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Pharmacognostic and analytical assessment for *Pterolobium hexapetalum* (Roth.) Santapau and Wagh. – A dynamic Folklore therapeutic plant

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ABSTRACT

The aim of the current study was to assess a scrupulous pharmacognostic cram is to enhance the constructive information with regard to its species identification, characterization and standardization of a dynamic folklore therapeutic plant *Pterolobium hexapetalum*. It is one of the largest scrambling shrubs belonging to the Leguminosae family having significant medicinal properties. The aerial part of the fresh and healthy plant materials was collected from the Maruthamalai hill, Coimbatore District with a strong traditional background. The confined name of the species is "Karuindu" used as medicine in Siddha, Ayurveda and folk medicine for treating various illnesses. The external morphology of study species, the anatomy of leaf and stem, physicochemical examination of plant powder, powder with diverse chemical reagents, fluorescence analysis of therapeutic powder, ash values, mineral studies, heavy metal analysis, extractive values, phytochemical analysis, GC-MS analytical studies were examined with the aim of drafting pharmacopeial principles. The studied plant contains 19 bioactive chemical constituents which confirm the healing domination. The analytical assessment of study species authenticates the presence of various bioactive constituents with a broad range of remedial properties which is used to treat manifold muddle as well it provides a comprehensive insight regarding the phytoconstituents profile which could be browbeaten for the maturity of plant-supported drugs.

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INTRODUCTION

India is a conceivably distinct main manufacturer for medicinally aromatic herb as well as dubbed as "Medico-botanical garden of the world". It is one of the foremost countries enjoying chief global market share in therapeutic flora. Foliages have been used as a source of inspiration for the development of novel drug (Prakash *et al.*, 2011) (WHO, 1999) recommended estimative value to various urbanized countries, have a prominent fraction of population making use of the traditional practice, especially the use of curative plants for their wellbeing system. Current chief and thorny difficulties stumble upon in every conventional structure is the utilization of various botanical species beneath the similar medicine name, since their resemblance in the external typeset. Nowadays the recognition of those plant species is an extremely complicated one, particularly in dehydrated form. In such circumstances, pharmacognostic evaluation is the merely basis to recognize the genuine plant used, which avoid adulteration of drugs by using microscopical, standard-

ization and diagnostic methods.

The identification of phytochemical characteristics from plant species is important for the health care system. Our literature survey revealed that there are no scientific reports carried out regarding the screening of phytochemical constituents in the proposed study plant previously. To that end, the existing effort was taken on *Pterolobium hexapetalum* (Roth.) Santapau & Wagh, mainly used to construct a phytochemical profile and also standardize by GC-MS analysis.

Plant description

The species *Pterolobium hexapetalum* is one of the largest scrambling shrubs belonging to the Leguminosae family having significant medicinal properties (Plate 1). The confined name of the species is "Karuindu". The stem of the study plant is spiny, straggler with recurved prickles. The surface of the stem is glabrous, smooth and woody. The leaves are bipinnate and 8 - 10 cm long. The leaflets are oblong, obovate, apex rounded, base oblique, pubescent and rachis ends in bristle. Each leaf consists of 6 - 10 pairs of leaflets. The facade of the leaflet is fairly glabrous bar on the veins underneath, soft and astringent taste. The type of inflorescence is an axillary or terminal raceme with long pedicel. The flowers are 0.6 cm to 1.0 cm long, white with a pinkish tinge, sepals 5 with imbricate aestivation, tube short, cup-shaped, petals 5, unequal and clawed. The pod is indehiscent, 4.5 x 1.5 cm long and reddish colour. The fruit is one-seeded and 10 x 5 mm in size.

Plate 1: **Fruiting twig of *Pterolobium hexapetalum* (Roth.) Santapau & Wagh**

Traditional uses

The *Pterolobium* is used as medicine in Siddha, Ayurveda and folk medicine for treating various ailments. The leaves and stem bark are extensively

