ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Phytopharmacognostical Standardization on Indian Medicinal Plant Acacia Caesia (L.) Wild.

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ABSTRACT

Plants are being highly explored as a major source of medicinal compounds due to the presence of various phytochemical compounds. In the current investigation, a locally used medicinal plant species Acacia cassia was selected and used for analysis of major bioactive constituents of medicinally important plant. The plant A. cassia belongs to the family Mimosaceae is a glabrous, woody straggling shrub growing up to 6-10 m height. The local name of the species is 'Kari induct or Inhaul'. The plant species is used in Ayurveda, Sidda and Folk medicine. It is commonly known as 'Cabool' in India and ethno medicinally have long been used for the treatment of skin, sexual problems, wound, and stomach and tooth problems. The current study deals with the macroscopic, physic-chemical along with phytochemical analysis and fluorescence analysis of powdered crude drug were carried out for systemic identification and authentication of aerial plant parts. The different extracts of Acacia cassia was studied for its in vitro antioxidant activity using hydroxyl and superoxide anion radical. This study provides referential information for identification and characterization of A. cassia and its extracts.

Keywords: Acacia cassia, macroscopically, physic-chemical, phytochemical

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ISSN: 0975-8585

INTRODUCTION

Biodiversity is an asset, and the biological wealth of a region constitutes important resources for the region. The ever-increasing rate of tropical deforestation for various reasons in different parts of the world results in biodiversity loss, including those of medicinal plants. The concern increases because humans depend on nature for everything. To cope up with the increasing demand of medicinal plants, the viable option is commercial cultivation of these non-agricultural species to reduce collection pressure from the wild. Plant and its products are rich sources of a phytochemical and have been found to possess a variety of biological activities including antioxidant potential. The ethnomedical literature contains a large number of plants with potential antioxidant properties that can be used against diseases, in which reactive oxygen species (ROS) are thought to play a major role [1, 2].

For the present investigation, a locally used medicinal plant species, Acacia cassia (L.) Willd. Generally inhabiting the foot hills of Western Ghats of Coimbatore District and Erode District, Tamil Nadu, India was selected. Acacia cassia is an armed woody straggling shrub belongs to the family Mimosaceae. It is commonly known as 'Cabool' in India and ethno medicinally have long been used for the treatment of skin, sexual problems, wound, stomach and tooth problems [3]. Still many herbal products derived from Acacia species are sold in markets in pure or mixed forms like baboon tooth paste, ayur shampoo, nyle shampoo, etc. All Acacias are suitable materials for fuel, forage, soil fertility and soil conservation. In Indian systems of medicine Acacia cassia is a strong antioxidant medicinal plant. In this present study we reported that physicochemical, proximate analysis, phytochemical and antioxidant characterization of petroleum ether, ethanol and water extracts of locally available and medicinally valuable plant Acacia cassia.

MATERIALS AND METHODS

Chemicals and instruments

All the chemicals used for the study were of laboratory grade.

Collection and authentication of study plant material

Fresh and healthy leaves were collected from the Maruthamalai hill (arid; 540 m above mL; dry deciduous forest), Coimbatore District (a part of the Western Ghats of Western Tamil Nadu) and the voucher herbarium specimen was processed followed by standard methods [4]. The collected study plant was identified with the help of the existing Floras [5,6,7] and identity was authenticated compared with type specimens available in the herbarium of Botanical Survey of India, Southern Circle, TNAU, Coimbatore, Tamil Nadu (Voucher specimen No. BSI/SRC/5/23/2015/TECH/343) (Plate 1) and the type specimens were deposited for further reference.

Pharmacognistical studies macroscopically characteristics

The morphological characters of the stem and leaf such as colour, surface texture, taste and odour were examined as per [8, 9].

Shade drying and powdering of the collected plant material

Freshly collected aerial plant parts were cleaned to remove adhering dust and then shade dried. The shade dried plant materials were mechanically ground to coarse powder and passed through a Willy Mill to get 60-Mesh size and used for physic-chemical, fluorescence analysis, proximate analysis, phytochemical and antioxidant studies. Samples were stored in the good grade plastic containers which are maintained at room temperature until analysis [10].

