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QUALITY CONTROL STANDARDIZATION OF AQUEOUS AND ETHANOL EXTRACTS OF *CRATEVA ADANSONII* DC. STEM BARK

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ABSTRACT

Standardization is the process of making something conforms to a standard and it is necessary to establish Quality, Safety and Efficacy of a herbal product. *Crateva adansonii* DC (*Varuna*) is an evergreen tree from the Capparidaceae family and the stem bark of this plant is mainly indicated for urinary calculi (*mutra* ashmari). This study was designed to determine some selected standardization parameters of the stem barks of this plant. Organoleptic, physical, chemical and biological analyses were conducted. Under physical parameters, the foreign matter content (2%), total ash (1.88%), acid-insoluble ash (1.32%), water-soluble ash (4.45%) and loss on drying (9.66%) were within the standard limits. Water-soluble extractive value (10.5%) was higher than the alcohol-soluble extractive value (1.3%). Swelling index (7ml) shows low mucilage content and foaming index (<1000) shows low saponins content of the bark. Both aqueous and ethanol extracts of the bark were positive for alkaloids, tannins, flavonoids, steroids, glycosides and proteins. Only the aqueous extract was positive for saponins while only the ethanol extract was positive for terpenoids. Carbohydrates were not detected in both extracts. R_f values for the TLC of ethanol extract (MeOH: Chloroform 2:8, v/v) were 0.42, 0.73 and for the aqueous extract (Butanol: Dichloro methane: Water 4:1:5 v/v) 0.42, 0.67 and 0.83.

Keywords: *Varuna* stem bark, standardization, freeze-dried extract, TLC

INTRODUCTION: The increasing demand for herbal medicines emphasizes the importance of standardization to ensure quality, safety and efficacy. Standardization is the process of making something conforms to a standard [1]. Standardization involves setting quality benchmarks to meet regulatory standards, particularly crucial for Ayurveda formulations, where complex plant-derived ingredients and traditional preparation methods challenge large-scale production. Standardization includes organoleptic, physical, chemical, botanical and biological evaluations [2] to verify the identity, purity, potency and safety of herbal materials. These evaluations assess sensory attributes, moisture content, ash values, bioactive markers and plant authenticity.

Crateva adansonii DC (*Varuna*), an evergreen tree from the Capparidaceae family [3], is indigenous to India. Its stem bark is greyish with white patches, and its pharmacodynamic properties include litholytic and blood purification. This is used in Ayurveda to treat urinary disorders, digestive issues, and gout [4]. Its therapeutic significance underlines the need for quality control. This study standardizes *Crateva adansonii* DC. stem bark (FIGURE 1), ensuring its consistent quality and safety.

FIGURE 1: *Crateva Adansonii* DC Stem Bark

MATERIALS AND METHOD

Collection of the plant material

The stem barks of *Crateva adansonii* DC were collected in the month of July from a jungle in Galle district, Sri Lanka and authenticated at Department of Ayurveda Pharmacology, Faculty of Indigenous Medicine, University of Colombo.

Organoleptic evaluation

Fresh stem bark was examined for colour, odour, taste, texture, hardness, and brittleness through visual inspection, handling, chewing, and bending tests.

Physical Evaluation [5]

(A) Foreign Matter Content – 500g sample of *Varuna* stem bark was spread in a thin layer, and foreign matter was sorted visually using a magnifying lens and sieve. The foreign matter was weighed, and its percentage was calculated: Foreign Matter (%) = (Weight of foreign matter/Weight of sample) × 100%.

(B) Ash Values

Total Ash Value: Four grams of the sample were placed in a silica crucible, ignited at 550°C in a muffle furnace for 4-5 hours until white ash formed, cooled in a desiccator for 30 minutes, and weighed.

Acid-Insoluble Ash: The residue from the total ash was boiled with 25ml of 2M HCl, filtered, washed, transferred to a silica crucible, ignited at ≤450°C, cooled, and weighed.

Water-Soluble Ash: The above procedure was repeated using 25ml of distilled water.

