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PHARMACOGNOSTIC INVESTIGATION AND EVALUATION OF ANTIMALARIAL ACTIVITY ON THE LEAVES OF *CAESALPINIA CRISTA* (L.)ROXB., AN ANTIMALARIAL MEDICINAL PLANT OF ASSAM

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ABSTRACT

Objective

To investigate pharmacognostic parameters for establishing qualitative and quantitative standards on *Caesalpinia crista* leaves and to validate its traditional claim for treating malarial fever.

Methods

Pharmacognostic standardization parameters were established following WHO guidelines and other Pharmacopoeial monograph. The antimalarial efficacy of alcoholic extract of *C. crista* leaves was determined against RKL-2 *Plasmodium falciparum* strain by *in-vitro* method. The results were compared with chloroquine standard.

Result

C. crista leaves are bipinnately compound, bitter in taste, have anomocytic stomata and contains flavonoid and saponins. Total ash content (9.15 % ± 0.42), alcohol soluble extractive (14.4% ± 1.12) and water soluble extractive (23.6% ± 2.08) values were recorded. HPTLC fingerprint profile revealed presence of four major spots (R_f values 0.71, 0.77, 0.84 and 0.88) with chloroform: methanol: formic acid (8.8:0.5:0.2) solvent system. The alcoholic extract of *C. crista* leaves killed 18% asexual malaria parasites *in vitro* at 50 µg/ml doses.

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Conclusion

The established pharmacognostic parameters may be utilized for checking proper identity and purity of the plant materials. Alcoholic extract of *C. crista* leaves showed some degree of antimalarial activity.

KEY WORDS: *Caesalpinia crista*, Pharmacognosy, flavonoids, sterols, HPTLC fingerprint, antimalarial.

INTRODUCTION

Caesalpinia crista Linn.(Family: Fabaceae), commonly known as "Gray nicker bean" or "Fever nut" plant and locally known as 'Lataguti' in Assam is a famous medicinal plant for treating malarial fever (Kanilal *et al.* 1938; Chaudhri 1996; Anonymous 2000). The plant is an extensive woody climber or scrambling prickly shrub armed with fine but strong prickles. It is found in wild throughout the plains of India, up to an altitude of 1000m in the Himalayas. The plant is closely resembled with *C. bonduc* or *C. bonducella* and often confused and synonymously known by the same name in various regions (Kanilal *et al.* 1938; Chaudhri 1996; Anonymous 2000; Chopra *et al.* 1956). The seeds are an important ingredient of 'Ayush-64', an Ayurvedic compound preparation used as antimalarial drug (Chaudhri 2004). The finely powdered leaves are used as uterine tonic after child birth. The seeds are considered as tonic in intermittent fevers, antiperiodic, febrifuge, anthelmentic, antipyretic and specific in the treatment of hydrocele (Kirtikar and Basu 1976; Nadkarni 2007). Traditionally, the young leaves are used in the treatment of malarial fever in Assam (Gam Konwar 2005). The leaves and the fruits are reported to contain tannins and flavanoids. The seed kernels contain a number of diterpenoid type of compounds such as α, β, γ and δ-caesalpin. ζ-caesalpin and bonducellin have also been isolated from seed kernels (Rastogi and Mehrotra 1990; 1993). The dichloromethane extract of the seed kernels exhibited promising antimalarial activity against *Plasmodium burghei* infected mice *in-vivo*. The isolated diterpenoid (furanocassane type) also showed dose dependent inhibitory effects on *P. falciperum* FCR-3/A2 growth *in-vitro* (Awale et al. 2006; Linn et al. 2005).

Considering the traditional importance of the plant *C. crista*, especially in the treatment of malarial fever, a detail pharmacognostic investigation on leaf part of the plant has been carried out and presented in this communication. The study includes macro and microscopic features, physicochemical parameters, leaf constants, and HPTLC fingerprint profiles. These characteristics could be useful in proper identification of the plant.

MATERIALS AND METHODS

Chemicals & Reagents

All chemicals and reagents were obtained either from Qualigens fine Chemicals or E-Merck, India and were of analytical grade. The RPMI-1640 medium was obtained from Sigma Aldrich.

Collection of Plant materials and authentication

The leaves of *C. crista* were collected from the Sivasagar district of Assam in the month of March and identified by Dr. L. R. Bhuyan, Systematic Botanist, State Forest Research Institute (SFRI), Itanagar. A voucher specimen (No. DU/PS/HRB-03/2005) is preserved in the departmental herbarium of the Department of Pharmaceutical Sciences, Dibrugarh University. The plant material was shade dried, coarsely powdered and packed in a closed vessel for further use.

