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(RESEARCH ARTICLE)

Comprehensive pharmacogenetic and phytochemical analysis of Durva Ghrita: Identification, Authentication, Isolation, Extraction, and Standardization

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Abstract

A Cynodont dactylion grass is widely distributed throughout India. It is commonly known as Durva (Marathi), Durba (Bengali), Gurkhali (Kanarese), Durva or Hairtail (Sanskrit), Rampulla (Tamil), Gairanod (Telugu), and Huckabay (Punjabi). It is frequently referred to as harmalol, doob, or dub. This hardy perennial grass is native to warm temperate and tropical regions, but it can be found anywhere in the world. Cynodont dactylion may grow in any type of soil, particularly in dry areas. Flavonoids, alkaloids, glycosides, terpenoids, triterpenoids, steroids, saponins, tannins, resins, phytosterols, reducing sugars, carbohydrates, proteins, volatile and fixed oils, and more are among the many chemical substances it contains. It has key constituents that are Apigenin, Luteolin, Cynodin, p-coumaric acid, ferulic acid, β-sitosterol, Saponins. C dactylion has various chemical tests for its identification such as Mayer's, Dragendorff's, Shinoda test, Ferric chloride test, Froth test, Keller–Killiani test for cardiac glycosides, Salkowski, Liebermann–Burchard test, such that TLC, HPTLC, HPLC and GC-MS are confirmatory tests. C dactylion has long been used to treat microbial infections, dysentery, and urinary tract infections. It is used as antidiabetic in rats, diuretics, immunological and antiallergics and in various antimicrobials.

Keywords: Cynodont Dactylion; Antidiabetic Activity; P- Coumaric Acid; Chemical Constituents; TLC; Extraction

1. Introduction

A perennial herbaceous creeping grass found across India, Durva (Cynodont dactylion Linn. Pers., Family: Pinaceae) can grow up to 1500 meters in the lower Himalayas. It belongs to the group of 10 medicinal plants called "Atpudham," which is connected to Keralan medicine and culture.[1]

Durva or Hairtail (Sanskrit), Durva (Marathi), Durba (Bengali), Gurkhali (Kanarese), Rampulla (Tamil), Gairanod (Telugu), and Huckabay (Punjabi) are some of its common names.[2]

Medicinal plants contain a variety of possible medications and offer a safe, healthier alternative to manufactured pharmaceuticals. To extract various phytochemical ingredients, various parts are utilized, including leaves, roots, stems, fruits, seeds, and barks. Furthermore, a significant part of drug discovery is played by medicinal plants, which are abundant in physiologically active chemicals.[3]

Long used to treat urinary tract infections, diarrhea, and microbiological infections, Cynodont dactylion is a possible source of metabolites like flavonoids, alkaloids, glycosides, phenols, and β-sitosterol.[4]

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The present work used Gas Chromatography-Mass Spectrometry (GC-MS) analysis of an extract of Cynodont dactylion leaves to identify twenty chemical components.[5]

Cynodont dactylion can be found in nearly every type of soil, however it is more common in fertile soils like loamy soil. It is prevalent in disturbed environments including roadsides, gardens, trampled and overgrazed regions, uncultivated plains, and areas with high nitrogen levels. It is also frequently seen in damp locations beside rivers. It can be grown in dry land environments.[6]

Figure 1 Cynodont dactylion

2. Plant Profile

Table 1 Synonyms

Sr. No.	Language	Synonym
1	Marathi	Durva
2	Tamil	Rampulla
3	Hindi	Doob
4	Kanada	Gracehill
5	Telugu	GarikeandThellagariki
6	English	Bermuda and Bahama
7	Sanskrit	Durva
8	Other	Weed
9	German	Bermudagrass
10	Italian	Gramine
11	Portuguese	Capim-Bermunda
12	Spanish	Gamemaster
13	Swedish	Handlangers
14	Chinese	Gou yak gen
15	Afrika	Gewonekweek, Sweetgrass
16	Arabic	Thaiel, Najee, Tohma
17	French	Chinden pied-de-poule

2.1. Taxonomical Classification

- Kingdom - Plantae
- Division - Magnoliophyte
- Class - Liliopsid
- Order - Cereals
- Family - Phocaea
- Genus - cynodont
- Species - cynodont dactylion [8]

2.2. Botanical Description

It is a perennial creeping herb with wiry, slender stems (culms) that root at the nodes to form matted tufts. The leaves are delicate, glaucous-green, sharp, narrowly linear or lanceolate, and measure 2–10 cm by 1.25–3 mm. Spikes 2–6, emanating from a thin, purplish or green ascending peduncle. Grain is oblong and 1.05 mm long. Fruiting and Flowering: August through October (all year long).