(C) Extractive Values

Water-Soluble Extractive: Four grams of powder were soaked in 100ml of water, shaken for 6 hours, and left for 18 hours. The filtrate (25 ml) was evaporated, dried at 105°C for 6 hours, cooled, and weighed.

Alcohol-Soluble Extractive: The procedure was repeated using 100ml of ethanol.

(D) Loss on Drying/Moisture Content – Two grams of powder were placed in a pre-weighed moisture dish, heated at 105°C for 30 minutes, cooled in a desiccator, and weighed. The process was repeated until constant weight was achieved, and moisture content was calculated based on the air-dried material.

Preparation of the *varuna* stem bark decoction

The stem bark samples were washed, shade dried and ground to a coarse powder (pass 60#) using a grinder. Then 60g of the powder was mixed with 1920ml of water, heated until reduced to 240ml, and filtered through four folded cotton cloths into a separate glass vessel [6].

Preparation of the freeze-dried extract of *varuna* stem bark decoction

The decoction was freeze-dried using the freeze dryer at Industrial Technology Institute, Colombo, Sri Lanka for 48 hours. The extract stored in an air tight glass vial at 4^0C.

Preparation of the ethanol extract of *varuna* stem barks

Hundred grams of *varuna* stem bark powder was mixed with 200ml of 99% ethanol, stirred at 160rpm for 24 hours, filtered, and concentrated using a rotary evaporator at 40°C. The extract was stored in an airtight glass vial at 4°C.

Chemical Evaluation [7]

(A) Phytochemical screening

Table 1 shows the different tests carried out in the phytochemical screening.

Table 1: Phytochemical analysis of aqueous and ethanol extracts of *varuna* stem bark

Phytochemical	Procedure	Observation
Alkaloid	Mayer's reagent test: 2 drops of the reagent added to 2ml of each extract Wagner reagent test: 2 drops of the reagent was added to 2ml of each extract Hager's reagent test: 2 drops of the reagent was added to 2ml of each extract	Cream color precipitate Reddish color precipitate Yellow colour precipitate
Tannins	$Fecl_3$ Test: 5 drops of $Fecl_3$ were added to each extract and mixed well Lead acetate test: 3 drops of Lead acetate solution was added to 5ml of each extract and mixed well	Black precipitate Yellow precipitate
Saponins	Foam test: 5ml of each extract was mixed with 2.5ml of distilled water separately, shaken vigorously, and kept for 10 minutes	Stable foam of honey comb appearance
Flavonoids	Alkaline reagent test: 2ml of extract was mixed with few drops of 2% NaOH Shinoda test: 2ml extract mixed with few drops of Con. H_2SO_4 and few pieces of Magnesium	Yellow disappear after adding dil. HCl Pink red
Terpenoids	Salkowski test: 5ml of each extract was mixed with 2ml of Chloroform and 3ml of con. H_2SO_4 was added along the sides of the test tube	Reddish brown color
Steroids	Lieberman Burchard test: 2ml of Acetic anhydride and 2ml of con.H_2SO_4 were added to 2ml extract	Dark bluish green color

Cardiac glycosides	Keller Kiliani's test: 1ml of Glacial acetic acid added to 3ml of extract and con.H_2SO_4 was introduced to the bottom of the tube	Reddish brown ring at the interface
Carbohydrates	Benedict's test: 2ml of extract mixed with 3ml of reagent and boiled for 2 minutes.	Brick red precipitate
Proteins	Biuret test: 2ml of extract mixed with 2ml of 1% NaOH and few drops of $CuSO_4$. Ninhydrin test: 2ml extract mixed with few drops of reagent and boiled	Purple color Purple color

(B)Thin Layer Chromatography (TLC)

For the ethanol extract of *varuna* stem bark, a 2:8 (v/v %) methanol-chloroform solvent system was used, and for the freeze-dried aqueous extract, a 4:1:5 (v/v %) butanol-dichloromethane-water system. Extracts were spotted on a silica gel plate, developed, dried, and visualized under UV radiation at 254nm. R_f values were calculated for each spot.

