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PHYSICO-CHEMICAL STANDARDISATION AND REVIEW ON *CAPPARIS ZEYLANICA* LINN., A COMMON SIDDHA HERBAL DRUG

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ABSTRACT

Capparis zeylanica Linn. is a rigid, wiry and much branched shrub and widely distributed in India, Sri Lanka, Bangladesh and Malaysia. (Family: Capparidaceae; Syn: *Capparis horrida* Linn.; Sanskrit - Kantakalatha; Malayalam – Karthotti; Tamil – Adondai). The root and leaves of *Capparis zeylanica* Linn. are widely used in the indigenous system of medicine. In Indian traditional systems of medicine, *C. zeylanica* is considered as an important remedy for the treatment of several diseases. To assure the quality of the drug, standardisation has become very essential and need of the present hour. Hence it was thought worthwhile to explore this plant on the basis of standardization parameters for the quality control of the drug. In the present paper, a detailed pharmacognostic study on *Capparis zeylanica* Linn. (leaves) based on its physico-chemical and phytochemical studies were carried out to lay down the pharmacopoeial standards. The physico - chemical parameters such as moisture content, total ash, acid insoluble ash, water soluble ash, water soluble extractives, alcohol soluble extractives, pH of water extract, volatile oil and fibre content, were determined. The limit tests for arsenic & heavy metals were carried out and were found to be within the permissible limit which proves the safety of the drug material. The fluorescence characters of the drug in different solvents were detected in visible, short UV and long UV light. High Performance Thin Layer Chromatographic (HPTLC) and Thin Layer Chromatographic (TLC) studies were carried out. A preliminary phytochemical study was carried out using different extractives of the plant material. Apart from these studies, this paper attempts to encompass the available literature on *C. zeylanica* with respect to its traditional uses, pharmacological activity and phytochemical aspects.

KEYWORDS: *Capparis zeylanica*, *Adondai*, physicochemical, chromatographic, pharmacopoeial standards.

INTRODUCTION

Capparis zeylanica Linn., belonging to the family Capparidaceae (Syn: *C. horrida* Linn.) is a rigid, wiry and many branched climbing shrub grows in a moist habitat and is commonly distributed throughout the major parts of India, China, Nepal, Bangladesh, Sri Lanka, Malaysia and some parts of Pakistan.[1,2,3] In India, the plant is widely distributed in Mumbai, Delhi, Dehradun, South of Himalayas and Andaman.[4,5] The plants are 2-3 metres in height and armed with 3-6 mm long recurved thorns. *C. zeylanica* is a scandent shrub with young parts clothed with reddish-brown tomentum and hairy, which are soon falling off. Stem is woody, rough, young parts green, rusty tomentose with pungent smell. Leaves are elliptic-oblong, 3-10 cm long on 10-15 mm long petiole, rounded base and apex mucronate. Flowers are supra axillary, usually 2, sometimes 1 or 3, 2-4 cm across on up to 4 cm long pedicel. Sepals are subequal and tomentose. Petals are up to 18 mm long, white or slightly yellowish and hairy outside. Stamens are many, twice as long as petals and purple tinged in upper part. Fruits are globose, 3-4 cm in diameter and embedded in white pulp.[6,7,8] The vernacular names of the plant are Sanskrit – *karambha*, *tapasapriya*, *vyaghranakhi*; Hindi – *ardanda*, *jhiris*; Bengali – *kalokera*; Gujarati – *govindakal*, *kakhbilado*, *karrallura*; Kannada – *mullukattari*; Konkani – *vaghamti*; Rajasthani - *gitoranj* , Tamil – *adondai*, *karrotti*; Malayalam – *karthotti*; Marathi – *govindi*, *kaduvaghanti*, *vaghanti*; Panjabi – *garna*, *karwila*, *karwilun*; Telugu – *arudonda* and English – Indian caper.[3]

Review on Traditional uses

Traditionally *C. zeylanica* was first reported as vegetable.[5] Leaf extract of *C. zeylanica* with black pepper powder is taken twice daily for treatment of dysentery.[9] Leaf juice is taken orally with fresh goat milk for curing cough and cold.[10] For the treatment of diabetes ripe fruits are consumed twice for fortnight and during ingestion, stem bark extract is administered thrice daily.[11] *C. zeylanica* plant is also served as an appetizer prepared as a dipping paste with pepper, tamarind and garlic.[12] Fresh roots, onions and jaggery grinded together in the form of bolus is recommended twice daily for cure of convulsive seizures.[13] The root bark of *C. zeylanica* is used for cooling, cholagogue and as a bitter and is used traditionally as stomachic, sedative, antihydrotic and also in cholera, neuralgia, hemiplegia and rheumatism. The seeds and fruits are used in urinary purulent discharges and

