medicines. It is used for curing neurological disorders, headache, leucorrhoea, tuberculosis, diabetes, fever, uterine disorders and as an anti-rheumatic and anti-pyretic agent[1]. It is also reported to possess anti-tumor, anti-HIV, hepatoprotective, abortifacient, antimicrobial and immunostimulant properties. The Sanskrit literature identifies different species of Sida namely Bala, Athibala, Nagabala and Jysethbala[1]. The exact correlation between Sanskrit and Botanical names are however lacking, this has resulted in a situation where different species are used for the preparation of the same medicine at different places. In South Kerala *Sida alnifolia* is used as the drug while in North Kerala *Sida rhombifolia* sp *retusa* is the preferred species. The North Indian preparations mainly contain *Sida cordifolia*[2]. The chemical profiles of these different species have differences and naturally difference in activities may be expected. This study, reviews the secondary metabolites profile of the different species of Sida in use, their HPTLC results, the antioxidant properties of the different species and the respective Kashayams to signify the importance of exercising caution in the selection of plant species for the preparation of medicines.

MATERIALS AND METHODS

Materials

The five species of Sida under study were collected from Venjaramood, Nedumangadu and Kesavadasapuram of Trivandrum district, kerala. All the plant materials were identified by Department of Botany, University of Calicut, Thenjippalam, where voucher specimens have been deposited. The plants were washed with water, dried in shade; their root portion was separated and used for the study. Cumin and dried ginger were procured from commercial sources. DPPH and Trolox were purchased from Sigma Chemicals and the other chemicals were procured from Merck, India. A UV-Visible spectrophotometer BL180 was used for measuring the optical density.

Preparation of the extracts

Required amount of crude plant roots as well as cumin and ginger were crushed, weighed accurately and refluxed with definite volumes of respective solvents for 4 hours. It was kept overnight for cooling and the extract was collected by filtration. The solvents in each case were removed by a rotary evaporator and the dried extracts were used for preparing the samples for antioxidant study.

Preparation of the Kashayam

42 gram of Sida roots, 27.72g of cumin and 14.28g of dried ginger were weighed and taken in a clean earthen pot. To this 105ml of water was added and the level was marked. 745 ml of water was added to the mixture and boiled till the level reached the original mark. The extract was allowed to cool and it was filtered through linen cloth.

Antioxidant measurement

Antioxidant activity was estimated using DPPH as radical generator and Trolox as standard in a UV-Visible Bio spectrophotometer BL 198.

The standards and samples were prepared in 1:1 acetone water mixture and the measurements were made at 517 nm. From the absorbance values the IC_{50}, percentage inhibition and TEAC were calculated.

IC_{50} value is defined as the concentration of substrate that causes 50% loss of the DPPH activity.

The percentage inhibition is calculated as

$$\% \text{ inhibition} = \frac{A_{DPPH} - A_{Trolox\ /\ Sample}}{A_{DPPH}} X\ 100$$

Trolox equivalent antioxidant capacity (TEAC) is the ratio of percentage inhibition of sample to percentage inhibition of trolox at the same concentration of sample and trolox

$$\text{TEAC} = \frac{\%\ inhibition\ \ of\ \ sample}{\%\ inhibition\ \ of\ \ Trolox}$$

RESULTS AND DISCUSSION

Due to confusion between botanical name and local name and hence a detailed description of each of the species used is presented here.

***Sida acuta* Burm.**
Syn: *S. carpinifolia, S. lanceolate, S. herbaceae*
Common name: Kurumthotti
Description
S.acuta is a profusely branching annual herbaceaous weed, cosmopolitan in distribution. It is found as a major weed throughout the hotter parts of India and Sri Lanka. The bark is smooth, greenish, the root is thin, long, cylindrical and very rough; leaves are lanceolate, nearly glabrous, peduncles equal to the petioles, the flowers are yellow, solitary or in pairs; seeds are smooth and black[9,13].