Plate 2: **T.S. of *Pterolobium hexapetalum* Leaf**

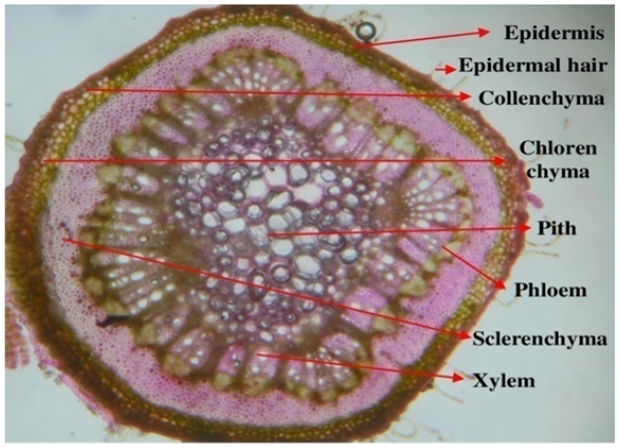

Plate 3: **T.S. of *Pterolobium hexapetalum* Stem**

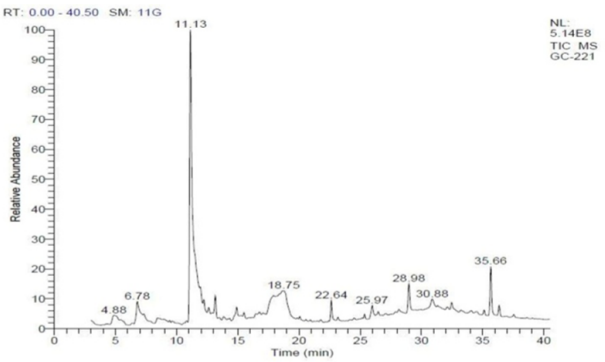

Figure 1: GC-MS chromatogram of *Pterolobium hexapetalum* ethanolic extract

ragged as curing agent to cure cough for children and delivery pains. The other plant parts acquire a large number of therapeutic activities are used to cure different diseases like fever, toothache, cough, dog bite, vomits, heat boils, chest pain, diarrhoea, constipation and piles, bone fracture, jaundice, skin infection, wound healing, venereal diseases and an ulcer (Anjaneyulu and Sudarsanam, 2013; Kumar, 2007).

Table 1: Organoleptic characters of plant powder

Characters	Observations
Colour	Greenish yellow
Texture	Coarse
Savor	Pungent
Odour	Characteristic smell

Table 2: Organoleptic characters of successive extracts

Extraction medium	Colour	Consistency	Odour
Petroleum ether	Light green	Semi solid	Characteristic smell
Ethanol	Blackish green	Semi solid	Characteristic smell
Water	Light brown	Semi liquid	Characteristic smell

Table 3: Powder their behaviour with different chemical reagents

Powder + Reagents used	Colour of the powder
Powder for itself	Soft green
Powder + Concentrated HCl	Pale pink
Powder + Concentrated H_2SO_4	Fluorescent yellow
Powder + Acetic acid	Dark green
Powder + Ethanol	Light green
Powder + Ferric chloride	Brownish green
Powder + concentrated HNO_3	Yellowish brown
Powder + water	Yellowish green

Table 4: Fluorescence behaviour of aerial plant powder

Reagents	Behaviour of plant powder		
	Visible light	UV light	
		Short (250-270)	Long (365-390)
Powder for itself	Soft green	Dark green	Black
Powder + 1 N NaOH in water	Dark green	Brownish green	Dark black
Powder + 1 N NaOH in ethanol	Pale green	Green	Bluish green
Powder + 1 N HCl	Green	Yellowish green	Dark green
Powder +50 % H_2SO_4	Dark green	Blackish green	Black
Powder + 50 % HNO_3	Brown	Greenish yellow	Black

MATERIALS AND METHODS

Chemicals and instruments

For all the studies, we used laboratory grade chemicals. Compound microscope, watch glass, glass slides, coverslips, Petri dishes and universal glasswares were used. Photographs were taken with using Photonic microscope (Model A X 70 TRF, Olympus optical) and trinocular microscope. Formalin, acetic acid, ethyl alcohol and safranin staining reagent were procured from The Precision Scientific Co., Coimbatore, India. All methodical grade solvents were obtained from E-Merck Ltd., Mumbai, India.