dysentery.[14, 15] In Northern India, the leaves are used as a rubefacient[10], counterirritant and as a cataplasm in boils, swellings and piles.[8] *C. zeylanica* fruit has been considered as an anti dot for snake bite.[16-18] Traditionally it is used to cure swelling of testicle, small pox, boils, cholera, colic, hemiplegia, neuralgia, sores, pneumonic & Pleurisy.[16, 19-21] *C. zeylanica* root bark was used in small pox and swelling of testicles.[22] Root bark is ground with water, boiled and taken orally to treat indigestion.[23] *C. zeylanica* stem and leaf are used as spasmolytic. It is suggested that the root bark preparation can be used for sedative purpose.[2] The leaf juice has been used by the folklore in the treatment of diarrhea.

Review on pharmacological activity

The ethanol and methanol extracts of root of *C. zeylanica* extracts showed strong *in vitro* antioxidant activities by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and by reducing power assay methods. Antioxidant properties of methanolic extracts of raw floral buds have been shown in various *in vitro* models and the potential use in oxidative stress-based pathological conditions has been suggested.[24] *C. zeylanica* root powder extract showed antioxidant activities.[25]

The leaves of *C. zeylanica* were found to exhibit immune stimulant activity. Oral administration of ethanolic and water extracts at doses of 150 and 300 mg/kg in mice, dose dependently potentiated the delayed type hypersensitivity reaction induced by sheep red blood cells. The extracts also prevented myelosuppression in mice treated with cyclophosphamide drug.[26] The crude extract of plant was reported to have CNS depressant activity. The steam volatile fraction of flowers and seeds were highly antimicrobial. The 50% alcoholic extract of aerial parts reported as spasmolytic.[27]

The ethanol and water extracts of *C. zeylanica* leaves showed dose dependent increases in pain threshold in tail-immersion test. Moreover, both the extracts exhibited a dose-dependent inhibition of writhing and also showed a significant inhibition of both phases of the formalin pain test. The water extract significantly reversed yeast-induced fever[28] in rodents. The aqueous extract from total aerial parts of the plant has been used for its antifungal, antiinflammatory, antidiabetic, and antihyperlipidemic activities and is among the constituents of polyherbal formulations to treat liver ailments.[29-33]

Chloroform, ethanol and water extracts of *C. zeylanica* root exhibited *in vitro* antibacterial activity against Gram positive and Gram negative bacteria, whereas petroleum ether extract

exhibited antibacterial activity against selected bacterial strains.[34] The cytotoxic activities of crude extract and fatty acid are also explored.[35]

Pharmacological study revealed Antirheumatic, antiinflammatory & *in vitro* antibacterial activities.[36, 37] *C. zeylanica* constituents flavonoids have been known to possess antioxidant, antineoplastic, antiulcer, antiinflammatory and antimicrobial activities. Ethanolic extract of *C. zeylanica* root showed promising antiaggressive activity qualitatively comparable to that of diazepam.[38] The Methanolic extract of *C. zeylanica* plant has significant antipyretic activity.[39]

Review on Phytochemistry

C. zeylanica was found to have variety of chemical constituents. Whole plant showed the presence of saponin, p-hydroxybenzoic acid, syringic acid, vanillic acid, ferulic acid and p-coumanic acid. Leaves & seeds showed presence of β-carotene, thioglycoside, glycocapparin, n-triacontane, α-amyrin & fixed oil where as root bark showed presence of an alkaloid, a phytosterol, a water soluble acid and a mucilaginous substance.[36, 37]

The roots are reported to contain alkaloid, phytosterol, acids and mucilage. A new fatty acid E-octadec-7-en-5-yonic acid has been isolated from the chloroform extract of roots. Fatty acids like ricinolenic acid, malvalic acid, sterculic acid, linoleic acid etc. has also been identified.[38] The elemental analysis was performed by EDX and found that it contains Al, Si, Cl, K, Ca, Fe, Cu and Zn.[39] In the present paper, a detailed pharmacognostic study on *C. zeylanica* (leaves) based on its physicochemical and preliminary phytochemical studies were carried out to lay down the standards.

MATERIALS AND METHODS

Plant Material

The present paper deals with a detailed pharmacognostic study on the leaves of *C. zeylanica* (Fig.1). The fresh leaves of *C. zeylanica* was collected, dried and supplied by Siddha Medicinal Plants Garden, Mettur Dam. The plant material was cut, crushed and kept in airtight containers and used for all experimental purposes.