Fig 1: ***Sida acuta***

Medicinal uses
In Indian traditional medicine, the root of *S. acuta* is extensively used as a stomachic, diaphoretic and antipyretic. It is regarded as cooling, astringent, tonic and useful in treating nervous and urinary diseases and also disorders of the blood, bile and liver[14]. It is also used to treat gonorrhea, elephantiasis and ulcers and is claimed to have aphrodisiac properties. The juice of the root is applied to wounds. The whole plant is used to treat snake bite and it lessened the hemorrhagic effect of *Bothrops atrox* venom[15]. *S.acuta* has significant antiplasmodial activity due to its alkaloid content[16]. The alkaloid cryptolepine is the antiplasmodial constituent[17]. The roots of *S.actua* are effective as hepatoprotective against paracetamol overdose-induced liver damage. Decoction of the plant is taken orally to cure urinary diseases and impotency. The aerial part of the plant is the most frequently used part. In Central America, the plant is used to treat asthma, renal inflammation, cold, fever, headache, ulcers and worms[18].

***Sida rhomboidea* Roxb.**
Description
It is an under shrub with a height of 1 to 2 feet with stellate hairs. Stem is circular; leaves ovate, toothed in the distal half; flowers solitary cymes, pedicels fused at the base; stamens filamentous and numerous; fruits capsule; carpals numbering is 6-10.
It is a variety of *S. rhombifolia* which is found as a weed of marshy places throughout India. It is known by different names- Bala, Mahabala, Pitabala etc. In Tamil the plant is popularly known as “Anaikurumthotti”. The local tribal groups of the Nilgiris district are using this plant as a very important medicinal herb to treat a wide array of inflammatory conditions. It is regarded as a variety of *S. rhombifolia*[19] and also as a distinct species.


Fig 2: ***Sida rhomboidea***

Medicinal uses
The roots and leaves of *S. rhomboidea* are aphrodisiac, tonic, useful in fever, heart diseases, burning sensations, piles and all kinds of inflammations. Munda tribes apply pounded leaves on swellings. Total alcoholic extract of the roots of *S. rhomboidea* has shown significant anti inflammatory, antipyretic and antibacterial activity[20]. The ethyl acetate extract of *S. rhomboidea* has got significant antinociceptive activity[21].

***Sida cordifolia* L.**
Syn: *S. herbacea, S. althaeitolia, S. rotundifolia*
Common name: Country mallow
Ayurvedic names: Vatyalaka, Sitapaki, Vatyodarahva, Bhadraudani, Samanga, Samamsa, Svarayastika
Description
The plant is found throughout the tropical and subtropical plains of India and Sri Lanka. The whole plant is velvety[8]. It is an erect perennial under shrub growing up to a height of 1m. Stem ascending, terete or suleate, softly villious and densely stellate-pubescent all over. Leaves ovate or ovate-oblong, obtuse or sub-acute at apex. Flowers yellow, peduncles auxiliary, jointed much above the panicles, upper flower nearly sessile and fasciculate towards the tip of the branches forming sub-spicate inflorescence. Fruits sub-discoid, 6-8mm across, mericarps 10, 3 sided. Seeds are trigonous, glabrous and tufted-Pubescent near the hilum[9].


Fig 3: ***Sida cordifolia***

Medicinal uses
The roots are used to treat a variety of ailments including pulmonary tuberculosis, rheumatism, hematuria, urinary and heart diseases. The roots have recently been used to cure Parkinson’s disease and as a food supplement for fat loss[10]. It is a tonic, astringent, emollient, aphrodisiac and it is used in the treatment of leucorrhoea, gonorrhea and general debility[11,12]. Expressed juice of the whole plant is useful for sexual strength and in diuretic spermatorrhoea. The juice obtained from the roots is applied to unhealthy sores. Decoction of the roots bark is given in sciatica and rheumatism[1]. The paste of its leaves is applied in ophthalmic diseases. It is also rejuvenative, nutritive and stimulant to the heart. It is especially anabolic to muscle

tissues and augments the seminal fluids and promotes reproduction. It also boosts the fetal growth. The plant is analgesic, anti-inflammatory and tonic in nature. It effects on central nervous system and provides relief from anxiety. It is used to reduce the body weight. It lowers the blood pressure and improves cardiac irregularity. It is used in the popular medicine for the treatment of stomatitis of asthma and nasal congestion

