

Variation in Pharmacognostic Characters and Polyphenolic Contents among Four Species of Medicinal Plants from the Genus *Spermacoce* (Rubiaceae)

G. M. ANKAD, R. T. KONAKERI¹, H. V. HEGDE* AND S. ROY

Regional Medical Research Centre, Nehru Nagar, Belgaum-590 010, ¹Department of Dravya Guna, Shri C. B. Guttal Ayurvedic Medical College, Dharwad-580 008, India

Ankad, *et al.*: Pharmacognostic Analysis of *Spermacoce*

Spermacoce hispida L. is one of the important medicinal plants used in traditional systems of medicine. It is observed that, several times it is difficult to differentiate the plant from the other allied species from the same genus, *Spermacoce*, especially, when they are in drug form. Therefore, the present study aims to document the differences in the pharmacognostic characters, preliminary phytochemical analysis and polyphenolic contents from the leaves of four species belonging to the genus *Spermacoce*, viz. *S. hispida* L., *S. mauritiana* O. Gideon, *S. stricta* L. and *S. ocymoides* Burm. Transverse section passing through the midrib with lamina on either sides, epidermal characters, leaf constants, organoleptic characters, physicochemical analysis, extractive values and preliminary phytochemical analysis were carried out for all these species. Total phenolic content by Folin-Ciocalteu method and total flavonoids by AlCl₃ method were also estimated from the leaves of all these species. The results indicated that *S. hispida* can be clearly differentiated from the other selected species on the basis of size and number of epidermal cells, size of trichomes, leaf constants, physicochemical analysis and extractive values. However, it is also found that *S. hispida* possess total phenolic content at 6.88±0.34 mg CAE/g and 9.17±0.46 mg TAE/g. Total flavonoids was at 5.98±0.30 mg QE/g. The study will provide information with respect to identification and differentiation amongst selected species of genus *Spermacoce*.

Key words: Traditional medicine, *Spermacoce*, pharmacognostic study, phytochemical, total phenolic content, total flavonoids

Plants are utilized extensively as raw drugs for many formulations in traditional systems of medicine. Often, pharmacognosy is employed as a tool to check the genuineness of the raw drugs and to detect adulteration/substitution of these plant materials. Despite of several modern techniques, the QC of the raw drugs still rely upon the pharmacognostic studies by and large. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken^[1]. Usually, the identification of the medicinal plants in the field is based on morphological features or other traditionally known characteristics. In several such instances, there is a chance of selecting incorrect raw drugs/adulterants, especially because of their similar

morphological features with other plants. Therefore, an extensive microscopical and phytochemical screening is needed for raw drug to avoid any ambiguity and such a study will serve also as a reference for further studies^[2].

Traditionally, leaves of several species of *Spermacoce*, belonging to family Rubiaceae, are being used in various Indian systems of medicine. The major species among them is *Spermacoce hispida* L., which is locally known as 'Madanaghanti'^[3]. The

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*Address for correspondence

E-mail: harshavh@rediffmail.com

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leaves *S. hispida* are reported to be used in treating conjunctivitis, haemorrhoids, gallstones and to relieve headache^[4]. Generally, most of these activities are attributed to the secondary metabolites in the plant^[5]. In recent past, it is understood that, the phenolic compounds present in plants have a great deal of biologically active constituents and therefore have been studied extensively. One of the prominent properties of the phenolics is their excellent radical scavenging activity^[6], whereas flavonoids, a group of polyphenolic compounds, are known for series of properties such as free radical scavenging activity, inhibition of hydrolytic and oxidative enzyme and antiinflammatory action^[7]. However, no information is available on the total phenolic contents and flavonoids in the genus *Spermacoce*.

Meantime, it was observed that, it is difficult to differentiate the plants and the leaves morphologically, both in the field and in raw form, due to its similarity with other allied species of same genus viz. *S. mauritiana* O. Gideon., *S. stricta* L. and *S. ocymoides* Burm.^[3,8]. Therefore, the present study is designed to provide information on pharmacognostical characters, preliminary phytochemical analysis and polyphenolic contents from the leaves of selected species from the genus *Spermacoce*, in the process of standardizing parameters for correct identification of *S. hispida* leaves and for their differentiation from other allied species of the same genus.

