ORIGINAL ARTICLE

Phyto-physicochemical and high-performance thin layer chromatography investigation of root of *Cadaba farinosa*, Forsk

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Abstract

Background: *Cadaba farinosa,* Forsk belongs to family Capparidaceae, traditionally used for a variety of medicinal purposes. Despite of its popular medicinal utilization, still no conclusive study has been reported so far regarding the pharmacognostical standardization. **Objective:** The present investigation was focused to scientifically establish the standard monograph of *C. farinosa* on the basis of its physicochemical and phytochemical parameters. **Materials and Methods:** Various physicochemical parameters such as ash values, extractive values, foreign organic matter, moisture content, reaction of powdered drug with different chemicals, and fluorescence analysis of *C. farinosa* were determined for ascertaining the quality of crude drug. The preliminary qualitative and quantitative phytochemical analysis, various inorganic minerals, and heavy metals along with high-performance thin layer chromatography (HPTLC) of *C. farinosa* were performed. **Results:** The physicochemical parameters were established. The various phytochemicals such as phenolic compounds, alkaloids, flavonoids, glycosides, and tannins were present in both ethyl acetate and aqueous extract, while sugars and carbohydrates, protein, amino acid, saponin, gums, and mucilage were present in aqueous extract. Steroids are only present in ethyl acetate extract. Terpenoid, fixed oil, and fats were absent in both the extracts. Various inorganic minerals and heavy metals were present in *C. farinosa*. The HPTLC fingerprinting of various phytochemical in ethyl acetate extract was done. **Conclusion:** The obtained data would serve as a useful guide toward establishing pharmacognostic standards, identification, assessing purity, standardization, and preparation of monograph of *C. farinosa*.

Key words: *Cadaba farinosa* Forsk, Capparidaceae, Phyto-physicochemical, High Performance Thin Layer Chromatography

INTRODUCTION

Cadaba farinosa, Forsk belongs to family Capparidaceae, is commonly known as Indian Cadaba. The plant is unarmed shrub or tree growing up to 1–2 m in height. Older stems are smooth and purplish in color while the young stems are pubescent and yellowish-brown in color. The leaves are 12–25 mm long and 8–12 mm wide with simple entire, elliptical oblong, and ovate in shape. The flowers are corymbose type and are dirty white in color. The fruits are cylindrical in shape and glabrous or pubescent while seeds are strait, surrounded by orange red aril. Wood of tree is white and yellow on aging.[1,2] Conventionally, various parts of *C. farinosa* such as root, leaves, flower buds, and fruit are used as medicine. Roots are used as anthelmintic, emmenagogue, uterine obstruction, dysentery, and female fertility. Leaves are used in amenorrhea, dysmenorrheal, purgative, rheumatism, uterine obstruction, and in the preparation of medicated oil. Flower buds have stimulant, purgative, antiphlogistic, and anthelmintic property. Fruits have edible property while ash of plant is rubbed into skin to relieve the general body pain.[3,4] The earlier claims showed that *C. farinosa* has hepatoprotective and antioxidant,[5] antiprotozoal,

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schistosomicidal and antifungal,[6,7] anticancer,[8] antibacterial and cytotoxic,[9] antidiabetic,[10] and wound healing[11] property. Besides its well accepted medicinal uses, no conclusive data are available regarding its phytophysicochemical properties and HPTLC analysis. Hence, the present study was designed to focus on the investigation of phytophysicochemical properties and HPTLC analysis of *C. farinosa*.

MATERIALS AND METHODS

Collection and Authentication of Plant

The plant *C. farinosa* was collected from the campus of Shri Venkateswara University, Tirupati, Andhra Pradesh., India, during the month of July 2008. The plant was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai. A voucher specimen no. PARC/2008/239 has been deposited in the institute.

Instruments

The UV-Visible spectrophotometer, model UV-1800, Shimadzu, Japan and HPTLC, Camag, Muttenz, Switzerland was used for the study.

Chemicals and Reagents

All chemicals and reagents used for the investigation of physicochemical and phytochemical property of *C. farinosa* were of analytical grade.