***Sida alnifolia* L**
Syn: *S. Spinosa, S. alba*
Description
It is a weed of waste places distributed at higher and lower altitudes in South India. It is an annual herb seen in Deccan and Karnatic Districts. The leaves are obtuse, pedicels jointed near the flower.


Fig 4: ***Sida alnifolia***

Medicinal uses
It is used as tonic and for the treatment of asthma and other chest ailments. The ethanolic extract of *S.alnifolia* possesses hypoglycemic activity. It also depressed the normal blood pressure. It is a tonic in wasting diseases, cures ulcer and biliousness, useful in urinary infection, leprosy and skin infection. The leaves possess demulcent and refrigerant properties and useful in gonorrhea and scalding urine. The roots are used as tonic diaphoretic and useful in treatment of fever, debility as demulcent in irritability of bladder[14].

***Sida rhombifolia* L. var. *rhomboidea* Mast**
Syn*: Sida retusa, S.canariensis, S.compressa, S.orientalis*
Common name: Arrow leaf sida, Cuban jute, Indian hemp, Broom weed
Ayurvedic names: Jysethbala, Katambara, Kesaruha, Kesarika, Mrigadani, Sarimi, Sahadevi, Devahara, Gandhavallari, Pitapuspi, Purasani.[1]
Description


Fig 5: ***Sida rhombifolia***

It is a weed of waste places in all plain districts. It grows in over 70 countries throughout the tropical, subtropical and warm temperate regions. It is a perennial, woody, fibrous stemmed shrub, deeply rooted and grows up to height of 2m. The plant usually has a single stem unless disturbed but may branch near the ground. The twigs are slender green and semi woody. They have small green leaves broad at base and tapering to a point, alternate, 3-7cm long and fine hairs on both sides. Flowers are small orange - yellow in clusters at end of branches. Pods have fine bristles breaking up into segments.
Medicinal uses
Pounded leaves of the plant are applied as a paste to reduce swelling and as a cure for boils and headaches. Root decoction is taken as tea to treat diarrhea[3]. In India the plant is used in the treatment of gonorrhea. In Europe it is used as anti tubercular agent[1]. Decoction of the plant is used to treat rheumatic pain, cardiac problems and biliary problems in children. Fresh plant juice is used as demulcent and diuretic. The methanolic extracts of the aerial parts are anti-inflammatory and the powdered roots, aerial parts and their aqueous extract are hepatoprotective4. The ethyl acetate extract of the plant growing in Bangladesh has cytotoxic and anti-bacterial activities5. It is widely used in different traditional medicine for the treatment of various types of ailments like malaria, chest pain, fever, abdominal pain and as a tonic[6,7,8].
Secondary metabolite profile
The phytochemical literature of these plants is voluminous. The important phytoconstituents are alkaloids, phytosterols, fatty acids, carbohydrates and flavonols. Phenyl ethyl amine base such as ephedrine, quinazoline bases such as vasicine, choline bases such as betaine and thioester bases are universally present in all species. Phytosterols such as sitosterol, stigmasterol, campasterol etc are also very common where as ecdysteroids enjoy distribution in all species except *S.alnifolia*.
The chemical profiles of five species were compared using HPTLC with hexane extracts and methanol extracts of the plants. The plates were developed with 100% chloroform for hexane extract and 85% chloroform-methanol mixture for methanol extract. The Rf were tabulated results are shown. It is clear that there are considerable differences in the distribution of phytochemicals in the five species. (Table 1).
There is high degree of qualitative similarity in the distribution profile of the compounds but quantitative differences may exist. Radical scavenging activity is a useful quantitative tool to compare the ability of the sample to interact with redox processes, the significance of which in biological systems needs little emphasis. Antioxidant property which is a quantitative assessment of the reducing power hence should be related to the pharmacological activity of the medicine. Studies on the antioxidant potential of the different plant extracts and Kashayams however reveal that there are differences in the activities of Kashayams prepared from different species of Sida. The selection of the correct species in the preparation of the medicine assumes significance in this context.
Antioxidant properties of the plant species and the corresponding Kashayams
The antioxidant measurement in this study was performed by measuring the ability of the samples to decolourize DPPH. The activity was compared by evaluating IC_{50}, percentage inhibition and TEAC values. The antioxidant