MATERIALS AND METHODS

Fresh leaves of *S. hispida*, *S. mauritiana*, *S. stricta* and *S. ocymoides* were collected from Belgaum, India (N 15.71369°, E 074.37958°) and were authenticated. Herbaria were prepared and deposited in herbarium repository (Voucher No. RMRC-530, RMRC-531, RMRC-532, RMRC-533, respectively) at RMRC Belgaum for future reference.

Transverse section of midrib and epidermal characters:

Fresh leaves of selected species of *Spermacoce* were used for microscopic sectioning. Sharp razor blades were used to take transverse section passing through midrib with lateral extensions of lamina on either side. Phloroglucinol and conc. HCl were used to observe lignified tissues. Upper and lower epidermal layers were peeled to study the epidermal characters.

Numerous temporary and permanent mounts of the sections were made and examined^[9,10].

Determination of leaf constants:

Leaf constants were determined according to standard methods with the help of camera lucida after calibrating the microscope using stage and ocular micrometers. Leaf constants were observed from leaves collected from different plants to ensure the variations of characters and average result of characters were mentioned^[10].

Powder characteristics:

The collected leaves were washed, shade dried and pulverized. The powder was then passed through # 85 mesh and collected fine powder is used for powder microscopy. Fragments of powder were cleared with chloral hydrate. Phloroglucinol and conc. HCl were used to observe lignified tissues, iodine to observe starch grains, acetic acid and dil. H₂SO₄ to detect calcium oxalate crystals and glycerine was used as mountant. The diagrams of different structures were drawn with the help of camera lucida mentioning the scale^[10].

Microphotographs:

Microscopic descriptions of tissues were justified with microphotographs mentioned with appropriate scale bars, photographs were taken with Olympus BX-41 microscope.

Organoleptic characters, physicochemical analysis and extractive values:

Fine powder obtained by pulverization was used for organoleptic characters to evaluate color, texture, odour and taste. Physicochemical parameters were done to evaluate the percentage of loss on drying, total ash content, acid insoluble ash and water soluble ash. Extracts of powdered leaves were prepared with different solvents for the study of extractive values^[11].

Preliminary phytochemical analysis:

Aqueous and ethanol extracts were prepared to find out the presence of secondary metabolites performing various tests^[11].

Total phenolic content:

Total phenolic content (TPC) was quantified using modified Folin-Ciocalteu method^[6]. The assay mixture was prepared using 0.5 ml of distilled water, 0.125 ml

different concentrations of standard tannic acid and/or caffeic acid with 0.125 ml of Folin–Ciocalteu reagent, incubated for 10 min in dark. After 10 min 1.25 ml 7% aq. sodium carbonate and 1 ml of distilled water was added and the reaction mixture was incubated in dark for 90 min at 37°. The absorbance of blue colour was read at 760 nm using distilled water instead of standards in the reaction mixture as blank on double beam spectrophotometer. Similarly, extracts prepared (10% w/v in methanol) were also quantified and the results were compared to the standard curves and expressed as mg/tannic or caffeic acid equivalent per gram dry powder for the samples.

Total flavonoids:

Total flavonoid (TF) contents were quantified using method given by Luxmon-Ramma *et al.*^[12]. One milliliter of 2% methanolic AlCl₃ was reacted with 1 ml of different concentrations of standard quercetin for 10 min in dark. Absorbance was measured at 367 nm on double beam spectrophotometer using 2% methanolic AlCl₃ as blank. Standard was replaced with extracts prepared (10% w/v in methanol) and results were compared to the standard curves obtained. The results were expressed as mg/quercetin equivalent per gram dry powder for the samples and the data was represented as showed by Sandeep *et al.*^[13].

RESULTS

Transverse section of midrib:

The characters of transverse section (TS) of midrib with lateral extensions of the lamina on its either sides are shown in fig. 1a-d. All the species were dorsiventral in nature, plano convex shaped with a depression on middle of dorsal side. Detailed TS showed rectangular shaped upper and lower epidermis. Lamina showed two layers of palisade parenchyma underneath the upper epidermis and 3 to 5 rows of spongy parenchyma were found beneath palisade layer. The rest of the midrib was occupied by the cortical parenchyma with collateral vascular bundle embedded in the middle. Xylem was towards center and phloem towards periphery. Parenchymatous tissue was thin walled with prominent intercellular spaces.