- **Roots -** The primary roots give rise to tiny, hair-like, fibrous, cylindrical roots that are cream in color and up to 4 mm thick.
- **Stem -** slim, erect, jointed, leafy, up to 1 mm thick, extremely smooth, and yellowish-green in hue (color).

Figure 2 Cynodont dactylion

- **Leaf** - Soft, smooth, narrowly linear or lanceolate, finely acute, more or less glaucous, typically glaringly distichous in the barren shoots and at the base of the stem; light, glabrous, or occasionally bearded sheath; ligule, a very fine ciliate rim; 2 to 10 cm long and 1.25 to 3 mm wide.[6]

3. Pharmacological Activity

3.1. Anti- diabetic Activity

An aqueous extract of cynodont dactylion significantly reduced blood sugar levels in healthy rats for as long as six hours. Up to 500 mg/kg bow, a dose-dependent impact was observed. The effect slowed when the dosage was raised to 1000 mg/kg bow.

A Diabetes has long been treated orally with a variety of medicinal herbs and their compounds. According to a phytochemical analysis, dog grass, or cynodont dactylion, includes flavonoids and sterols that have hypoglycemic effects and the capacity to help the pancreatic beta cells grow again. In animal studies, cholesterol has also been demonstrated to lower blood sugar levels.[8]

3.2. Diuretic Activity

When given orally to hydrated male Wistar rats at varying concentrations (i.e., 0.125, 0.250, and 0.500 g/kg of body weight), C. dactylion extract exhibits considerable diuretic activity; the 0.500 g/kg dose yields the most notable effect.

Significant diuretic action is demonstrated by the increased sodium, potassium, and chloride ions from the body when the root stalk of C. dactylion was extracted using the aqueous extract method and given orally at various dosages, such as 100, 250, 500, and 750 mg/kg body weight.[2]

3.3. Immunological and antiallergics

Compound 48/80 caused mast cell activation and the amount of nitric oxide in serum and rat peritoneal mast cells were used to assess cynodont dactylion's potential ant anaphylactic and mast cell stabilizing mechanism.

The findings demonstrated that a cynodont dactylion compound (CDC), which was obtained using bioassay-guided fractionation, significantly ($p<0.01$) inhibited the anaphylactic reaction elicited by compound 48/80 and ($p<0.001$) activated mast cells. This CDC also significantly reduced Compound 48/80, which raised nitric oxide levels in rat serum and peritoneal mast cells.

The humoral antibody response was used to test cynodont dactylion's immunomodulatory effects in mice. Oral administration of the juice at 250 and 500 mg/kg in mice improved humoral antibody response upon antigen challenge, as evidenced by a dose-dependent, significant increase in antibody titer in the hemagglutination antibody assay and plaque-forming cell assay.[9]

3.4. Antimicrobial Action

3.4.1. Antiviral Activity

cynodont dactylion aqueous extract has strong antiviral activity against the white spot syndrome virus at 100 mg/kg of animal body weight, with no mortality or signs of white spot disease.[10]

3.5. Anti- inflammatory Activity

The anti-inflammatory properties of Cynodon dactylon were determined to be positive. The phytochemical substance found in Cynodon dactylon may have an inhibitory effect on inflammation or disrupt the process of crystal-induced epithelial cell injury.[10]

3.6. Antiarrhythmic activity

When applied during 30 minutes of ischemia and 30 minutes of reperfusion, the hydroalcoholic extract of C. dactylon's rhizome exhibits antiarrhythmic properties against I/R-induced arrhythmias. It also significantly lowers the incidence of total VF at reperfusion time and the length of reversible VF. The antiarrhythmic effects of C. dactylon were inversely correlated with extract concentration, with lower concentrations exhibiting stronger effects throughout both the ischemia and reperfusion phases.[10]