Physicochemical Analysis

The various physicochemical parameters of the powdered *C. farinosa* such as ash values, extractive values, foreign organic matter, moisture content, physicochemical analysis of powder, and fluorescence analysis were performed.[12]

Fluorescence Analysis

The fluorescence characteristics of powdered *C. farinosa* with different chemicals were observed in daylight and UV light. The powdered *C. farinosa* was treated with the various neutral solvents (hydroalcoholic and water), acidic (1 M hydrochloric acid, 80% sulfuric acid, conc. sulfuric acid, 50% nitric acid, and 5% ferric chloride), and alkaline solvents (1 M sodium hydroxide and alcoholic 1 M sodium hydroxide).[13]

Preparation of Extracts

Shed dried powder of root of *C. farinosa* was exhaustively extracted with ethyl acetate and aqueous solvent. The extracted was collected and concentrated by under vacuum and semisolid mass was obtained. The dried extracts were weighed and the required quantity of the same was dissolved in appropriate solvents for further investigations.[14]

Phytochemical Investigation

The ethyl acetate and aqueous extracts of *C. farinosa* were subjected to the preliminary qualitative phytochemical investigation.[15]

Quantitative Estimation of Constituents

Various constituents such as fat, carbohydrate,[16] protein,[17] total alkaloid,[18] and total flavonoids[19] were determined quantitatively.

Inorganic Minerals and Heavy Metals Estimation of *C. farinosa*

The various inorganic minerals such as sodium, potassium, phosphate, magnesium, chromium, copper, selenium, and iron were determined in powdered *C. farinosa*.[20]

High Performance Thin Layer Chromatography (HPTLC)

The HPTLC is the better means to separate the various phytochemicals present in extract.[21] The acetyl acetate extract of *C. farinosa* was found to have a number of phytoconstituents present in it. Hence, further attempt was taken to separate the individual components with the help of HPTLC. The condition was maintains as follows:

Sample: Ethyl acetate extract

Stationarphase

- Plate size: 10 × 10 cm
- Material: HPTLC plate Silica gel 60 F254 (E. MERCKKgaA)
- Solvent: Methanol
- Drying device: Oven

Sample Applicator: CAMAG Linomat 5

Instrument: CAMAG Linomat 5 "Linomat5_120222 (1.00.12)

Application parameters for Linomat 5

- Spray gas: Inert gas
- Sample solvent: Ethanol
- Dosage speed:100 nl/s
- Syringe size: 100 µl
- No. of Track: 3
- Mode of Application: Band
- Band length: 6.0 mm

Development – Glass tank

- Development chamber: Twin Trough Chamber 20 x 10 cm
- Mobile phase: $CHCl_3$: MeOH: H_2O (6:3.25:0.75, v/v)
- Solvent front position: 50.0 mm

- Volume: 10 ml
- Drying device: Oven
- Temp: 60°C
- Time: 5 min
- Development Mode: Ascending

Detector/Scanner: CAMAG TLC Scanner 3. "Scanner 3_111115" S/N 111115 (1.14.21)

- Number of tracks: 3
- Optimize optical system: Light
- Scanning speed: 20 mm/s
- Wavelength: 254 nm
- Measurement mode: Absorption

RESULTS AND DISCUSSION

Physicochemical Analysis

The physicochemical constants such as ash values, extractive values, foreign organic matter, and moisture content of *C. farinosa* were determined for ascertaining the quality of crude drug [Table 1]. The results revealed that *C. farinosa* was having 6% w/w total ash, 1% w/w acid insoluble ash, and 4% w/w water soluble ash. The aqueous extractive value was higher (73.60% w/w) as compared to ethyl acetate extractive value (25.65% w/w). The foreign organic matter was found to be 0.1% w/w and the moisture content was 9% w/w.

Reaction of Powdered Drug with Different Reagents

The behavior of powdered *C. farinosa* with various reagents under ordinary light by naked eye was determined and given in Table 2.

Fluorescence Analysis

The results of fluorescence analysis of powder *C. farinosa*is summarized in Table 3 which showed the presence of various chemical constituents.

Phytochemical Investigation

The preliminary phytochemical tests were carried out on ethyl acetate and aqueous extract of *C. farinosa*, which revealed the presence of various phytoconstituents. The phytochemicals such as phenolic compounds, alkaloids, flavonoids, glycosides, and tannins were present in both ethyl acetate and aqueous extract, while sugars and carbohydrates, protein, amino acid, saponin, gums, and mucilage were present in aqueous extract. Steroids are only present in ethyl acetate extract. Terpenoid, fixed oil, and fats were absent in both the extracts.