Extraction of plant materials

For the purpose of qualitative phytochemical tests, successive extractives in petroleum ether, chloroform, methanol and water were obtained by continuous hot extraction using Soxhlet apparatus. Alcohol extracts obtained by cold maceration method was used for evaluation of antimalarial activity.

Pharmacognostic and Phytochemical Studies

The macroscopical and microscopical description of matured fresh leaves was done according to terms outlined by Wallis (1995). Various leaf constants such as stomatal number, stomatal index, vein-islet number, vein-termination number were determined following standard literature (Wallis 2005; Johnson 1940; Anonymous 1996; Mukharjee 2002; Khandelwal 2004). Phytochemical studies such as qualitative phytochemical tests, determination of ash and extractive values were studied following standard procedures. All the successive extractives were subject to qualitative phytochemical screening for the presence of various phytoconstituents.The HPTLC fingerprint of successive extractives of powdered leaves with different organic solvents (petroleum ether, chloroform, acetone and methanol) was established (Kokate 1994; Wagner 1996). The samples were applied on a silica gel GF254 precoated plates (Merck) with a Cameg Linomet HPTLC applicator. The plates were developed in two different solvent systems, viz. Solvent1 (pet. ether: chloroform: formic acid; 7.5:2.5:0.1) and Solvent2 (Chloroform: methanol: formic acid; 8.8:0.5:0.2) and visualized under UV365nm and day light after spraying with vanillin-sulphuric acid reagent, heating at 105^{0}C for 5 min.

All the photographs were taken with Cannon Power Shoot S-80 digital camera and microscopic observations were made under Leica DM 1000 trinocular research microscope.

Antimalarial activity evaluation

Antimalarial activity of the alcoholic extract of *C. crista* was determined by the previously described procedure (Trager and Jensen 1976). The cold macerated alcoholic extract was concentrated at low temperature (40^0 C) on a Rotary Vacuum evaporator and kept at refrigerator (4^0 C) until further use. The malarial parasite *Plasmodium falciparum* RKL-2 strain was cultured in RPMI-1640 medium supplemented with 25mM HEPES, 10% dextrose, 0.23% sodium bicarbonate and 10% human serum. The parasite was subcultured to bring 0.8–1.5% parasitemia and synchronized with 5% d-sorbitol. The test drug was dissolved in DMSO and further diluted with culture medium to get different concentrations and added to 96 well micro-titer plates which already contain the cultured parasite in RPMI medium. The plates were incubated for 72 hours at 37^0 C under 5% CO_2. The results were expressed in terms of percentage death rings and schizonts counted against per 100 asexual parasites.

RESULT

Macroscopic features: The leaves are 12-18 inch long, bipinnately compound with large stipples, and bear numerous recurved prickles through out the rachis and its branches. The leaf comprises of 6-10 pairs of leaflets on each pinna that are oppositely arranged. The leaflets are 30-60 cm long, oblong or elliptic in shape, subacute or obtuse apex, entire margin and minute petioles with a pair of short hooked prickles (Fig.-1). The dried leaflets are greenish yellow in color, odorless but bitter in taste.

Microscopic features: The transverse section of the leaf of *C. crista* showed a single layer of epidermal cells followed by a single layer of elongated palisade cells just below the upper epidermis in the lamina region (Fig.-2). Both upper and lower epidermal cells are rounded or polygonal in nature and are thickly covered with warty cuticle. The palisade layer is densely filled with chlorophyll. Few unicellular covering trichomes are also present in the upper epidermal layer. The midrib presents a flat ventral surface and convex dorsal surface. The epidermal layers are continuous over the midrib. The cells of the lower epidermis however are small with thick cuticle. Collateral vascular bundles are prominent occupying the central portion of the midrib covered by a band of thick walled collenchymatous pericycle. Few sphaeraphides and vascular strands are seen in the spongy

parenchyma. The leaf constant parameters such as stomatal number, stomatal index, and vein-islet and vein termination number were determined and the values are tabulated in Table-1. The stomata were identified as ranunculaceous or anomosytic type (Fig.-3).