Epidermal characters:

The comparative results of upper epidermis are mentioned in Table 1 (fig. 2) and lower epidermis

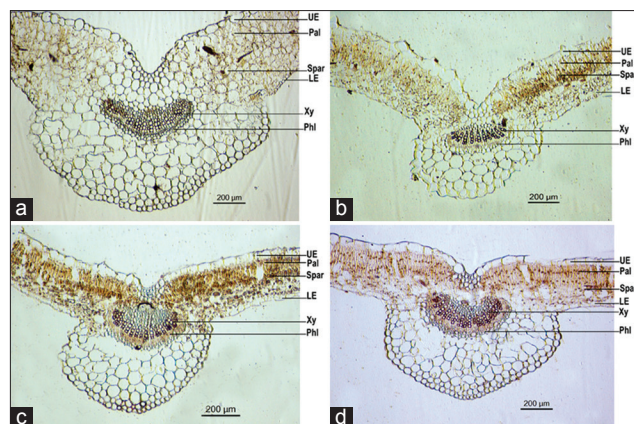


Fig 1: Transverse section of midrib.

Transverse section of midrib of *S. hispidum* (a), *S. mauritianum* (b), *S. strictum* (c), *S. ocymoides* (d). UE-Upper epidermis, Pal-palisade parenchyma, Spar-spongy parenchyma, LE-lower epidermis, Xy-xylem, Phl-phloem. Scale bar-200 µm.

are mentioned in Table 2 (fig. 3). The results revealed size and type of epidermal cells, size and type of trichomes, type of stomata and number of epidermal cells. The upper epidermal cells of *S. hispidum* were rectangular to polygonal shaped which were similar in *S. strictum* and *S. ocymoides* whereas *S. mauritianum* possessed irregular shape with papillae. *S. hispidum* has shown variation in size of epidermal cells and number of epidermal cells as compared to other species. Trichomes were absent in *S. hispidum* and were present in other species. The lower epidermal cells were irregular shaped with wavy margin in all species, *S. hispidum* has shown variation in size of epidermal cells and number of epidermal cells as compared to other species. Trichomes were present in *S. hispidum* and *S. mauritianum* and were absent in other two species. All species possessed Rubiaceous (paracytic) stomata in both upper and lower epidermal layer.

Leaf constants:

The comparative results of leaf constants are mentioned in Table 3. The leaf constants of *S. hispidum* showed differences with respect to stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio comparing with other species.

Powder characteristics:

Powders of all the species revealed the presence of fragments of upper and lower epidermal cells, lamina, mesophyll tissue, palisade parenchyma, trichomes, annular and spiral vessels and prismatic and acicular crystals of calcium oxalate (fig. 4).

TABLE 1: EPIDERMAL CHARACTERS FOR UE

Characters	<i>Spermacoce hispida</i>	<i>Spermacoce mauritiana</i>	<i>Spermacoce stricta</i>	<i>Spermacoce ocymoides</i>
Type of epidermal cells	Rectangle to polygonal shaped	Irregular shaped with papillae	Rectangle to polygonal shaped	Rectangle to polygonal shaped
Size of epidermal cells (μ)	149.86-368.42	200.67-367.17	164.11-228.22	98.54-213.63
Type of stomata	Rubiaceous	Rubiaceous	Rubiaceous	Rubiaceous
Type of trichome	-	Uniseriate	Thick walled, uniseriate	Thick walled, uniseriate
Size of trichome (μ)	-	50.99-202.62	59.09-82.25	85.90-110.28
Epidermal cells number	175-208	192-218	325-349	400-430

UE: Upper epidermis

TABLE 2: EPIDERMAL CHARACTERS FOR LE

Characters	<i>Spermacoce hispida</i>	<i>Spermacoce mauritiana</i>	<i>Spermacoce stricta</i>	<i>Spermacoce ocymoides</i>
Type of epidermal cells	Irregular shaped, wavy	Irregular shaped wavy	Irregular shaped, wavy	Irregular shaped wavy
Size of epidermal cells (μ)	133.10-369.89	165.33-391.64	93.50-280.59	100.25-209.11
Type of stomata	Rubiaceous	Rubiaceous	Rubiaceous	Rubiaceous
Type of trichome	Uniseriate	Uniseriate	-	-
Size of trichome (μ)	200.39-479.60	64.55-355.50	-	-
Epidermal cells number	236-252	350-380	496-520	530-560