Compilation and authentication of plant material

The aerial part of unsullied and strong plant resources was unruffled from the Maruthamalai hill (arid; 540 m above msl; dry deciduous forest), Coimbatore District (a part of the Western Ghats of Western Tamil Nadu). (Plate 1). The collected

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Table 5: Physico-chemical and extractive values of study plant powder

Parameters	Values in Percentage
Moisture content (Loss on drying)	2.95±0.02
Total ash	45.7±0.60
Acid insoluble ash	4.05±0.01
Water soluble ash	24.6±0.30
Extractive values	8.25±0.14
a. Petroleum ether	14.23±0.09
b. Ethanol	6.74±0.14
c. Water	

[#]Values are expressed as means of triplicate determinations ± Standard Deviation

Table 6: Estimation of minerals in the plant powder

Name of the minerals	Values (mg/100g)dry Weight
Macroelements	
Calcium	47.81±1.06
Magnesium	5.89±0.93
Sodium	125.6±0.54
Microelements	
Zinc	2.57±0.01
Iron	1.98±0.10
Copper	0.56±0.06

[#]Values are expressed as means of triplicate determinations ± Standard Deviation

Table 7: Estimation of heavy metals in aerial plant powder

Heavy metal	Values (ppm)
Lead	<2 ppm
Mercury	In traces
Chromium	In traces

Table 8: Extractive values of powder in various solvents

Method of extraction	Solvents used	Yield (%)
Continuous hot percolation using Soxhlet apparatus	Petroleum ether	8.5
	Ethanol	10
Hot and cold maceration	Water	7.4

species was acknowledged with the help of The Flora of the Tamilnadu Carnatic (Matthew, 1982) and the Flora of the Presidency of Madras (Gamble, 1915). Further, the identity was authenticated with the type specimens existing in the herbarium of Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore, Tamil Nadu (voucher specimen number–BSI/SRC/5/23/2016/Tech./2824). The herbaria were deposited for further reference in Department of Botany, Vellalar College for Women (Autonomous), Thindal, Erode. Photographic images were too taken to appendage the herbarium. The medicinal properties of the plant were checked by the earlier report published by (Ganesh and Sudarsanam, 2013).

Macroscopic study and microscopic examination

The macroscopic and microscopic revision of this plant species were conceded out according to the method of (Trease and Evans, 1983).

Microscopical studies

Fresh stem and leaf of *Pterolobium hexapetalum* were removed from the plant, furthermore used for

Table 9: Qualitative phytoconstituents screening of *Pterolobium hexapetalum*

Name of the constituents	Name of the extracts		
	Petroleum ether	Ethanol	Water
Test for carbohydrate			
Barfoed's Test	-	-	-
Tests for proteins and amino acids			
Biuret test	-	-	-
Ninhydrin test	-	-	-
Tests for alkaloid			
Mayar's test	+	+	+
Wagner's test	+	+	-
Tests for tannin and phenolic compound			
Ferric chloride test for tannin	+	+	+
Sulphuric acid- sodium hydroxide solution test	-	+	-
Test for flavonoid			
Alkaline reagent Test	-	-	+
Test for triterpenoid			
Libernann-Burchard test	-	-	-
Test for steroid			
Salkowskis test	+	+	+
Test for saponin			
Foam formation test	-	-	-
Test for glycoside			
Borntrager's test	-	-	-
Test for anthraquinone			
Borndragers test	+	+	+
Test for quinine	+	+	+
Test for coumarin	+	+	+
Test for fixed oil	-	-	-

Note: '+'indicate the presence and '-' indicate the absence of compounds

Table 10: Estimation of alkaloid content

Name of the solvent	Total alkaloid (mg atropine /g extract) #
Petroleum ether	61.6 ± 2.51
Ethanol	94.3 ± 3.51
Water	57.0 ± 2.65

Values are expressed as means of three independent analysis ± Standard Deviation

free-hand slice cutting. Further sliced sections were preset in FAA (Formalin - 5 ml + acetic acid - 5 ml + 70 % Ethyl alcohol - 90 ml) as per the methodology of (Sass, 1940). Thin, freehand transverse sections were made with the aid of pointed blade and cleaned with chloral hydrate solution. The appropriate skinny sections were stained with safranin and mounted in glycerin as per the method of (O'Brien *et al.*, 1964). Photographs of different magnifications were taken and recorded on a Photonic microscope (Model A X 70 TRF, Olympus optical) with a camera in magnification power (40x and 100x). The vascular bundle arrangement of leaf and stem were classified according to (Metcalfe and Chalk, 1979) and the anatomical features are referred by standard anatomy book (Esau, 1965).