Physico-phyto chemical studies

The organoleptic evaluation of leaf powder and the extracts, such as colour, texture, odour and taste were carried out as per [8]. The parameters such as actions of plant powder with different chemical reagents, fluorescence analysis, total ash, acid insoluble ash, water soluble ash and extractive values of petroleum ether,

ethanol and water were studied according to the official method [8, 11]. The determination of moisture content (Loss on drying) was followed by [12].

Estimation of volatile oil

The volatile oil was extracted from 100 g of powder by hydro distillation for 3 h, using a Clevenger-type apparatus, extracted with ethanol and dried anhydrous $Na_2 SO_4$ according to the method of [13].

Estimation of fibers and sugars

Fiber content was extracted in 5 g of plant powder followed by the method of [14]. The total sugars were estimated following the phenol-sulphuric acid method described by [15]. The reducing sugars were estimated by employing arsenomolybdate reagent introduced by [16] for colorimetric determination of the cuprous oxide formed in the oxidation of the sugars by alkaline cooper tartarate reagent.

Successive solvent extraction

Dried and powdered plant powder (50 g) was filled in the thimble and extracted successively with petroleum ether and ethanol (50 g/250 ml) using a Soxlet extractor for 5–6 h. Extracts thus obtained will be concentrated in rot vapor and separated in glass vials and stored at 4°C in refrigerator for further use. Each time, before extracting with the next solvent, the powdered material was dried in a hot air oven at 40°C. Finally, the material was macerated using hot water with occasional stirring for 16 hrs and the water extract was filtered. All the three extracts were subjected to phytochemical analysis and antioxidant studies [17].

Preliminary phytochemical studies

Phytochemical screening of different successive solvent extracts was carried out following the methods of [18, 19, and 20]. Carbohydrates, proteins and amino acids, alkaloids, anthroquinones, flavonoids, glycosides, phenols and tannins, saponins, steroids and terpenoids were qualitatively analyzed.

Quantification of secondary metabolites

The total phenolics and tannins were determined following the procedure of [21]. The determinations of total flavonoids were determined following the procedure of [22].

In vitro antioxidant studies

Hydroxyl radical scavenging activity [23]

Various quantities of extracts (250–1250 µg) were added with 1 ml of iron-EDTA solution, 0.5 ml of EDTA solution and 1 ml of DMSO. The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22 %) and incubated at 80-90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1 ml of ice-cold TCA (17.5 % w/v). Three milliliters of Nash reagent was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the colour formed was measured spectroscopic ally at 412 nm against reagent blank.

HRSA % = 1 - (difference in absorbance of sample / difference of blank) x 100

Superoxide radical scavenging activity [24]

Each 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 mg riboflavin, 12 mM EDTA, 0.1 mg NBT and various concentrations (50–250 µg) of sample extracts. Reaction was started by illuminating the reaction mixture with sample extract for 90 seconds. Immediately after illumination the absorbance was measured at 590 nm. The entire reaction assembly was enclosed in a box lined with aluminum foil. Identical tubes with reaction mixture kept in dark served as blank.

% Inhibition = (control OD - sample OD / control OD) x 100

ISSN: 0975-8585

RESULTS AND DISCUSSION

Macroscopic characteristics

Standardization is an essential measure of quality, purity and authenticity. Macroscopically features as one of the effective parameters for the Pharmacognistical identification of several plant derived crude drugs [25, 26]. The macroscopic characters of fresh aerial plant parts of Acacia cassia are presented in Table 1. The results revealed that the species habit is armed woody straggling shrub growing up to 6-10 m in height. Branch lets glabrous and intermodal thorns are hooked. The leaves are alternate, querulous, margin entire and apex obtuse. The petiole grows up to 6 cm and prickled on the underside. The flower is ceramist-white in colour, inflorescence is terminal panicles. The calyx-tube is 5-lobed and pubescent. The petal is 5 in number and it is cream in colour. The stamens are numerous and basally connate. The Pod is stipulate, thin, flat, glabrous base, apex obtuse and horned (Plate 1).