Phytochemical Studies: The percentage of total ash, water soluble and acid insoluble ash, alcohol soluble and water soluble extractive, successive soxhlet extractive (in petroleum ether, chloroform, methanol and water) were determined. All the determinations were in triplicate and their mean values ± S.D. was calculated (Table-2). All the successive extractives were subjected to qualitative phytochemical screening for the presence of alkaloids, glycosides, free sugars, steroids, tannins, flavonoids, and saponins. It was observed that mainly flavonoids and free sugars were detected in alcoholic and water extract while the presence of sterols was detected in petroleum ether, chloroform, and alcoholic extract (Table-3). The TLC Fingerprint profiles of different extractives were documented by recording R_f values and color of the bands under day light and UV365 nm (Table-4, Fig.-4).

Antimalarial activity: The alcoholic extract of *C. crista* leaves showed concentration dependent antimalarial activity against *Plasmodium falciparum* RKL-2 strain (Table-5).

DISCUSSION

In spite of common use of *C crista* seeds and leaves in traditional medicine in Assam, no pharmacological and pharmacognostical investigations have been carried out on this plant. The results of macro-microscopic studies, phytochemical tests, HPTLC fingerprint profile and leaf constant parameters are reported first time in this communication. Dorsiventral type, anomocytic stomata, co-lateral vascular bundles, presence of sphaeraphides (crystals) in the mesophyll region are some of the characteristic microscopic features of *C. crista* leaves. Further, presence of flavonoids, sterols and terpenoids were confirmed by various qualitative tests. Contrary to the expectations the extent of in vitro antimalarial activity recorded by the ethanolic extract of the leaves of *C. crista* was very marginal (18 %). This is surprising in view of the successful use of this plant for the traditional treatment of malarial fever. It is likely that this plant may possess antipyretic activity which justifies its use in fever. However, this assumption needs scientific validation. Moreover, since the seed kernel extracts as well as isolated compounds of Indonesian

species of *C crista* have recorded promising antimalarial activity (Linn et al. 2005), there is a need to make a detailed phytochemical investigation on various parts of this plant and subsequent evaluation of antimalarial efficacy on islolated compounds.

CONCLUSION

The plant *Caesalpinia crista* is an important medicinal plant which is used in many parts of the world for treating various disorders including malarial fever. The crude extracts as well as isolated compounds are reported to have promising antimalarial activity on Indonesian species. The preliminary study on leaf extract showed some degree of antimalarial activity. The pharmacognostic study may be useful for proper identification of plant specimen and will serve as reference guide for future studies.

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REFERENCES

Anonymous. *The Wealth of India*- a dictionary of Indian raw materials, vol-1: A-Ci, NISCOM, CSIR (1st supplementary series), 2000, pp. 6-11.

Anonymous, *The India Pharmacopoiea*, Govt. of India, Ministry of Health and Family Welfare, vol.-II, 1996, pp.

Awale S, Linn TZ, Tezuka Y, Kalauni SK, Banskota AH, Attamimi F, Ueda JY, Kadota S. Constituents of *Caesalpinia crista* from Indonesia. *Chem Pharm Bull* (Tokyo), 2006, 54(2) : 213-218.

Chaudhri RD. *Herbal Drug Industry- A Practical Approach to Industrial Pharmacognosy*, Eastern Publishers, New Delhi, 1st ed., 1996 (3rd reprint 2004) pp. 86, 142-143.

Chopra RN., Nayar SL. and Chopra IC. *Glosary of Indian Medicinal Plants*, PID, New Delhi, India, 1956, pp. 43.

Iyengar MA and. Nayak SGK. *Anatomy of Crude Drugs*, Published by Dr. M.A. Iyengar, Kasturba Medical Collage, Manipal, India, 3rd ed.,1984, pp. 42-54.

Johanson DA. *Plant Microtechnique*, Mc. Graw Hill Book Co. Inc., New York and London, 1st edn., 1940, pp. 45

Kanjilal UN., Kanjilal PC. and Das A. *Flora of Assam*, Avon Book Company, Delhi, India, 1938, reprinted 1982, vol-II, pp. 120-121.

Khandelwal KR. *Practical Pharmacognosy- techniques and experiments*, Nirali Prakashan, Pune, India, 12th edn., 2004, pp. 146-160.

Kirtikar KR., and Basu BD. *Indian Medicinal Plants*, vol.-II, International Book Distributors, Dehradun, India, 2nd ed., 1976 (reprinted 1981), pp. 842-844.

Kokate CK. *Practical Pharmacognosy*, Vallabh Prakashan, Delhi, India, 4th edn., 1994 (reprinted 2001), pp. 112 -135.