Shade drying and powdering of the collected plant material

Freshly collected aerial plant parts were washed

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Table 11: Phytocomponents identified from the ethanolic extract of *Pterolobium hexapetalum* by GC-MS analysis

RT (Min.)	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)	Nature of the Compound	Biological activity
4.86	Phenol (CAS)	C6H6O	94	2.07	Aromatic alcohol	Antioxidant
5.10	Benzenesulfonic acid, 4-hydroxy- (CAS)	C6H6O4S	174	2.07	Aromatic acid	Surfactant on hair and skin
5.66	Cis-aconitic anhydride	C6H4O5	156	0.46	Acid anhydride	Anti-tumour and breast cancer treatment
6.78	1-Butanol, 3-methyl-, formate (CAS)	C6H12O2	116	6.64	Ester	Flavouring agent
8.43	L-Serine, O-(phenylmethyl)- (CAS)	C10H13NO3	195	2.25	Amino acid	Biosynthesis of purines and pyrimidines
11.13	1,2,3-Benzenetriol	C6H6O3	126	46.09	Trihydricalhocol	Antiseptic, antioxidant, antidermatitic, fungicide and insecticide
12.22	á-Selinene (CAS)	C15H24	204	0.57	Organic selenium Compound	Anti-ulcerogenic and anti-inflammatory
12.62	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (CAS)	C15H22	202	0.56	Benzene derivate	Antioxidant and antiulcer
14.90	(-)-Caryophyllene oxide	C15H24O	220	2.97	Bicyclic sesquiterpene	Anticancer and analgesic property
15.50	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-,[1R-(1R*,3E,7E,11R*)]-	C15H24O	220	0.68	Alkene	Anti-tumor, analgesic, antibacterial, sedative, fungicide and anti-inflammatory
17.72	Mome inositol	C7H14O6	194	2.86	Cyclo hexane derivative	Antialopecic, anticirrhotic, antineuropathic, cholesterolytic, lipotropic and sweetner
22.64	Hexadecanoic acid (CAS)	C16H32O2	256	2.26	Aromatic carboxylic acid	Anti-inflammatory and antioxidant
25.35	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS)	C20H40O	296	0.55	Alkene	Antimicrobial, anticancer, anti-inflammatory and diuretic

Table 12: Phytocomponents identified from the ethanolicextract of *Pterolobium hexapetalum* by GC-MS analysis

RT (Min.)	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)	Nature of the Compound	Biological activity
25.97	9,12-Octadecadienoic acid (Z,Z)-(CAS)	C18H32O2	280	2.22	Saturated fatty Acid	Anti-inflammatory and anti-cancer
32.10	Hexadecadienoic acid, methyl ester (CAS)	C17H30O2	266	0.48	Ester	Anti-inflammatory and cancer preventive
32.46	24,25-Dihydroxycholecalciferol	C27H44O3	416	1.39	Alcohol	Synthesis of vitamin D3
35.13	4-Hydroxymethyl[2.2.2]paracyclophane	C25H26O	342	0.56	Aromatic hydrocarbon	Diamine derivatives and dihalo derivatives
35.66	9-Octadecenamide	C18H35NO	281	6.10	Amide	Induce drowsiness and reduce anxiety
36.35	Squalene	C30H50	410	1.26	Aromatic hydrocarbon	Synthesis of cholesterol, steroid hormones and vitamin D

thoroughly with distilled water to eliminate adhering dust and then shade dried at 31°C for 15 days. The shade dried plant materials were instinctively ground to loutish powder and passed through a Willy Mill to get the 60-Mesh size (Harborne, 1973).

Analysis of physicochemical constituents

The parameters, for example, organoleptic evaluation of powder and the extracts, actions of plant powder with diverse chemical reagents, fluorescence performance of the powder with different chemical reagents, moisture content of the powder, total ash, acid-insoluble ash, water-soluble ash and extractive values were studied according to the official method (Kokoshi *et al.*, 1958; Trease and Evans, 1983).

Soxhlet extraction

The air-dried, aerial plant powder (50 g) was filled in the thimble (50 g/250 ml) and extracted using a Soxhlet extractor sequentially with different solvents (Petroleum ether (60-80°C) and Ethanol (78.5°C)) in the escalating order of polarity for 5–6 h. Extracts thus obtained will be concentrated in rotavapor, separated in goblet vials and stored at 4°C in the refrigerator for further use.

