Pharmacognostical, phytochemical screening and acute toxicity study of *Crateva nurvala* stem bark

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ABSTRACT

The aim of this research was to study the pharmacognostic, phytochemical parameters & acute toxicity of *Crateva nurvala* stem bark (family: Capparidaceae). Its stem bark was extracted with non-polar and polar solvents by simple maceration method.TLC were also carried out to identify the chief chemical constituent. The acute toxicity study of these extracts was also carried out in female albino rats (50mg to 5000 mg/kg body weight) as per OECD guidelines. Macroscopic study showed that it was yellowish brown powder with slight characteristic smell and bitter taste. Powder microscopy showed the presence of collenchyma, starch grains, pericyclic fibres, cork cells, stone cells, calcium oxalate crystals and phloem fibres. Phytochemical screening reported the presence of triterpenoids, Flavonoids, tannins & steroidal compounds. In the acute toxicity study, oral administration of 5g/kg of *Crateva nurvala* stem bark various extracts produced neither mortality nor changes in behaviour or any other physiological activities.

Keywords: *Crateva nurvala*, phytochemical, microscopy, acute toxicity.

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INTRODUCTION

Since ancient times, plants and herbal preparations have been used as medicine. During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs (1).

Crateva nurvala (Family: Capparidaceae) is a small tree with a much branched head. Leaves are deciduous 3 foliolate; petioles 3.8–7.6 cm long; leaflets 5–15 ovate, lanceolate or obovate, acute or acuminate, attenuate at the base, entire, glabrous on both surfaces, pale beneath and reticulately veined. It is usually cultivated in the vicinity of temples in Central India, Bengal & Assam. Its bark is hot, bitter at first and then sweet sharp taste, easy to digest, stomachic, laxative, antilithic, anthelmintic, expectorant and antipyretic. Researches shown that it bark contains saponins and especially useful in urinary complaints such as kidney and bladder stones (2–3).

MATERIALS

Plant material

Crateva nurvala stem bark was collected from local forest area of sirsi in Western Ghats, Karnataka and authenticated by Prof. G. S. Naik (Botanist) of Department of Botany, G. C. Science and Art College, Ankola. A voucher herbarium specimen number GCSAC/CN/01 was also preserved in the same college. The collected material was chopped into small pieces and powdered to coarse consistency in cutter and grinder mill respectively. The powder passed through .40 # mesh particle size and stored in an airtight container at room temperature.

Animals

Healthy young female albino rats (Sprague-Dawley Strain) of weighing 150 to 250 g were selected for experiment & were obtained from animal house of KLES College of Pharmacy, Belgaum and ethical clearance was granted by institutional ethical committee in resolution no. 1/18/2007 held on 23rd November 2007 at J N Medical college, Belgaum (Ethical committee IAEC reg. no. : 627/02/a/CPCSEA). The animals were fed on a standard pellet diet (Goldmohar rat feed, Mumbai) & water ad libitum. All the protocols were performed in accordance with institutional animal ethical committee as per the direction of the CPCSEA (Committee for the purpose of control and supervision of experiments on animals). Alloxan monohydrate was obtained from S. D. Fine chemicals limited, Mumbai. The other chemical reagents were of analytical grade or better.

METHODS

Standardization of crude & powdered drug(4)

Physical evaluation is the primary step adopted in the identification and standardization of crude drugs. It helps in the determination of adulterants and validates the authenticity of crude drug. Various parameters like organoleptic characters, powder microscopy, soluble extractives, loss on drying, ash values & total foreign organic matter were considered for the measurements.

Extraction(5)

The fresh air-dried, powered crude drug was extracted with petroleum ether (60–80°), benzene, chloroform, 95% ethanol & chloroform water I. P. by following simple maceration procedure at room temperature for seven days in a 2000–5000 ml conical flask with occasional shaking and stirring. The extract was filtered and concentrated to dryness at room temperature to avoid the decomposition of the natural metabolites. The dried extract was stored carefully for phytochemical investigation.

Phytochemical screening(4)

Preliminary phytochemical screening was carried out to study the presence of alkaloids, steroids, triterpenoids, essential oil, flavonoids, tannins, carbohydrates, and amino acids in the crude drug & their various extracts.

Thin Layer Chromatography of Major Chemical Constituents in Extracts(6)

TLC of extracts was carried out by using Silica gel GF 254 (activated) as adsorbent. Its slurry was prepared & poured on the clean, dried glass plate and spreaded on it as a uniform coating with thickness of 0.4 mm using glass rod. Plates were activated in hot air oven & maintained at 110°C for 30 minutes. The spots of 20µl of extracts were applied by using fine capillary tube of diameter less than 1 mm on the activated plates at the distance of 2 cm from one end of the plate. Chromatograms were developed by one way ascending TLC. Number and position of various constituents present in the drug was determined by spraying the plate with different spraying reagent. Sprayed plates were heated at 100°C for 10 minutes. Spots were marked and R_f value was calculated for spots.