LE: Lower epidermis

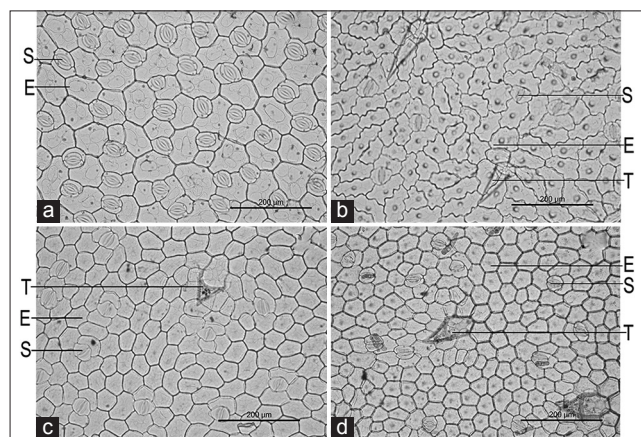


Fig 2: Epidermal characters for upper epidermis.
Epidermal characters for upper epidermis of *S. hispida* (a), *S. mauritiana* (b), *S. stricta* (c), *S. ocymoides* (d). S-Stomata, E-epidermal cell, T-trichome. Scale bar-200 μ m.

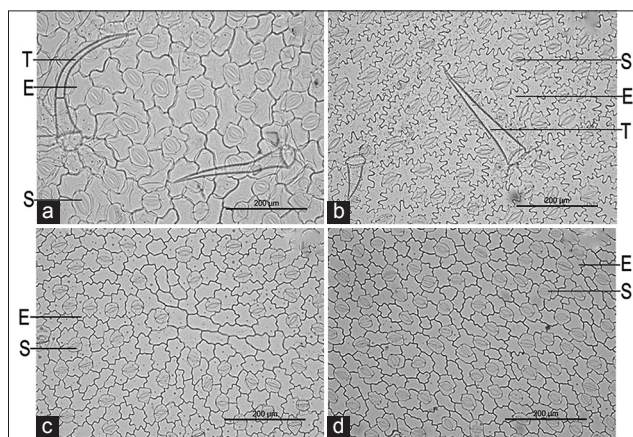


Fig 3: Epidermal characters for lower epidermis.
Epidermal characters for lower epidermis of *S. hispida* (a), *S. mauritiana* (b), *S. stricta* (c), *S. ocymoides* (d). S-Stomata, E-epidermal cell, T-trichome. Scale bar-200 μ m.

Organoleptic characters, physicochemical analysis and extractive values:

The results of organoleptic characters are mentioned in Table 4, they were similar in all species. Physicochemical analysis and extractive values results are mentioned in Table 5. The results of *S. hispida* shown difference in terms of loss on drying (5.7263% w/w), total ash content (9.6457% w/w), acid insoluble ash (0.0794% w/w) and water soluble ash (7.0412% w/w) with other selected species. The results of extractive values revealed, *S. hispida* shown difference in terms of water soluble extractive (21.9649% w/w) and ethanol soluble extractive (6.9464% w/w) with other selected species.

Preliminary phytochemical analysis:

The results of tests for the detection of phytochemicals are mentioned in Table 6. Ethanol and aqueous extracts were treated with various reagents to detect the phytochemicals. All the species showed the presence of alkaloids and glycosides, tannins and phenolic compounds, carbohydrates and amino acids. *S. hispida* and *S. mauritiana* showed presence of flavonoids and in other two species it was absent. Steroids and proteins were absent in all species.

Total phenolic content:

The TPC of selected *Spermacoce* species were expressed in terms of caffeic acid/tannic acid equivalents using the standard curve