Biological Evaluation [8]

(A) Swelling Index

Coarsely powdered 1gof *varuna* stem bark was added to 25ml of water in a 25ml glass stoppered measuring cylinder. The mixture was shaken every 10 minutes for 1 hour, and then allowed to stand for 3 hours. The volume occupied by the powder was measured in ml.

(B) Foaming Index

One gram of stem bark powder was mixed with 100ml water, boiled at 80-90°C for 30 minutes, and cooled. The decoction was filtered, diluted, and poured into 10 stoppered test tubes. After shaking for 15 seconds, the foam height was measured. The foaming index was calculated as 1000/a, where 'a' is the foam height.

RESULTS

Table 2: Results of physical and biological evaluation

Parameters	Results
Foreign Matter Content	2%
Total Ash Acid-insoluble Ash Water-soluble Ash	1.88% 1.32% 4.45%
Water-soluble extractive value Alcohol-soluble extractive value	10.5% 1.3%
Loss on drying at 105^0C	9.66%
Foaming index	<1000
Swelling index	7ml

Table 3: Results of phytochemical analysis

Phytochemical	Ethanol extract	Aqueous extract
Alkaloid	+	+
Tannins	++	++
Saponins	-	++
Flavonoids	+	+
Terpenoids	++	-
Steroids	+	++
Cardiac glycosides	++	+
Carbohydrates	-	-
Proteins	+	+

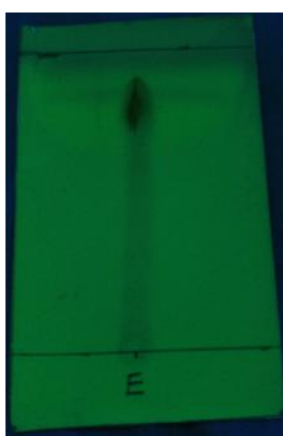

FIGURE 2: Chromatogram of the freeze-dried aqueous extract (Butanol: Dichloro methane: Water 4:1:5, v/v %)

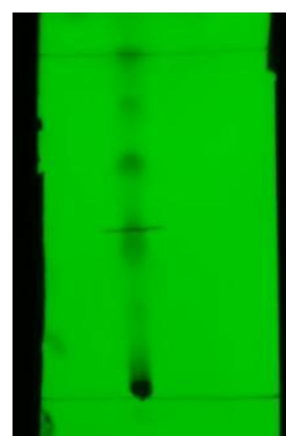

FIGURE 3: Chromatogram of the ethanol extract (MeOH: Chloroform 2:8, v/v %)

DISCUSSION

The foreign matter content of the sample was 2%, indicating high purity. Ash values, which indicate contamination, were low, with total ash at 9.88%, acid-insoluble ash at 1.32%, and water-soluble ash at 4.45%. The water-soluble extractive value (10.5%) was higher than the alcohol-soluble value (1.3%), showing that the bark's constituents were more soluble in water. The moisture content was 10.66%, essential for drug stability by preventing bacterial or fungal growth. The foaming index was below 1000, indicating low saponin content, and the swelling index was 7ml, suggesting mucilage content.

Both freeze-dried aqueous and ethanol extracts were positive for alkaloids, tannins, flavonoids, steroids, glycosides, and proteins. Only the aqueous extract was positive for saponins, while the ethanol extract contained terpenoids. Carbohydrates were not detected. These bioactive compounds contribute to the plant's pharmacological activities such as anti-diabetic, anti-inflammatory, and anti-cancer properties [9]. The chromatograms revealed two spots (R_f values 0.42 and 0.73) for the aqueous extract (FIGURE 2) and three spots (R_f values 0.42, 0.67, and 0.83) for the ethanol extract (FIGURE 3),

indicating the presence of different chemical constituents.

CONCLUSION

From the selected standardization parameters for the study, it can be concluded that the crude drug sample is matching with the quality standards. From the present study it was concluded that the preliminary phytochemical analysis of *Crateva adansonii* DC indicated the presence of Alkaloids, Tannins, Saponins, Terpenoids, Flavonoids, Glycosides, Steroids and Proteins.

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