Physico-chemical parameters

The physico-chemical parameters like determination of moisture content, ash content, acid insoluble ash, water soluble ash, volatile oil, solubility in water and alcohol, pH of water extract, fibre content and test for Arsenic and Heavy metals were carried out by standard methods.[40, 41]

Fluorescence characters

The fluorescence characters of the drug in different solvents were detected in visible, short UV and long UV light.[42]

Preliminary phytochemical study

In order to examine the presence of different natural products in the plant, characteristic phytochemical tests for sugar, starch, poly phenols, saponin, mucilage, steroid, alkaloid and flavonoid were performed using different extractives of the plant material.[43, 44] For this the plant material was extracted using pet.ether, chloroform, ethyl acetate and methanol and these extracts were subjected to phytochemical tests.

Sample preparation for TLC and HPTLC

Extract of the plant was prepared by boiling 1g each of the drug in 10 ml methanol. The filtrate obtained was concentrated on a water bath to 1 ml. This methanol extract was used for TLC and HPTLC studies.[45]

Development of thin layer chromatographic (TLC) profile

TLC profile of the methyl alcohol extract of the plant material was performed on silica gel G 60 F_{254} pre-coated aluminium sheet and the plate was developed in toluene: ethyl acetate (1:1). Then the plate was air dried and visualized under UV light (short) & UV light (long) and then dipped in vanillin-sulphuric acid reagent and the plate was then heated at 105ºC till

the colour of the spots appeared. R_f values of the spots obtained in UV 254, UV 366 and using vanillin-sulphuric acid as derivatising reagent were recorded.

Development of High Performance Thin Layer Chromatographic (HPTLC) profile

Methanol extract of the plant was spotted in the form of bands with Camag microlitre syringe on a precoated silica gel plate with Camag Linomat V applicator. Mobile phase used was toluene: ethyl acetate: Formic acid (8:2:2drops). Linear ascending was done in twin trough glass chamber saturated with mobile phase.[46] The plate was dried and visualized in UV 254 & 366 nm. The plate was dipped in anisaldehyde- sulphuric acid and heated at 105°C till the colour of the spots appeared.

RESULTS AND DISCUSSION

The analytical data of physico-chemical parameters of the leaves of *C. zeylanica* obtained are given in Table 1. Total ash value of the material indicated the amount of minerals and earthy material attached to the plant material. Acid insoluble ash usually represents the amount of silica present as sand and dust. Loss on drying at 105^0C (14.88%) was showing the presence of moisture content and volatile oil (if any) present in the drug. The water soluble extractive value indicates the presence of polar constituents such as tannin, sugar, plant acid, mucilage and glycosides. Alcohol soluble extractive is an approximate measure for their chemical constituents. The pH value (6.05) indicated that the water extract of the drug was slightly acidic. These values are specific for each drug. All the parameters obtained together provide dependable standards for the drug.

Table 1: Physico-chemical parameters of *C. zeylanica*

Sl.No.	Tests	Results
1	Moisture content (%)	10.50
2	Total ash (%)	11.66
3	Acid insoluble ash (%)	1.08
4	Water soluble ash (%)	4.08
5	Water soluble extractives (%)	25.76
6	Alcohol soluble extractives (%)	10.05
7	pH	6.57
8	Volatile oil (%)	Nil
9	Fibre content (%)	18.50

The arsenic and heavy metals - lead, cadmium and mercury - were found to be within the permissible limit which proves the safety of the plant material.

The fluorescent characters of the plant material in different solvents were detected in visible, UV short light and UV long light and the results are given in Table 2.

Table 2: Fluorescence behaviour of different extracts of *C. zeylanica*

Sl.No.	Extractives	Visible light	Short UV	Long UV
1	Petroleum ether	Light yellow	Light green	Green
2	Benzene	Light brown	Greenish brown	Purple
3	Acetone	Light brown	Light green	Light brown
4	Ethyl acetate	Brownish yellow	Light green	Light brown
5	Ethyl alcohol	Brownish yellow	Greenish brown	Reddish brown
6	Methyl alcohol	Brownish yellow	Greenish brown	Reddish brown
7	Distilled water	Light green	Green	Greenish brown

Preliminary phytochemical screening of the methanolic extract of *C. zeylanica* revealed the presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpenoids, phenols and absence of fixed oils and steroids. Earlier phytochemical reports show the presence of fatty acids, flavonoids, alkaloids, saponins, glycosides, terpenoids, tannins, proteins and carbohydrates in the leaves.[22] These results are in agreement with our observations. The presence of these biochemical compounds might be making *C. zeylanica* medicinally important and nutritionally valuable.