Finally, the material was macerated by hot water (99.98°C) with periodic stirring for 16 hrs and water extract was filtered. The solvent extracts in a different order were concentrated, vacuum dried and weighed. The yield percentage was expressed in terms of the air-dried sample. The extracts were dried over anhydrous sodium sulfate, stock up in sealed vials in the refrigerator (5-8°C) until analysis (Anonymous, 1989).

Proximate analysis and Trace metal studies

The macro and microelement investigation were conceded by using Atomic Absorption Spectrophotometer (Model ECIL AAS 4127) (Tandon, 1993).

Extractive values and qualitative phytochemical screening

The extractive values were performed following the procedure of (Trease and Evans, 1983). Phytochemical screening of diverse solvent extracts was carried out in a successive manner using the standard procedure described by (Harborne, 1984; Kokate *et al.*, 1995).

Quantification of phytoconstituents

The total alkaloids were determined following the procedure of (Fadhil *et al.*, 2008).

GC/MS investigation

The ethanol in crude (1 μl) form holding diverse compounds was used for (GC-MS) investigation.

GC/MS conditions

The ethanol in crude form was subjected to GC-MS on the instrument - THERMO GC – TRACE ULTRA VER: 5.0, THERMO MS DSQ II, DB 35 - MS CAPILLARY STANDARD NON - POLAR COLUMN and the GC-MS trace ultra-version 5.0 software employing the following conditions: RT x 5 MS column (30 Mts x 0.25 mm ID x 0.25 μM df, composed of 100 % Dimethyl poly diloxane). Initially, the oven temperature was maintained at 70ºC for 2 minutes and the temperature was steadily increased up to 260ºC for 6 minutes. One μl of the sample was injected for investigation. Helium gas 99.995 % of purity was used as a carrier gas as well as an eluent. The flow rate of helium gas was set to 1 ml/min. The sample injector temperature was maintained at 260ºC and the split ratio is ten throughout the experiment periods. The ionization mass spectroscopic analysis was done with 70 eV. The mass spectrum was recorded for the mass range 40-1000 m/z for about 37.50 minutes.

Detection of chemical compounds was based on comparison of their mass spectra. The recognition of compounds was based on the comparisons of their mass spectra with NIST Library 2008 WILEY8, FAME. The total GC running time was 37.51 minutes (Massada, 1976). The name, molecular weight and structure of the mechanism for components were ascertained in test materials.

Statistical studies

The values are expressed as mean $\pm$ SD.

RESULTS AND DISCUSSION

Anatomical studies of Leaf

The present anatomical study provides characters which would facilitate quick identification and differentiation of the drug from alike material, often used indiscriminately with genuine herbal medicines (Sharmila *et al.*, 2018). The transverse section of the leaf has thick broad midrib region, lateral smooth veins and dorsiventral thin lamina. Towards the lower region, it is plano-convex in shape and it is slightly knobbed towards the upper region. Both the epidermal cells are rectangular in outline, covered by means of thick cuticle and also consist of extended unicellular trichomes. In the bulged midrib portion, 2-3 layers of tightly arranged, elongated palisade cells are presently followed by parenchymatous cells (1-2 layers). The centre core region is occupied by the vascular bundle and it is differentiated into xylem and phloem. The entire vascular bundle is bounded by a sclerenchymatous sheath and embedded in the mesophyll portion. There are 6-8 xylem strands are present. Irregular rectangular cells are noted in the lower epidermal region followed by spongy parenchymatous cells arranged in 3-5 layers. These spongy cells are closely arranged in midvein, loosely arranged in lateral veins and it contains huddled calcium oxalate crystals and small starch grains.

Leaf through lamina region in transverse section confirmed elongated pointed trichomes. The palisade cells are arranged in a single layer, whereas spongy tissues are 2-4 layered. Spongy cells in lamina region also contain clustered calcium oxalate crystals and they are loosely arranged. The lower region consists of stomata and it is a paracytic type (Plate 2). From the anatomical point of view, the foliar epidermal studies are one of the most noteworthy taxonomic studies (Jones, 1986; Mownika *et al.*, 2019).