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Test solution: Reflux 5 gm of powdered drug with 25 ml of hexane for 1 hour. Filter and evaporate to dryness. Dissolved the residue in 2 ml chloroform.

Solvent system[7]: n-hexane: Ethyl acetate (9:1), Visualization of spots: Sprayed the plate with Liebermann burchard reagent.

Acute oral toxicity studies (8)

The acute oral toxicity studies of extracts were carried out as per the guidelines of Organization for Economic Co-operation Development (OECD) guidelines, draft guidelines 423 adopted on 17th December 2001 received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of social justice and empowerment, Government of India.

Health young albino female rats of weighing between 150 to 250 gms (8 to 12 weeks old) were used for acute toxicity study to determine LD50 of various extracts. Three animals were used in each group and the starting dose lied in the range of 50 mg–5000mg/kg body weight. Totally 06 groups, each with three animals were used for assessing the toxicity study. Five groups received test dose and one group was selected as control group. Animals were observed at regular time intervals at least once during the first 30 minutes of initial dosing during the first 24 hrs. Additional observations like behavioural changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behaviour pattern.

RESULTS

Macroscopic study of stem bark of *Crateva nurvala* revealed that it was 8–14 cm long and 4–7 cm wide with thickness

TABLE 1: PHYSICAL TEST OF CRUDE DRUGS

	Physical Test			
Crude drugs	**Nature**	**Colour**	**Odour**	**Taste**
Crateva nurvala	Coarse powder	Yellowish brown	Characteristic	Bitter

TABLE 2: EXTRACTIVE VALUES

Crude drugs	**Pet-ether% w/w**	**Benzene% w/w**	**Chloroform% w/w**	**Alcohol% w/w**	**Aqueous% w/w**
Crateva nurvala	1.46	3.90	3.62	10.65	20.45

of 6–10 mm. The thinner bark pieces were channelled but the thick bark pieces were flat and slightly curved. The outer surface was ash colored and rough due to presence of lenticels and transverse wrinkles. Inner surface was comparatively smooth and whitish brown to buff colored. Fracture was found to be short and splintery. The smoothened internal surfaces showed small yellowish dots indicating presence of stone cells.

Table 1 shows organoleptic characters of the crude drug. Powder microscopy of the drug showed the presence of collenchyma, starch grains with globular or elliptical in shape, lignified pericyclic fibres, brown coloured cork cells, stone cells with pitted thickening, prismatic calcium oxalate crystals and phloem fibres.

Other physical properties necessary in quality control of drug e.g. extractive values, loss on drying, total ash, acid insoluble ash, water soluble ash values and extract organoleptic features were also determined and results were tabulated (Table 2–5).

The preliminary phytochemical screening revealed the presence of triterpenoid, steroids chiefly in petroleum ether & benzene extracts; tannins, flavonoids, and carbohydrates in alcoholic and aqueous extracts; traces of volatile oil & glycosides (Table 6)

TABLE 3: LOSS ON DRYING

Crude drugs	**Loss on drying (% w/w)**
Crateva nurvala	4.37

TABLE 4: TOTAL ASH, ACID INSOLUBLE ASH AND WATER SOLUBLE ASH VALUES

Crude drugs	**Total ash value% w/w**	**Acid insoluble ash value% w/w**	**Water soluble ash% w/w**
Crateva nurvala	9.76	0.647	1.56

TLC of *Crateva nurvala* major Phytoconstituents of stem bark revealed a bright brownish red spot ($\mathbf{R_f = 0.30}$), which turns magenta on keeping. Other spots were $\mathbf{R_f = 0.71}$(Magenta color) $\mathbf{R_f = 0.15, 0.90}$ (light violet spots) also noted.

In acute toxicity study, all the extracts of *crateva nurvala* did not show significant toxicity signs when observed for

TABLE 5: PHYSICAL TEST OF EXTRACTS

Crude drugs	**Extracts**	**Nature**	**Colour**	**Odour**	**Taste**
Crateva nurvala	Pet-ether	Semisolid	Yellowish brown	Characteristic	Astringent
	Benzene	Solid	Yellowish brown	Characteristic	Astringent
	Chloroform	Semisolid	Reddish Brown	Characteristic	Astringent
	Alcohol	Semisolid	Reddish Brown	Characteristic	Astringent
	Aqueous	Semisolid	Reddish Brown	Characteristic	Astringent