capacities of the different fractions of the extracts of the five plants are represented in the Table 5, 6. IC_{50}, percentage inhibition and TEAC were evaluated for comparing the antioxidant activity.

Comparison of antioxidant activity

The root of the five species were collected, dried and 20 grams of each were extracted successively with hexane, chloroform, ethyl acetate, methanol and finally with water.

Five Kashayams were also prepared by conventional procedure using each of the five roots. All the extracts and Kashayams were concentrated, solvent removed and weighed amount was dissolved in suitable solvents for measuring the antioxidant activity. DPPH was the radical generator and TROLOX the standard for measuring activity. DPPH concentration was 50 ppm and the sample concentrations ranged from 100 to 200 ppm.IC_{50}, percentage inhibition and TEAC values were used to compare the extracts and Kashayams prepared from different species of Sida. The results clearly demonstrate the variation in the distribution of antioxidant principles and the activity difference it create in the case of the Kashayams. (Table 4)

It is the polar fraction of the secondary metabolites that are making the highest contribution to the activity and the very close similarity in the IC_{50} values of the water extract and the Kashayams point to the possibility that these compounds may be the active principles of the Kashayam also. The higher activity of the Kashayam observed in these cases may be due to the contribution from cumin and ginger, the other ingredients of the Kashayam. The difference in values is however a clear indication that the five plant species are contributing differently to the total activity.

Percentage inhibition follow a similar trend. Water is the most efficient medium for the extraction of the antioxidant compounds, but the different species are found to show differences here also. The lipophilic fraction of *Sida acuta* has highest antioxidant character while in the case of *Sida cordifolia* and *Sida alnifolia* the hydrophillic part has the highest activity. The results in the case of Kashayams are also noteworthy. The values vary over a wide range from the lowest value in the case of *Sida rhombifolia* to a 132 % increase in the case of *Sida alnifolia*. (Table 5)

TEAC values also follow similar trend. A leveling effect observed in the case of the Kashayam may be due to the contribution from other ingredients. (Table 6)

The considerable differences in the values merit special attention. The antioxidant properties of the Kashayams are represented in Table IV. The variation in antioxidant property is observed here also. The differences in the antioxidant capacities point to the possibility that the pharmacological effects may also be different. However direct pharmacological evaluation is necessary to arrive at definite conclusion .

Table 1: Comparison of the phytochemical profile of five species of sida

Constituent	*S. acuta*	*S. rhomboidea*	*S. cordifolia*	*S. alnifolia*	*S. rhombifolia*
Phenyl Ethyl amine base	X	X	X	X	X
Quinazoline base	X		X	X	X
Choline base	X		X	X	X
Phytosterols	X				X
Saponins			X		
Fatty acids	X	X	X	X	X
Carbohydrates	X				
Hydrocarbons	X				
Terpenes	X				
Flavonones			X		
Amino acids		X			X