Anatomical studies of young stem

Transverse section of the young stem shows the initial stage of secondary growth and it is smoothed circular in outline. The epidermal layer is lean continuous all around the stem, made up of thin-walled cells and trichomes also noted. The cortex is wider and is differentiated into an external zone of about three layers of collenchyma, followed by 2-3 layers of chlorenchyma and interior zone of about eight stratums of sclerenchyma. The continuous sheath of phloem and rays encircling the xylem with 4-5 layers of radial files made up of small cells in a narrow zone. Xylem cylinder is present in the middle region, which is thick, dense and elements are traversed by narrow rays. The xylem cylinder is frequently accompanied on the outside by a sheath of cells containing solitary crystals. Xylem cylinder consists of xylem elements like fibres in the ground tissue; it is thick-walled, lignified and arranged in a narrow lumen. The vessels arise in radial lines of uniseriate and it is widely separated from each other. The vessel lines expand from the inner to outer boundaries of the cylinder and vessel cells are thickly walled. In the middle region, delicate, thin-walled parenchymatous cells are present (Plate 3). Due to lack of anatomical data on the genus *Pterolobium*, comparisons should be made with related taxa. Some simi-

lar works were carried out earlier in the members of Leguminosae (Karthikeyan *et al.*, 2012; Ganesh *et al.*, 2014).

Physico-chemical, proximate and phytochemical analysis

The plant powder for itself showed attributed odour and pungent taste. The colour of shade-dried powder changed from gloomy green to greenish golden yellow, as shown in Table 1. The organoleptic characters such as colour, consistency and odour of consecutive solvent extracts are given in Table 2. Powder with various reagents their behaviour was observed and depicted in Table 3. Powder treated with acetic acid, ethanol, ferric chloride and water showed greenish shades. Whereas in powder reacts with concentrated HCl gives a pale pink colour and fluorescent yellow colour with concentrated H_2SO_4. Similar tests were noted and recorded by (Parvathy and Gopalakrishnan, 1991).

The fluorescence behaviour of day and UV lights are used to check the identity of powdered drugs (Kokoshi *et al.*, 1958). Plant powder fluorescence behaviour with diverse chemical reagents was observed in daylight as well as UV light at short (250-270) and long (365-390) wavelengths (Table 4). Fluorescence examination denotes the occurrence of different phytoconstituents that are helpful for the evaluation of vigorous constituents of a drug, that are accountable for various pharmacological action and also useful for the preparation of genuine Ayurvedic drugs (Kokoshi *et al.*, 1958).

The physicochemical characters lend a hand to set up definite principles for dried drugs and used to judge their quality and purity also avoid batch to batch variation (Karthikeyan *et al.*, 2012). The characters like a loss on drying, ash and extractive values of study plant powder are analyzed (Table 5). The 2.95 ± 0.02 % of moisture, 45.7 ± 0.60 % of total ash content, 24.6 ± 0.30 % of water-soluble ash and 4.05 ± 0.01 % of acid-insoluble ash was noted in the plant powder. The ethanol solvent showed high percentage of extractive value (14.23 ± 0.09 %) chased by petroleum ether (8.25 ± 0.14 %) and aqueous (6.74 ± 0.14 %) extracts.

Mineral studies

The contents of calcium, magnesium and sodium found in the study plant were 47.81 ± 1.06 mg/100 g, 5.89 ± 0.93 mg/100 g and 125.6 ± 0.54 mg/100 g, respectively (Table 6). Magnesium, calcium and sodium are essential for the development of red blood cells and for body mechanism (WHO, 1996). The microelement zinc was noted in elevated amount (2.57 ± 0.01 mg/100 g) and is necessary for insulin discharge, encourage the discharge of vitamin-A from the liver and also needed for wound healing, normal expansion and reproduction (Sharmila, 2019). The powder had 1.98 ± 0.10 mg/100 g iron, which proves the effectiveness of drugs and also had 0.56 ± 0.06 mg/100 g of copper.

Estimation of Trace metals

The concentration of lead was less than two ppm, mercury and chromium occur only in trace amounts (Table 7), representing the water pollution free edaphic stipulation of the study area. This is in agreement with the outcome of (Begum *et al.*, 2017) who exanimate the heavy metals in recurrently consumable therapeutic plants.

Solvent extraction (Successive)

Percentage yield

The quantity of components in a given amount of unprocessed material extracted with appropriate solvents fortitude the extractable matter (Gopalasatheeskumar, 2018). The ethanol extract showed the highest (10 %) percentage of yield tracked by petroleum ether extract (8.5 %). The water extract showed the lowest yield (7.4 %) (Table 8).