Table 1: Macroscopic characteristics of Acacia cassia

S. No.	Macroscopic characters
1.	**Stem:** Height: 6-10 m in height Surface: Glabrous Thorns: Intermodal hooked thorns Texture: Rough due to the presence of hooked thorns Taste: Bitter Odour: Characteristic Colour: Dark green
2.	**Leaves:** Alternate, 10-15 cm, pinnate ca. 6 pairs, 3-8 cm Leaflets: 10-15 pairs, oblong, querulous, margin entire, apex obtuse Surface: Quite glabrous except on the veins underneath Petiole: Up to 6 cm, prickled on the underside with a convex elongate gland at base, rachis prickled Texture: Smooth Taste: Bitter Odour: Characteristic Colour: Dark green
3.	**Flowers:** Creamish-white heads in terminal panicles, peduncle up to 5 cm, bracts lanceolate, bracteoles up to 1 mm, calyx-tube 5-lobed, petals 5, Stamens α.
4.	**Fruits:** A stipulate pod, 7-15 x 1.5-2 cm, thin, flat, horned; dark brown when mature; seeds more than 10. Fruiting December onwards.

Plate 1: Snapshot of Acacia cassia in blooming stage

ISSN: 0975-8585

Physic - chemical analysis

Organoleptic characters of powder and successive extracts

Sensory or organoleptic characters describe colour, odour, taste and fracture of the raw materials used for drug preparation, identification and adulteration. The organoleptic evaluation of plant powder showed characteristic odour and bitter taste. Upon drying and powdering the colour of the powder changed from dark green to greenish black as shown in Table 2. The organoleptic characters such as colour, consistency and odour were noted in the successive leaf extracts of Acacia cassia (Table 3). This is correlated with the work of [27].

Table 2: Organoleptic characters of powder

S. No.	Characters	Observations
1.	Colour	Dark green
2.	Texture	Slightly course powder
3.	Taste	Bitter
4.	Odour	Characteristic smell

Table 3: Organoleptic characters of plant successive extracts

S. No.	Extraction Medium	Colour	Consistency	Odour
1.	Petroleum ether	Greenish yellow	Semi solid	Characteristic smell
2.	Ethanol	Brownish black	Semi solid	Characteristic smell
3.	Water	Yellowish green	Semi liquid	Characteristic smell

Behaviour of powder with different chemical reagents

The behaviour of leaf powder with various reagents were observed and presented in Table 4. Slight difference (pale green to dark green) was noted in the powder as such and treated with concentrated HCl, acetic acid, ammonium solution and ferric chloride when compared to other reagents used. Plant powder treated with concentrated H_2SO_4, iodine solution, sodium nitroprusside and potassium hydroxide indicates the brownish shade.

Table 4: Behaviour of powder with different chemical reagents

S. No.	Powder + Reagents used	Colour of the powder
1.	Powder as such	Dark green
2.	Powder + Concentrated HCl	Greenish yellow
3.	Powder + Concentrated H_2SO_4	Greenish brown
4.	Powder + Concentrated HNO_3	Wood brown
5.	Powder + Acetic acid	Greenish yellow
6.	Powder + Ammonium solution	Yellowish green
7.	Powder + Ferric chloride	Greenish yellow
8.	Powder + Iodine solution	Pale brown

9.	Powder + Sodium nitroprusside	Yellowish brown
10.	Powder + Potassium hydroxide	Yellowish brown

Fluorescence behaviour of powder with different chemical reagents

Fluorescence behaviour of the powdered plant material after treating with different chemical reagents was observed in visible light as well as under UV light at 254 nm and the observations is depicted in Table 5. Plant powder as such showed green to dark green in visible and UV light. The same observations were noted in powder treated with 50 % sulphuric acid. Powder treated with NaOH in water, NaOH in ethanol and HCl showed slight colour differences like pale greenish yellow to yellowish green, yellowish green to greenish yellow and greenish yellow to yellowish green. There is no distinct colour differences was seen in visible and UV light. Similar fluorescence profiling had been undertaken by [28, 29].