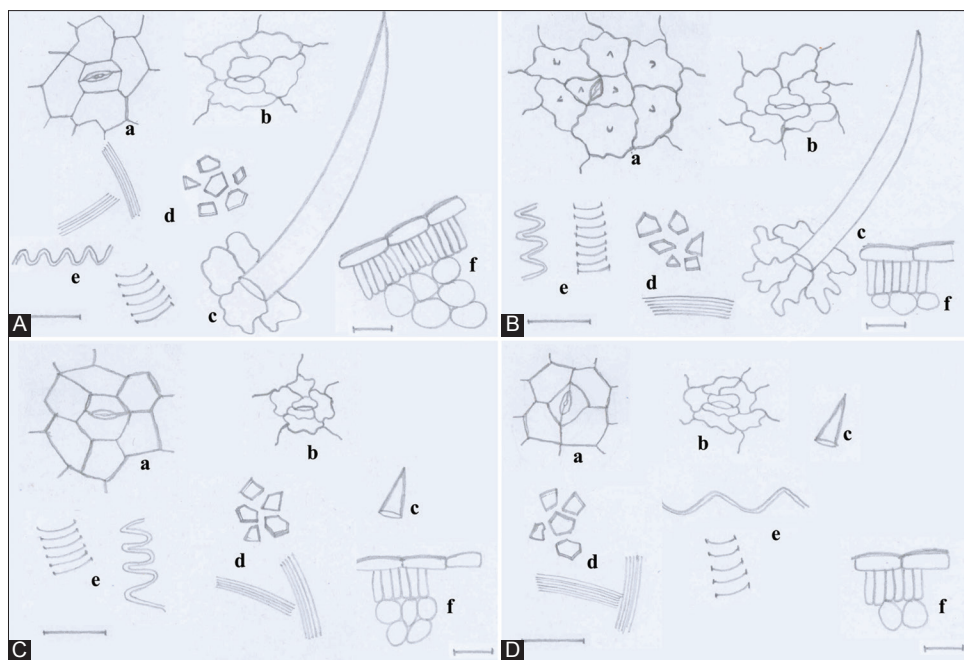


Fig 4: Powder characters.

Powder characters of *S. hispida* (A), *S. mauritiana* (B), *S. stricta* (C), *S. ocymoides* (D). Where (a) upper epidermis with rubiaceous stomata, (b) lower epidermis with rubiaceous stomata, (c) uniseriate trichome, (d) prismatic and acicular crystals of calcium oxalate, (e) annular and spiral vessels, (f) fragment of lamina. Scale bar-100 μm .

TABLE 3: LEAF CONSTANTS

Characters	<i>Spermacoce hispida</i>	<i>Spermacoce mauritiana</i>	<i>Spermacoce stricta</i>	<i>Spermacoce ocymoides</i>
Stomatal number				
UE	47-55	26-28	23-28	32-38
LE	65-74	80-92	105-130	150-170
Stomatal index				
UE	20.45-21.88	11.86-12.32	5.69-8.61	7.07-9.09
LE	20.96-22.69	18.18-20.81	17.47-20.00	21.73-24.28
Vein islet number	2-4	2-5	6-8	4-6
Vein termination number	2-5	4-7	3-4	4-6
Palisade ratio	7	7	5	6

UE: Upper epidermis, LE: lower epidermis

TABLE 4: ORGANOLEPTIC CHARACTERS

Name of the test	<i>Spermacoce hispida</i>	<i>Spermacoce mauritiana</i>	<i>Spermacoce stricta</i>	<i>Spermacoce ocymoides</i>
Color	Green	Green	Green	Green
Texture	Fine	Fine	Fine	Fine
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Pungent	Pungent	Pungent	Pungent

equation as shown in Table 7. Results of TPC are presented in Table 8. TPC of *S. hispida* was 6.88 ± 0.34 mg CAE/g and 9.17 ± 0.46 mg TAE/g. The selected *Spermacoce* species can be arranged on basis of TPC from lowest as in *S. mauritiana* < *S. hispida* < *S. stricta* < *S. ocymoides* to highest. Tannic acid equivalent TPC were higher than the caffeic acid equivalent TPC in all species.

Total flavonoids:

The TF of selected *Spermacoce* species are expressed in terms of quercetin equivalent using the standard curve equation as shown in Table 7 and results of TF are presented in Table 8. TF of *S. hispida* was 5.98 ± 0.30 mg QE/g. The selected *Spermacoce* species can be arranged on basis of TF from lowest as in *S. mauritiana* < *S. hispida* < *S. stricta* < *S. ocymoides* to highest.

DISCUSSION

S. hispida is widely used in traditional systems of medicine for various ailments^[8]. It is also reported

to be useful in reducing obesity, to control bloody diarrhea, urinary infections, oliguria, bone disease and fracture healing^[14]. The methanol extract of the plant was also reported for its antibacterial activity^[15] and hydroxyl radical scavenging and nitric oxide radical scavenging activities^[16]. Efforts were also made earlier to identify the chemical constituents of *S. hispida*^[17], *S. ocymoides*^[18] and other non-Indian species^[19] of the genus.

In spite of its wider and known utility, the pharmacognostic information on *S. hispida* in comparison with other allied species is not available. Hence, the present study forms the first report on comparative pharmacognostic and

phytochemical analysis of leaves from the genus *Spermacoce*.