Linn TZ, Awale S, Tezuka Y, Banskota AH, Kalauni SK, Attamimi F, Ueda, JY, Asih PB, Syafruddin D, Tanaka K, and Kadota S. Cassane and norcassane-type diterpines from *Caesalpinia crista* of Indonesia and their antimalarial activity against the growth of *Plasmodium falciperum*. *J. Nat. Prod.*, 2005, 68(5): 706-10.

Mukharjee PK. *Quality Control of Herbal Drugs- An approach to evaluation of Botanicals*, Business Horizone Pharmaceutical Publishers, New Delhi, 2002, pp. 183-189.

Nadkarni KM. *The Indian Meteria Medica*, Popular Prakashan, Bombay, India, vol.-I, 2007, pp. 226-229.

Personal communication (2005) with Shri Gam Konwar, Ranugaon, Simuluguri, Sivasagar, Assam.

Rastogi RP. and Mehrotra BN. *Compendium of Indian Medicinal Plants*, vol.-1, NISCOM & CDRI, New Delhi, 1990, pp. 67.

Rastogi RP. and Mehrotra BN. *Compendium of Indian Medicinal Plants*, vol.-3, NISCOM & CDRI, New Delhi, 1993, pp. 113.

Trager W. Jensen J B. (1976) Human Malaria parasite in continuous culture, Science, 193: 673-675.

Wagner H. and Bladt S. *Plant Drug Analysis- A Thin Layer Chromatography Atlas*, Springer, 2nd edn., 1996, pp. 349-352.

Wallis TE. *Text Book of Pharmacognosy,* CBS Publishers and Distributors, New Delhi, 5th edn., (1st Indian edn.),1985 (reprint 2005), pp.

Table-1: Leaf constant values of *C. crista*

Parameters	Values (in range)/mm^2
Stomatal Number (lower epidermis)	120 – 128
Stomatal Index (lower epidermis)	10.5 – 11.25
Vein-Islet Number	12.5 – 15
Veinlet termination	4.25 – 5.5

Table-2: Quantitative standards of *C. crista*

Parameters		Mean values (in %) ± S.D.
Ash Value	Total ash	9.15 ± 0.427
	Water soluble ash	4.73 ± 0.076
	Acid insoluble ash	1.11± 0.104
Extractive Value	Alcohol soluble	14.41± 1.127
	Water soluble	23.58± 2.081
Successive soxhlet extractive		
	Petroleum Ether extractive	4.56± 0.061
	Chloroform extractive	6.63± 0.550
	Methanol extractive	11.83± 0.202
	Water extractive	3.76± 0.251

Table-3: Phytochemical screening of *C. crista* extracts

Chemical Groups	Observations			
	Pet. Ether	Chloroform	Methanol	Water
Carbohydrate (free sugar)	-	-	+	+
Glycoside	-	-	-	-
Flavanoids	-	-	+	+
Sterols	+	+	+	-
Alkaloids	-	-	-	-
Saponins	-	-	-	-
Terpenoids	+	+	+	-

'+' indicates positive and '–' indicates negative result in the test.

Table-4: TLC fingerprint profile of *C. crista* leaf extracts

Solvent system	Extract	No. of bands (Under day light)*	R_f value
1. Chloroform: Methanol:Formic Acid (8.8:0.5:0.2)	Petroleum Ether	4	0.71, 0.77, 0.84 & 0.88
	Chloroform	4	- do -
	Acetone	4	- do -
	Methanol	No bands	- - -
2. Pet.Ether: Chloroform: Formic acid (7.5:2.5:0.1)	Petroleum Ether	5	0.17, 0.42, 0.55, 0.69 & 0.78
	Chloroform	5	- do -
	Acetone	5	- do -
	Methanol	No bands	- - -

* After spraying with vanillin sulphuric acid reagent

Table-5: Antimalarial activity of alcoholic extract of *C. crista* leaves.

Sample Code	Concentration of Test drug (µg/ml)	Activity (% dead rings + schizonts)
CC-1	50	18.0
CC-2	25	3.2.