Table 5: Fluorescence behaviour of plant powder

S. No.	Reagents	Behaviour of powder	
		Visible light	UV light
1.	Powder as such	Green	Dark green
2.	Powder + 1 N NaOH in water	Pale greenish yellow	Yellowish green
3.	Powder + 1 N NaOH in ethanol	Yellowish green	Greenish yellow
4.	Powder + 1 N HCl	Greenish yellow	Yellowish green
5.	Powder +50 % H_2SO_4	Green	Dark green

Proximate analysis and extractive values of leaf powder

The physic-chemical characters like moisture content, ash values and extractive values of powder were analyzed, and presented in Table 6. The results indicate that the sample contained 7.88% of moisture. The total ash content of the sample was 4.28%. The acid insoluble ash value (3.45%) was lower than water insoluble ash (10.21%). The volatile oil of the plant powder showed minimum percentage (0.002). The study plant possesses strong fiber content (5.89). The total sugars and reducing sugars are ranged between 20.59 and 4.21. The percentage of extractive value was maximum in ethanol extract (90.1%) followed by petroleum ether (89.4%) and water (65.8%) respectively. This is in accordance with the results of [26].

Table 6: Physic - chemical and extractive values of plant powder

S. No.	Physic-chemical properties	Values in Percentage (%)
1.	Moisture content (Loss on drying)	7.88
2.	Total ash	4.28
3.	Acid insoluble ash	3.45
4.	Water soluble ash	10.21
5.	Volatile oil	0.002
6.	Fiber content	5.89
7.	Total sugars	20.59
8.	Reducing sugars	4.21
9.	**Extractive values** a. Petroleum ether b. Ethanol c. Water	 89.4 90.1 65.8

Successive solvent extraction

Percentage yield

The air dried, powdered sample was extracted with different solvents for the phytochemical and antioxidant studies (Table 7). The percent yield was maximum in ethanol extract (11%) followed by petroleum ether extract (9.5%). The water extract yield more or less equal to petroleum ether extracts (9.0%). Total cash value, fluorescence analysis, and extractive values will be helpful in identification, authentification, and useful for the determination of the exhausted or adulterated drug [30, 31].

Table 7: Extractive values of powder in various solvents

S. No.	Method of extraction	Solvents used	Yield (%)
1.	Continuous hot percolation using Soxlet apparatus	Petroleum ether	9.5
		Ethanol	11
2.	Hot and cold maceration	Water	9.0

Preliminary phyto chemical analysis

To investigate the chemical constituents of powder, the successive solvent extracts were subjected to qualitative phytochemical screening. The preliminary phytochemical screening of all the three extracts revealed the presence of carbohydrates, proteins, amino acids, alkaloids, glycosides and phenols. From the twelve identified compounds petroleum ether and ethanol extraction showed best results. The petroleum ether and ethanol extracts were more efficient than water extract (Table 8). This is in accordance with the results of [32, 33].

Table 8: Qualitative phytochemical screening of Acacia cassia

S. No.	Constituents	Petroleum ether	Ethanol	Water
1.	Carbohydrates	+	+	+
2.	Proteins	+	+	+
3.	Amino acids	+	+	+
4.	Alkaloids	+	+	+
5.	Anthroquinones	+	+	-
6.	Flavonoids	+	+	-
7.	Glycosides	+	+	+
8.	Phenols	+	+	+
9.	Tannins	+	+	-
10.	Saponins	+	+	-
11.	Steroids	+	+	-
12.	Terpenoids	+	+	-

Quantitative phytochemical evaluation -Total Phenolic and tannin content

Plant Phenolic compounds have been found to possess potent anti-inflammatory activity [34]. Total Phenolic and tannin content of different solvent extracts of Acacia cassia were studied and expressed as tannic acid equivalent. As shown in Table 9, the total Phenolic content was maximum in ethanol extract (5.34 mg/g) followed by petroleum ether extract (10.8 mg/g). The minimum was recorded in water extract (1.45 mg/g). When compared with other solvent extracts, petroleum ether extract registered higher levels of tannin content (12.5 mg/g) followed by ethanol extract (6.91 mg/g). The minimum was recorded in water extract (1.89 mg/g).