Table 1: Physicochemical parameters of powdered *Cadaba farinosa*

Parameters	Yield (% w/w)
Ash value	
Total Ash	6.0
Acid insoluble	1.0
Water soluble	4.0
Extractive value	
Ethyl acetate extract	25.65
Aqueous extract	73.60
Foreign organic matter	0.1
Moisture content	9.0

Table 2: Physicochemical analysis of powder of *Cadaba farinosa*

Treatments	Colour
Powder as such	Yellowish white
Powder+Conc. Sulfuric acid	Brownish black
Powder+Conc. Nitric acid	Reddish brown
Powder+Conc. Hydrochloric acid	Brownish yellow
Powder+5% I_2	Brownish yellow
Powder+5N NaOH	Pale yellow
Powder+Glacial Acetic acid	Pale yellow
Powder+80% H_2SO_4	Reddish brown

Table 3: Fluorescent analysis of powder of *Cadaba farinosa*

Treatments	Day light	UV light
Drug Powder	Greenish Yellow	Green
Drug Powder+1M NaOH	Brownish green	Green
Drug Powder+alcoholic 1M NaOH	Greenish yellow	Pale Green
Drug powder+1M HCl	Yellowish Brown	Faint yellow
Drug Powder+50% HNO_3	Greenish Yellow	Green
Drug powder+5% $FeCl_3$	Brown	Green
Drug powder+80% H_2SO_4	Yellowish Brown	Dark Green
Drug powder+Conc. H_2SO_4	Black	Yellowish green
Drug powder+Water	Faint yellow	Yellowish green
Hydroalcoholic extract	Brownish yellow	Faint green

Quantitative Estimation of Constituents

The quantitative estimation of various phytochemical was performed. The data reveal that *C. farinosa* was

Table 4: Qualitative chemical tests of *Cadaba farinosa*

Chemical Constituents	Ethyl acetate Extract	Aqueous extract
Alkaloids		
Mayer's reagent	+	+
Dragendorff's reagent	+	+
Hager's reagent	+	+
Wagner's reagent	+	+
Sugars and Carbohydrates		
Molish's reagent	-	+
Barfoed's test	-	+
Fehling's solution test	-	+
Benedict's test	-	+
Glycosides		
Keller-Killiani test	-	+
Borntrager's test	-	-
Legal's test	+	-
Baljet's test	-	-
Protein		
Million's test	-	+
Biuret test	-	+
Xanthoproteic test	-	+
Amino acid		
Ninhydrin test	-	+
Saponin		
Foam test	-	+
Flavonoids		
Shinoda test	+	+
Tannins		
Ferric chloride solution	+	+
Lead acetate test	+	+
Gelatin solution test	-	+
Bromine water	-	+
Potassium dichromate	+	+
Phenolic compounds		
Ferric chloride solution	+	+
Lead acetate solution	+	+
Terpenoid		
Noller's test	-	-
Fixed oil and fats		
Spot test	-	-
Gums and mucilage		
Swelling test	-	+
Steroids		
Salkowski reaction	+	-

having 1.48% w/w of fat, 4.22% w/w protein, 7.03% w/w carbohydrate, 0.0786% w/w of total alkaloids, and 0.2156% w/w of total flavonoids [Table 5].

Inorganic Minerals and Heavy Metals Estimation of *C. farinosa*

The various inorganic minerals and heavy metals were estimated in *C. farinosa*. The result showed that *C. farinosa* contains inorganic minerals such as sodium (57.34), potassium (49.32), phosphate (9.58), and magnesium (0.787) while heavy metals such as chromium in trace amount, copper (2.126), selenium below detectable level, and iron (0.1009) [Table 6].

Table 5: Quantitative estimation of constituents of *Cadaba farinosa*

Phytoconstituent	Yield (% w/w)
Fat	1.48
Protein	4.22
Carbohydrate	7.03
Total alkaloids	0.0786
Total flavonoids	0.2156

Table 6: Inorganic minerals and heavy metals estimation of *Cadaba farinosa*

Inorganic Mineral	Yield (% w/w)
Sodium	57.34
Potassium	49.32
Phosphorous	9.58
Magnesium	0.787
Chromium	In traces
Copper	2.126
Selenium	Below detectable level
Iron	0.1009