Presence is indicated by X

Table 2: R_f values of the hexane extract of sample 1-5

R_f values				
Sample1	Sample 2	Sample3	Sample4	Sample5
0.07	0.03	0.16	0.06	0.04
0.10	0.07	0.21	0.09	0.07
0.16	0.09	0.24	0.19	0.09
0.36	0.15	0.34	0.23	0.17
0.68	0.24	0.59	0.27	0.29
0.76	0.35	0.76	0.38	0.41
	0.67		0.59	0.61
	0.76		0.63	0.69
			0.74	0.75

Table 3: R_f values of the methanol extracts of sample 1-5

R_f values				
Sample1	Sample 2	Sample3	Sample4	Sample5
0.01	0.02	0.03	0.04	0.04
0.3	0.09	0.07	0.32	0.11
0.75	0.3	0.18	0.39	0.33
0.87	0.49	0.30	0.46	0.52
	0.61	0.39	0.61	0.64
	0.74	0.47	0.72	0.75
	0.87	0.59	0.87	0.88
		0.65		
		0.76		
		0.87		

Table 4: Comparison of the IC_{50} (µg/ml) values of extracts of five species of Sida and the respective kashayams

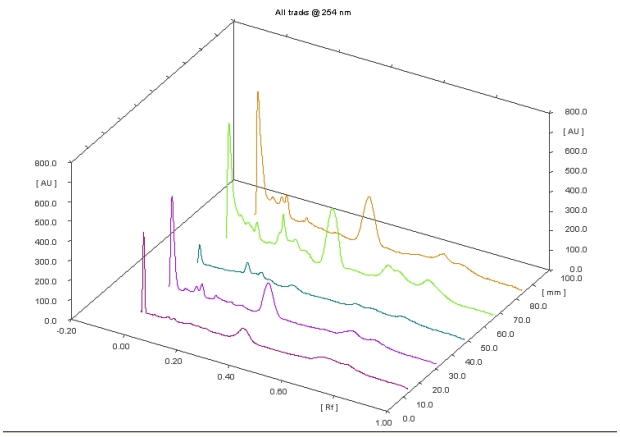
	S.acuta	*S.rhomboidea*	*S.cordifolia*	*S.alnifolia*	*S.rhombifolia*
Hexane	281.6319	472.0013	120.9969	120.9969	159.9099
Chloroform	12.16274	192.4656	113.2256	113.2256	17.35876
Ethyl acetate	93.0431	148.6493	86.40766	86.40766	64.52929
Methanol	39.86904	65.16343	95.28971	95.28971	120.3692
Water	5.38755	20.64595	5.789883	5.789883	7.355496
Kashayam	7.232979	4.075963	5.723015	4.048847	7.955805

Table 5: Comparison of the percentage inhibition values of extracts of five species of Sida and the respective kashayams

	S.acuta	*S.rhomboidea*	*S.cordifolia*	*S.alnifolia*	*S.rhombifolia*
Hexane	30.16113	19.9739	25.37374	3.326452	17.74327
Chloroform	51.34966	24.05918	20.46887	10.95938	24.66601
Et. Acetate	20.49478	10.95324	25.51831	0.950715	25.71704
Methanol	45.22318	39.18348	35.01213	42.38247	42.82604
Water	52.94366	13.78522	59.71542	41.53772	36.21947
Kashayam	42.59418	67.34357	59.13385	74.46317	32.07325

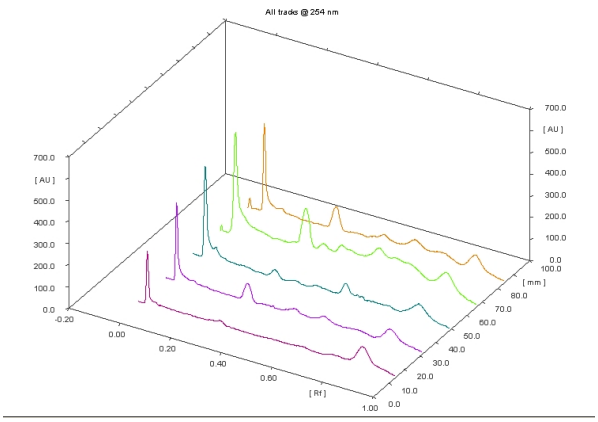
Table 6: Comparison of the TEAC values of extracts of five species of Sida and the respective kashayams