3.7. Antiulcer activity

The ulcer-healing effect of the plant extract was comparable to that of the popular drug ranitidine (H2-antagonist), which may be due to its antisecretory function associated with an improvement in the local healing process. Flavonoids have been found to possess antiulcer effects. Secondary syphilis and irritation of the urinary organs are treated with root decoctions.[10]

3.8. Wound healing activity

On the tenth day following their investigation using the incision wound healing model, the aqueous and alcoholic extracts of Cynodon dactylon were measured. The results were statistically significant ($p < 0.01$) and are shown in Table 3. Alcoholic extract's ability to cure wounds was on par with that of the conventional medication. When compared to the alcoholic extract, the aqueous extract had less of an impact.[10]

4. Chemical Constituents

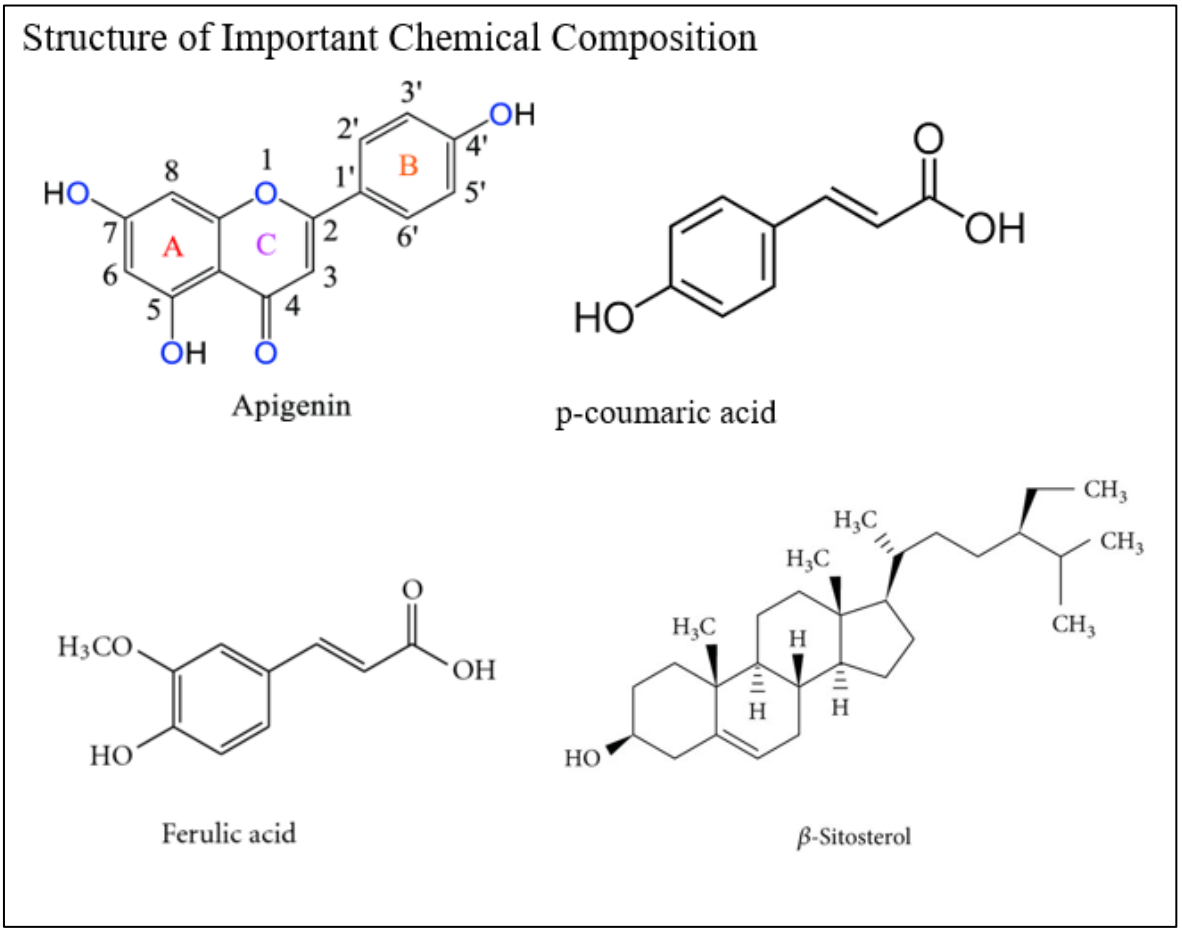
The plant contained flavonoids, alkaloids, glycosides, terpenoids, triterpenoids, steroids, saponins, tannins, resins, phytosterols, reducing sugars, carbohydrates, proteins, volatile and fixed oils, and more, according to the phytochemical analysis. Glycosides made up 12.2%, tannins 6.3%, alkaloids 0.1%, resins 1.0%, free reducing sugar 10%, and total reducing sugar 12%, according to the quantitative assessment of phytoconstituents.