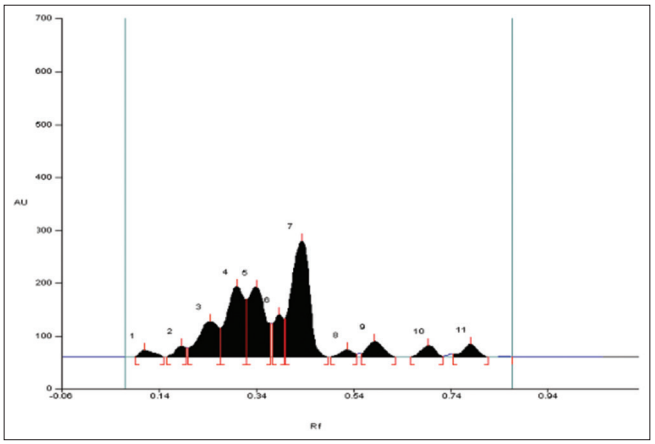

Figure 1: HPTLC fingerprint profile of ethyl acetate extract of *Cadaba farinosa*

HPTLC

The ethyl acetate extract of *C. farinosa* was subjected for HPTLC screening for the qualitative analysis of phytoconstituents. The numbers of solvent systems from lower to higher polarity were tried, but the solvent system which showed good resolution was used. The results are shown in Tables 7 and 8 and Figures 1 and 2. The results of HPTLC fingerprint scanned at 366 nm for ethyl acetate

Table 7: R_f values of ethyl acetate fractions of *Cadaba farinosa* by HPTLC

Sr.No.	Name of the extract	Solvent system	Detection wavelength	No. of spots	R_f value
1	Ethyl acetate extract	chloroform: methanol: water (6:3.25: 0.75, v/v)	254 nm	11	0.15, 0.20, 0.27, 0.32, 0.37, 0.40, 0.49, 0.55, 0.63, 0.72, and 0.82

Table 8: HPTLC profile of ethyl acetate fraction of *Cadaba farinose*

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.09	0.3	0.11	13.1	1.72	0.15	0.1	293.0	1.48
2	0.16	0.4	0.19	20.9	2.76	0.20	17.8	342.0	1.73
3	0.20	17.4	0.25	67.1	8.84	0.27	54.8	2155.6	10.88
4	0.27	55.1	0.30	133.9	17.63	0.32	108.7	3732.0	18.83
5	0.32	108.9	0.34	132.9	17.51	0.37	63.7	3585.3	18.09
6	0.37	63.3	0.39	81.0	10.66	0.40	71.3	1311.0	6.61
7	0.40	71.9	0.43	219.8	28.95	0.49	0.0	6314.2	31.86
8	0.49	0.1	0.53	14.2	1.87	0.55	5.1	276.1	1.39
9	0.56	6.4	0.58	29.9	3.93	0.63	0.1	759.0	3.83
10	0.66	0.0	0.69	21.7	2.86	0.72	1.4	490.4	2.47
11	0.75	5.1	0.78	24.9	3.28	0.82	0.1	562.1	2.84

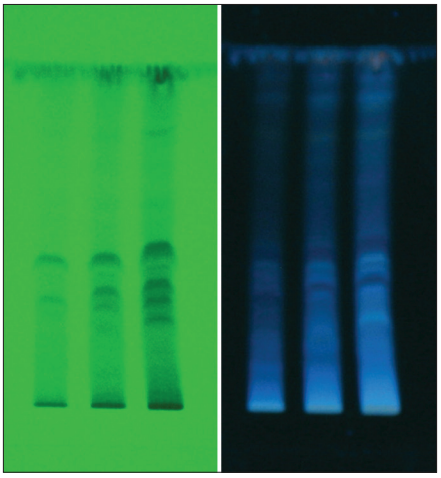

Figure 2: HPTLC of ethyl acetate extract of *Cadaba farinosa*

extract of *C. farinosa* revealed 11 spots at *Rf* value 0.15, 0.20, 0.27, 0.32, 0.37, 0.40, 0.49, 0.55, 0.63, 0.72, and 0.82 with percentage area 1.48, 1.73, 10.88, 18.83, 18.09, 6.61, 31.86, 1.39, 3.83, 2.47, and 2.84 in solvent system of chloroform: methanol:water (6:3.25:0.75, v/v) as solvent system.

CONCLUSION

The various physicochemical parameters such as ash values, extractive values, foreign organic matter, moisture content, reaction of powdered drug with different chemicals, and fluorescence analysis of powdered *C. farinosa* were determined for ascertaining the quality of crude drug. The phytochemical analysis of *C. farinosa* revealed the presence of various phytoconstituents. The various phytoconstituents were separated with the help of HPTLC and their respective *Rf* values have been accounted. Hence, the present study will provide useful information regarding correct identity, purity, and standardization of *C. farinosa*. The results of the present study could be useful for preparation of a monograph of *C. farinosa*.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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