ISSN: 0975-8585

Table 9: Estimation of total Phenolic, tannin and total flavonoids content of powder

S. No.	Extraction Medium	Total Phenolic (mg TAE/g extract) #	Tannin (mg TAE/g extract) #	Total flavonoids (mg RE/g extract) #
1.	Petroleum ether	10.8	12.5	2.13
2.	Ethanol	5.34	6.91	2.23
3.	Water	1.45	1.89	8.52

Values are means of three independent analysis ± Standard Deviation
TAE - Tannic acid equivalent; **RE -** Rustin equivalent

Total flavonoids content

The flavonoids have been found to possess anti-inflammatory properties in various studies [35]. Total flavonoids content of different solvent extracts of Acacia cassia leaf powder was studied and expressed as ruin equivalent and shown in Table 9. The total flavonoids content was maximum in water extract (8.52 mg/g) followed by ethanol (2.23 mg/g) and water (2.13 mg/g).

In vitro hydroxyl radical scavenging activity

Oxidative stress has been implicated in etiology of a number of human ailments [36]. Hydroxyl radical scavenging activity was assessed by generating the hydroxyl radicals using ascorbic acid - iron EDTA. The hydroxyl radicals formed by the oxidation, reacts with diethyl sulfoxides to yield formaldehyde, which provide a convenient method to detect hydroxyl radicals by treatment with Nash reagent. The hydroxyl radical scavenging activities of petroleum ether and ethanol extracts of Acacia cassia leaf powder are presented in Table 10. In the present investigation, all the samples and standard (BHA) exhibited percentage activity between 7.88 % and 47.52 %. All the extracts studied exhibited a dose dependent increase in scavenging activity. Among the extracts, the ethanol extract appears to have the highest potential for hydroxyl radical scavenging activity at 50, 100, 150, 200 and 250 µg/ml concentrations (Table 15).

Table 10: Hydroxyl radical scavenging activity of Acacia cassia

S. No.	Sample	Concentration (µg/ml)	% activity #
1.	Petroleum ether	50	07.88 ± 4.3
		100	09.88 ± 2.5
		150	12.91 ± 1.3
		200	15.33 ± 0.6
		250	24.06 ± 1.2
2.	Ethanol	50	42.63 ± 0.7
		100	43.38 ± 0.5
		150	44.79 ± 0.2
		200	45.44 ± 0.7
		250	47.52 ± 0.2
3.	BHA	2	15.63 ± 2.9
		4	18.64 ± 4.0
		6	22.64 ± 2.6
		8	27.46 ± 1.3
		10	33.78 ± 0.4

Values are expressed as means of triplicate determinations ± Standard Deviation

Superoxide anion radical scavenging activity

The superoxide anion is biologically important radical since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals. It is very harmful to the cellular components in a biological system. The petroleum ether and ethanol solvent extracts of Acacia cassia were found to scavenge the superoxide generated by riboflavin photo reduction method (Table 11). All the extracts studied exhibited a dose dependent increase in scavenging activity. The highest percentage of inhibition was noted in ethanol extract (49.3055 + 0.1324 %) which is compared well with the BHT standard (60.3467+0.3456 %). This is in accordance with the results of [37].

Table 11: Superoxide anion radical scavenging activity of Acacia cassia

S. No.	Sample	% of inhibition[#]
1.	Petroleum ether	14.4303+0.1496
2.	Ethanol	49.3055+0.1324
3.	BHT	60.3467+0.3456

[#]Values are expressed as means of triplicate determinations ± Standard Deviation
Values within the same row not sharing common superscript letters (a-c)
Differ significantly at $p<0.05$ by DMRT.

CONCLUSION

The information obtained from Pharmacognistical studies will be used for supplementary pharmacological and therapeutically evaluation of the species and will assist in standardization for quality, purity, and authentication with the help, of which adulteration and substitution can be prevented. The phytochemical protect human from a host of diseases. From the results of our analysis, it can be concluded that useful information about proximate composition and antioxidant properties of A. cassia, which are used for the therapeutic purposes. The low moisture content indicates good quality of plant material and its prolonged shelf life. The findings of this study support the fact that A. cassia possess promising sources of potential antioxidants. A more research has taken up to examine the incredible therapeutic properties of the medicines.

ACKNOWLEDGEMENT

The authors of this paper thanks to University Grants Commission, Hyderabad, for funding as Minor Research Project.

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