TABLE 6: QUALITATIVE CHEMICAL INVESTIGATION OF CRUDE DRUG EXTRACTS

	TEST	*Crateva nurvala*				
	Extracts	Pet- ether	Benzene	Chloroform	Alcohol	Aqueous
1.	**Test for steroids**					
a)	Salkowski test	+	+	–	+	–
b)	Liebermann-burchard test	+	+	+	–	–
c)	Liebermann reaction	+	+	–	–	–
2.	**Test for steroidal glycosides**	–	–	–	–	–
3.	**Test for triterpenoids**					
a)	Salkowski test	+	+	–	–	–
b)	Liebermann-burchard test	+	+	+	–	–
4.	**Test for glycosides**					
a)	Legal test	–	–	–	–	–
b)	Keller killani test	–	–	–	–	–
c)	Modified Borntrager test	–	–	–	+	+
5.	**Test for saponins**					
a)	Foam test	–	–	–	–	–
b)	Haemolysis test	–	–	–	–	–
6.	**Test for carbohydrates**					
a)	Molisch's test	–	–	–	+	+
b)	Barfoed's test	–	–	–	–	–
c)	Benedicts test	–	–	–	+	+
d)	Fehling solution test	–	–	–	+	+
7.	**Test for alkaloids**					
a)	Mayer's reagent test	–	–	–	–	–
b)	Dragondroff's reagent test	–	–	–	–	–
c)	Hager's reagent test	–	–	–	–	–
d)	Wagner reagent test	–	–	–	–	–
8.	**Test for Flavonoids**					
a)	Shinoda test	–	–	–	+	+
b)	Zinc/Hcl reduction test	–	–	–	+	+
9.	**Test for tannins**					
a)	5% Ferric chloride test	–	–	–	+	+
b)	Lead acetate test	–	–	–	+	+
c)	Potassium dichromate test	–	–	–	+	+
10.	**Test for proteins**					
a)	Biuret test	–	–	–	–	–
b)	Million reagent test	–	–	–	–	–
11.	**Test for amino acids**					
a)	Ninhydrin test	–	–	–	–	–
12	**Test for mucilage**					
a)	Ruthenium red	–	–	–	–	–

TABLE 7: ACUTE ORAL TOXICITY STUDIES OF PLANT EXTRACTS

Sl.No	Extracts	LD50 Cut-Off	Vehicle
1	*Crateva nurvala* - Ethanolic extract	5000 mg/kg b.w	Tween 80
2	*Crateva nurvala* - Aqueous extract	5000 mg/kg b.w	Tween 80
3	*Crateva nurvala* - Chloroform extract	5000 mg/kg b.w	Tween 80
4	*Crateva nurvala* - Pet-ether extract	5000 mg/kg b.w	Tween 80
5	*Crateva nurvala* - Benzene extract	5000 mg/kg b.w	Tween 80

$1/10^{th}$ of this lethal dose will be used as effective dose (Therapeutic Dose) for pharmacological screening.

the parameters during the first four hours and followed by daily observations for 14 days and no mortality was also observed, the drug was found to be safe at the tested dose level of 5000 mg/kg b.wt. (Table 7).

DISCUSSION

The study of nature, colour, odour and taste of powdered & crude drug under investigation constitute an important feature of organoleptic evaluation. The determination of extractive values with range of solvents gives information about extractable non polar and polar as well as total extractable plant constituents. The determinations such as loss on drying and ash values indicate the status of air-dried drugs used for studies. The total ash values when comes in acceptable range it simply shows that no inorganic adulteration is present. Total ash value, acid insoluble ash and water-soluble ash were determined and results were in acceptable limits. The results of qualitative chemical investigations of *Crateva nurvala* bark extracts have revealed the presence of various chemical constituents mainly triterpenoids and flavonoids which may be responsible for its antilithic and antidiabetic property respectively. The results obtained were comparable and satisfied the standard literature.

CONCLUSION

In present study the pharmacognostic, phytochemical parameters & acute toxicity of *Crateva nurvala* stem bark were studied and results were in accordance with its pharmacopoeial standards and standard literature. Phytochemical screening reported the presence of triterpenoids, Flavonoids, tannins and steroidal compounds which support its action in traditional use in urolithiasis, skin disorders, anthelmintic, expectorant and antipyretic activity. Pharmacological screening of these extracts is under process and activity guided fractionation & formulation is the future aspect.

REFERENCES

1. Bruneton, J., *"Pharmacognosy, Phytochemistry, MedicinalPlants"*, 2nd edition, Lavoisier Publication France (1999).
2. Kirtikar, K.R., Basu, B.D., *Indian medicinal plants*, Vol. 1, 2nd Edition, International book publisher, Dehradun, pp.190–92(2005).
3. Nadkarni, K.M., *Indian Materia Medica*, vol.1, Popular Prakashan Pvt. Ltd, Mumbai, pp. 387–88(2005).
4. Kokate C. K., *Practical Pharmacognosy*, 7th edition, Vallabh Prakashan, (1994).
5. *Pharmacopoeia of India*, Ministry of Health, Government of India, N. Delhi, p.948,650(1982).
6. Stahl, E., *Thin Layer Chromatography*. 2nd Edition, Springer International student Edition, New York, (1969).
7. Harborne, J.B., *Phytochemical methods- A guide to modern techniques of plant analysis*, Chapman and Hall Ltd, New Fetter Lang, London, (1984).
8. OECD/OCDE, *OECD Guidelines for the testing of chemicals, revised draft guidelines 423: Acute Oral toxicity- Acute toxic class method, revised document, Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA)*, Ministry of Social Justice and Empowerment, Government of India (2000).