Qualitative phytochemical evaluation

The outcome of the preliminary phytoconstituents screening showed the existence of subsequent phytochemicals such as alkaloids, tannins and phenolic compounds, steroid, anthraquinone, quinines and coumarin in all the extracts treated. The positive results for flavonoids are noted only in the water extract. The ethanol extract has more constituents, followed by petroleum ether and water extracts. The results revealed the absence of carbohydrates, proteins and amino acids, saponins, glycoside and fixed oil (Table 9). Similar work was carried out earlier by (Dash *et al.*, 2014) in *Acacia suma* species of Caesalpiniaceae family.

Quantification of alkaloid phytoconstituent

The total alkaloid content for different solvent extracts exhibited higher alkaloid content in ethanol extract (94.3 ± 3.51 mg/g) pursued by petroleum ether extract (61.6 ± 2.51 mg/g) and it is expressed as atropine equivalent (Table 10).

Gas Chromatography / Mass Spectrometry (GC / MS) analysis

The GC-MS investigation revealed the presence of phytoconstituents such as aromatic alcohols, aromatic acid, acid anhydride, esters, amino acids, trihydric alcohol, selenium group, benzene derivative, bicyclic sesquiterpene, alkene, cyclohexane derivative, aromatic carboxylic acid, saturated fatty acid,

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aromatic hydrocarbon and amide group.

The GC-MS ethanolic extract characterization showed 19 chemical compounds, which are indicated in (Tables 11 and 12). From the above chemical compounds, 1,2,3-benzenetriol ($C_6H_6O_3$) expressed the high peak area percentage (46.09) with a retention time 11.13. The spectrum profile of GC – MS confirmed the presence of 3 major components with retention time 11.13, 6.78 and 35.66 (Tables 11 and 12). The methanol portion holds hexadecanoic acid methyl ester, also called as methyl palmitate is an aliphatic acid ester accounted to cause growth inhibition and apoptosis induction in human gastric cancer cells (Daniel *et al.*, 2011).

In terms of percentage amounts 1,2,3-benzene trial, 1-butanol, 3-methyl-, formate and 9-octadecenamide were predominant in the extract and had the property of antioxidant, antiseptic, antidermatitic, fungicide, insecticide, flavoring agent, induce drowsiness and reduce anxiety. Among the other identified phytochemicals cis-aconitic anhydride, L-serine, O-(phenylmethyl)-, á-selinene, (-)-caryophyllene oxide, 12-oxabicyclo [9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-[1R-(1R*,3E,7E,11R*)]-, momeinositol, hexadecanoic acid, 2-hexadecen-1-ol, 3,7,11,15-tetramethyl, [R-[R*,R*-(E)]]-(CAS), 9,12-Octadecadienoic acid (Z,Z)- (CAS) and aromatic hydrocarbon squalene have the property of anti-tumour, breast cancer treatment, biosynthesis of purines and pyrimidines, anti-ulcerogenic, anti-inflammatory, analgesic, anti-alopecic, anti-cirrhotic, anti-neuropathic, cholesterolytic, lipotropic, anti-microbial and diuretic activities. Likewise, (Sharmila, 2019) screened 41 most important compounds from Acacia caesia plant. In terms of percentage amounts, 1,8-diphenyl-3,4,10,11- tetrahydro[1,4]dioxino[2,3-g:5,6-g']diisoquinoline, 6-(chloromethyl)-4-(3,4-dimethoxy-2-(phenyl methoxy)-phenyl)-3-methyl-2-yridinecarboxylate and 2',4',6'-Trinitro-5'-phenyl-1,1':3',1"-terphenyl were predominant in the extract and have the property of antioxidant, antidepressant potential, antibacterial activity, cytotoxic, diabetic and also induced brain activity (Figure 1). (Sharmila *et al.*, 2019)

CONCLUSIONS

The pharmacognostical results obtained from the current investigation concluded that the macroscopic and microscopic characters, fluorescence studies, physicochemical and phytochemical evaluation of study plant could be used as a diagnostic tool and also authenticate the drug for future investigation. Together, it will provide the protocol for pharmacological and clinical studies. In addition to this, the GC-MS profile can also be used as pharmacognostical contrivance for identification of phytochemicals, which confirms the application of study plant for numerous ailments by traditional practitioners and also supports the usage of this straggling shrub to human civilization. Further, the isolation, identification, purification, characterization and structural elucidation of bioactive compounds from this species are taken under investigation. All characters obtained from the results are also necessary for database preparation in this digital world by which further experiments or research can be preceded.

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Conflict Of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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