The results of pharmacognostic studies revealed that, TS of midrib, leaf constants (Table 3) organoleptic characters (Table 4) and preliminary phytochemical analysis (Table 6) remains almost same for all the investigated species of *Spermacoce*. The results are comparable with the earlier reports for *S. hispida*^[20] and *S. ocymoides*^[21]. However, significant differences were observed in the epidermal characters (Tables 1 and 2), physicochemical analysis and extractive values (Table 5) and polyphenolic contents (Table 8).

The highest TPC and TF were observed in the leaves of *S. ocymoides*. The present study gains importance, as the TF content was linked to the antioxidant activity in *S. hispida*^[22].

The present study is an effort to fill the gaps in the pharmacognostic studies of the genus *Spermacoce*, along with providing the differentiating features between the allied species for their correct identification. These parameters, hopefully, shall serve as quality control parameters and also help in further studies on better and correct utility of the

TABLE 5: PHYSICOCHEMICAL ANALYSIS AND EXTRACTIVE VALUES

Name of the test (% w/w)	<i>Spermacoce hispida</i>	<i>Spermacoce mauritiana</i>	<i>Spermacoce stricta</i>	<i>Spermacoce ocymoides</i>
Loss on drying	5.7263	4.1420	6.7941	4.8378
Total ash content	9.6457	9.0938	10.6420	11.3207
Acid insoluble ash	0.0794	1.7031	2.7908	2.0841
Water soluble ash	7.0412	2.6844	2.4599	4.2453
Water soluble extractive	21.9649	27.5432	13.5298	15.0250
Ethanol soluble extractive	6.9464	9.0976	10.2009	12.8647

TABLE 6: PRELIMINARY PHYTOCHEMICAL ANALYSIS

Secondary metabolite	Tests	<i>Spermacoce hispida</i>		<i>Spermacoce mauritiana</i>		<i>Spermacoce stricta</i>		<i>Spermacoce ocymoides</i>	
		Aqueous extract	Ethanol extract	Aqueous extract	Ethanol extract	Aqueous extract	Ethanol extract	Aqueous extract	Ethanol extract
Alkaloids	Dragendorff's	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Hager's	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve
	Wagner's	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Glycosides	Legal's	+ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve
	Deoxysugars	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Flavonoids Tannins and phenolic compounds	Shinoda's	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
	5% FeCl ₃	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
	Lead acetate	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
	Bromine water	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Steroids	Dilute HNO ₃	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
	Salkowski reaction	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Liebermann-Burchard	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Carbohydrates	Molisch's	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve
	Fehling's	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve
	Benedict's	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve
	Barfoed's	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve
	Selwinoff's	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Proteins	Biuret's	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	Million's	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Amino acids	Ninhydrin	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
	Test for tryptophan	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve

TABLE 7: STANDARD CURVE EQUATION OF TPC AND TF

Activity	Concentration range ($\mu\text{g/ml}$)	Regression equation	Correlation coefficient (R^2)
TPC			
Caffeic acid	10-800	$y=0.0029x-0.0458$	0.9940
Tannic acid	10-400	$y=0.0022x+0.0225$	0.9977
TF			
Quercetin	10-400	$y=0.0059x-0.0506$	0.9914

TPC: Total phenolic content, TF: total flavonoids

TABLE 8: TPC AND TF FROM LEAVES

Activity	<i>Spermacoce hispida</i>	<i>Spermacoce mauritiana</i>	<i>Spermacoce stricta</i>	<i>Spermacoce ocymoides</i>
TPC				
CAE (mg/g)	6.88 \pm 0.34	6.67 \pm 0.33	7.08 \pm 0.35	8.17 \pm 0.41
TAE (mg/g)	9.17 \pm 0.46	8.90 \pm 0.45	9.44 \pm 0.47	10.88 \pm 0.54
TF				
QE (mg/g)	5.98 \pm 0.30	5.62 \pm 0.28	6.18 \pm 0.31	6.31 \pm 0.32

Figures in tables are represented as mean of three readings \pm SD. TPC: Total phenolic content, TF: total flavonoids, TAE: tannic acid equivalent, CAE: caffeic acid equivalent, QE: quercetin equivalent, SD: standard deviation

intended plant from the genus *Spermacoce* according to traditional system of medicine.

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Conflicts of interest:

There are no conflicts of interest.

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