Fig.1: Leaves of *C. crista*

Fig.2: T.S. of *C. crista* leaf (through midrib; 10x 20x)
Ue- upper epidermis, Le- Lower epidermis,
Xy- Xylem, Phl- Phloem,
Pc- Palisade cells, Tr- Covering trichome

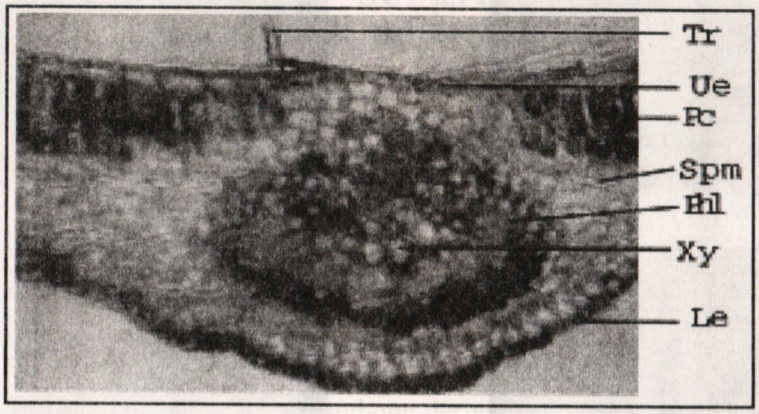
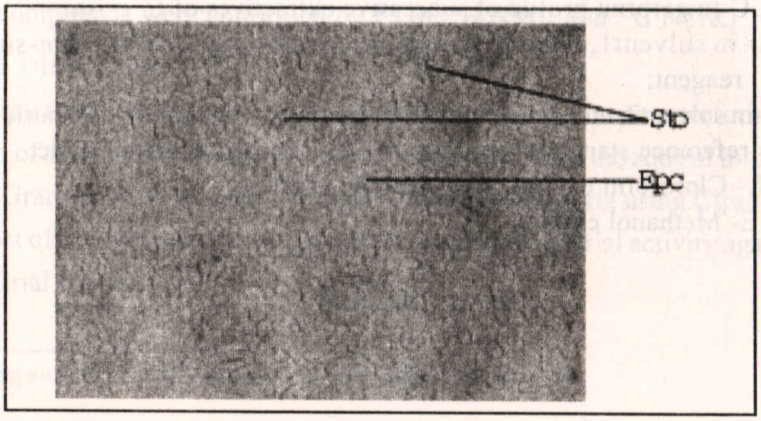


Fig.3: Photograph showing stomata and epidermal cells of *C. crista* leaf (Lower epidermis, 10x20x)
Sto- Stomata, Epc- Epidermal cells



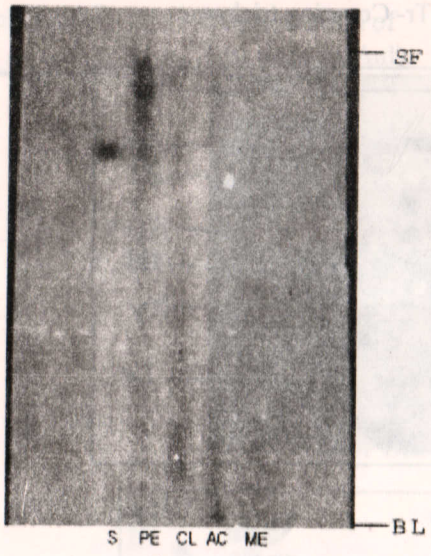


Fig.4(A)

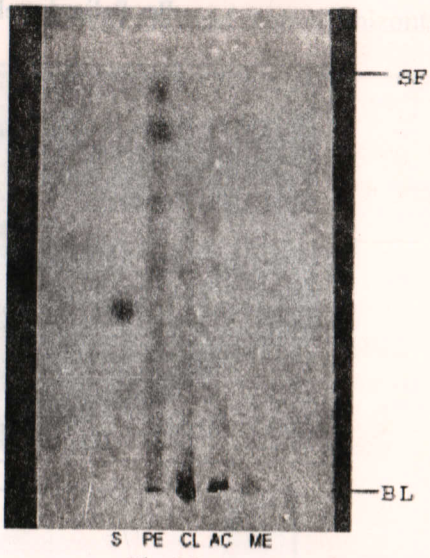


Fig.4(B)

Fig.4: TLC fingerprint profile of successive extractives of *C. crista*
[A- in solvent1, under day light after spraying with Vanillin-sulphuric acid reagent;
B- in solvent2, under day light after spraying with Vanillin-sulphuric acid reagent]
S- reference standard (Diosgenin), PE- Petroleum ether extract
CL- Cloroform extract, AC- Acetone extract
ME- Methanol extract

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