	S.acuta	*S.rhomboidea*	*S.cordifolia*	*S.alnifolia*	*S.rhombifolia*
Hexane	0.519988	0.229863	0.716634	0.084931	0.310072
Chloroform	0.876567	0.276877	0.578105	0.279816	0.43105
Et.acetate	0.358156	0.126052	0.445945	0.024274	0.449418
Methanol	0.790297	0.450929	0.611854	0.821994	0.748406
Water	0.925216	0.389338	0.926537	0.80561	0.965996
Kashayam	0.8261	0.775	0.917514	0.887274	0.855414



(X – R_f , Y- Track spacing , Z- Intensity)

Fig 6: HPTLC of hexane extracts of *Sida acuta* (1), *Sida rhomboidea* (2), *Sida cordifolia* (3), *Sida alnifolia* (4) and *Sida rhombifolia* (5)



(X – R_f , Y- Track spacing , Z- Intensity)

Fig 7: HPTLC of methanol extracts of *Sida acuta* (1), *Sida rhomboidea* (2), *Sida cordifolia* (3), *Sida alnifolia* (4) and *Sida rhombifolia* (5)

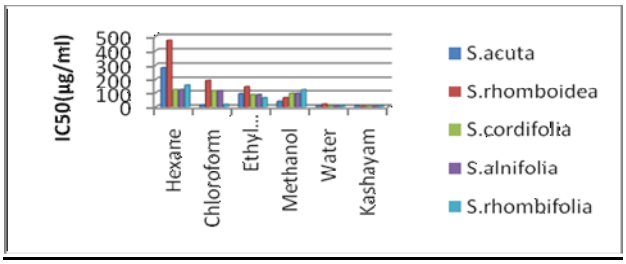


Fig 8: IC_{50} values of plant extracts and Kashayams

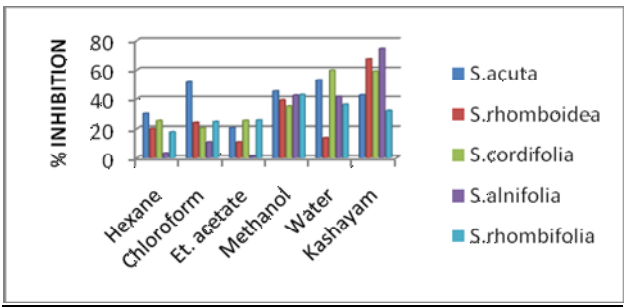


Fig 9: Percentage inhibition values of extracts and Kashayams prepared using five species

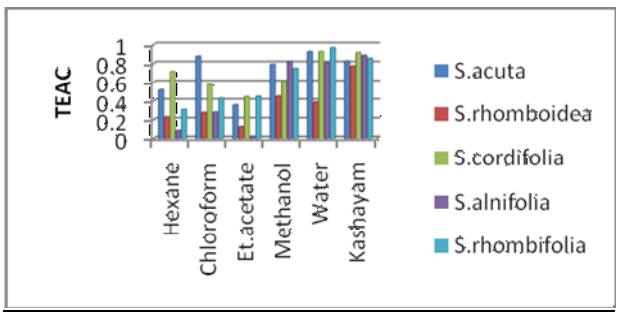


Fig 10: Comparison of the TEAC values of extracts and Kashayams

CONCLUSION

The practice of using different species of Sida for the preparation of same Ayurvedic medicines is not warranted by this observation. Care should be employed in the collection and identification of the raw materials in Ayurvedic preparations. However antioxidant property need not be directly related to pharmacological activity. Hence the choice of the species should be based on pharmacological evaluation of the medicines.

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