Table 3: Preliminary phytochemical tests

Sl. No.	Natural products	Test performed	Inference
1	Carbohydrates	Molischtest	+ve
2	Starch	Iodine test	+ve
3	Poly phenols	Neutral $FeCl_3$ test	+ve
4	Saponins	Foaming in water	+ve
5	Proteins	Millon's test	+ve
6	Steroids	Liebermann's test	+ve
7	Alkaloids	Mayer's reagent test	+ve
8	Flavonoids	Shinoda test	+ve
9	Oils and Fats	Filter paper test	+ve
10	Tannins	Lead acetate test	+ve
11	mucilage	Ethyl alcohol test	+ve

Physico chemical parameters only are not enough in establishing the standards of herbal drugs. Chromatographic methods give more information regarding the bioactive molecules. Thin layer chromatographic studies of the methanolic extract of *C. zeylanica* was carried out using toluene: ethyl acetate (1:1) as mobile phase. The R_f values and colour of the spots in UV short, UV long and using vanillin- sulphuric acid as derivatising reagent are observed and the results are given in Table 4.

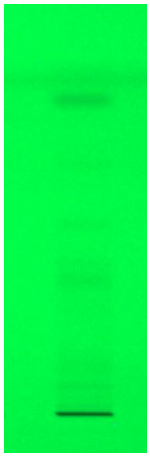
Table(4)-: R_f values and colour of the spots of methyl alcohol extract of *C. zeylanica* Linn., Leaf (TLC)

Sl.No.	UV 254 nm		UV 366 nm		After dipping in vanillin-sulphuric acid	
	R_f values	Colour	R_f values	Colour	R_f values	Colour
1	0.08	brown	0.05	Reddish brown	0.04	Green
2	0.14	Light purple	0.79	brown	0.09	’’
3	0.22	’’	0.95	Reddish brown	0.14	’’
4	0.32	’’	---	---	0.76	’’
5	0.53	purple	---	---	0.94	Dark green
6	0.62	Light brown	---	---	---	---
7	0.79	’’	---	---	---	---
8	0.96	brown	---	---	---	---

Solvent system: toluene: ethyl acetate (1:1); Adsorbent: Silica gel 60 F_{254} Aluminium sheets

HPTLC of *Capparis zeylanica* (Leaf)

HPTLC method is suitable for rapid decisive authentication of plant materials. The chemical fingerprint method developed in HPTLC study is able to ensure the quality of raw materials used for medicinal purposes and is widely accepted for the quality assessment of herbal drugs. In the present study, HPTLC fingerprint has been reported for the first time for *C. zeylanica* and the results provide referential information for standardisation. HPTLC photodocumentation of *C. zeylanica* is given in Fig.2 and R_f values of the spots obtained in Table 5.



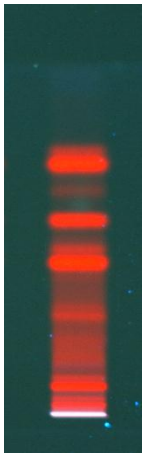


Fig 2: Under UV 254nm **Under UV 366nm** **After derivatisation**

Table(5): R_f values of the spots of methyl alcohol extract of *Capparis zeylanica* Linn., Leaf (HPTLC)

Visualization/ Detection	*Capparis zeylanica* R_f values
Under UV 254 nm	0.09, 0.15, 0.38, 0.43, 0.53, 70, 0.88
Under UV 366 nm	0.03, 0.08, 0.18, 0.28, 0.43, 0.46, 0.55, 0.63, 0.70, 0.74
After derivatisation	0.07, 0.16, 0.40, 0.44, 0.50, 0.55, 0.66, 0.70, 0.75, 0.89, 0.96

CONCLUSION

The present study on physicochemical parameters and preliminary phytochemical analysis provides important information which may be useful in authentication and adulteration for quality control of raw material. The present study adds to the existing knowledge of *Capparis zeylanica*. This paper gives evidence based information regarding the traditional uses, and physicochemical parameters, phytochemical investigations and pharmacological activities which help the researchers to investigate more about this important plant material.

C. zeylanica has been ethnomedicinally used as a therapeutic agent for a variety of diseases, as we have illustrated in this article. Moreover, numerous research works have proven its uses beyond the ethnomedicinal ones in experimental animals. Various compounds which were isolated from this plant may be responsible for its pharmacological activities. The road ahead is to establish specific bioactive molecules, which might be responsible for these actions. Nowadays the pharmaceutical industry is focussed towards the design and development of new plant based drugs through investigations from traditional system of medicine since they are well tested for their efficacy and generally beleived to be safe. *C. zeylanica* is found to be a popular remedy among the various traditional systems for curing a number of ailments. Being such an important medicinal plant it require more exploration in all aspects thoroughly for more therapeutically potential.

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