On a zero-moisture basis, 100 g contains 11.6 g protein, 2.1 g fat, 75.9 g total carbohydrate, 25.9 g fiber, 10.4 g ash, 530 mg Ca, 220 mg P, 112.0 mg Fe, 1630 mg K, and 28 g beta-carotene equivalent. The hydroalcoholic extract of all sections of Cynodon dactylon yielded a total of 20 chemicals. Hexadecanoic acid, ethyl ester linolenic acid, and ethy ester d-mannose were the main constituents of the hydroalcoholic extract; hexadecanoic acid ethyl ester was the most common (17.49%).

In contrast, 22 chemicals were found in the phenolic fraction of Cynodon dactylon whole parts. At 69.49%, hydroquinone was the most prevalent. Pantolactone 0.8977%, pentanoic acid, 4-oxo 0.7289%, 3-hydroxy-1-methylpyridinium hydroxide 1.4121%, hydroquinone 69.4771%, phthalic anhydride 1.3128%, vanillic acid 1.2001%, propanoic acid, 2-oxo 1.5939%, furfural 6.0224%, and syringic acid 1.1154% were among the identified chemicals.[9]

4.1. Key Constituents

Apigenin, Luteolin, Cynodin, p-coumaric acid, ferulic acid, β-sitosterol, Saponins.



5. Extraction Methods

5.1. Soxhlet Apparatus Extraction

After cleaning, the entire plant was left to dry in the shade for a week. The dried plants were pulverized with an electric blender and passed through a 20 μ mesh filter. Ethanol, methanol, and petroleum ether were among the organic and

aqueous solvents that were extracted using the Soxhlet system. The extraction was done at 60 degrees Celsius for 24 hours. A Scietek, model RE 300, rotating vacuum evaporator was used to concentrate the extracts at 45°C. Until they were used again, the concentrated extracts were stored at 4°C in the refrigerator.[11]

5.2. Maceration assisted extraction (MAE)

An Erlenmeyer flask containing 20 g of C. dactylon was left at room temperature for 24 hours while 400 ml of 80% ethanol was added. To stop photosensitive molecules from degrading, aluminium foil was used to completely cover the Erlenmeyer flasks. With solvent renewal, this maceration is carried out three times. Following the recovery of the hydro-alcoholic extract with a N°01 filter paper, the filtrate is evaporated in a rotary evaporator at a lower pressure to extract the ethanol. Following a minimum 48-hour oven drying period at a temperature below 40°C, the filtrate is gathered in amber glass bottles and stored at refrigerator temperature.[12]

5.3. Flavonoid estimation

Using the reagent AlCl3, we were able to ascertain the flavonoid content of our samples. In short, 1 milliliter of a freshly made AlCl3 solution (2% methanol) is combined with 1 milliliter of extract or standard (made in 80% methanol). After 10 minutes of treatment, the absorbance at 430 nm is measured using a spectrophotometer (SpectraMax PC 340). The regression formula (y = 11.198x - 0.0189; R2 = 0.9932) was used to estimate the absorbance for 1 mg of dry extract, and quercetin (0-0.2 mg/ml) was used to calibrate the absorbance in an aqueous medium. The findings were expressed as mg of quercetin equivalent (mg EQ/g E) per gram of dry vegetable material that was extracted.A [12]

6. Phytochemical Test

6.1. Test for alkaloids

6.1.1. Mayer's Test

- A test tube was filled with the acid layer. After adding a few drops of Mayer's reagent (potassium mercuric chloride), everything was well combined. It produced a creamy precipitate.[13]

6.1.2. Dragondroff's Test

- A test tube containing the acid layer was used. A few drops of the Solution of Potassium Bismuth Iodide, often known as Dandruff's reagent, were added. The precipitate turned reddish-brown.[13]

6.1.3. Test for flavonoids

- A 5 ml alcoholic extract containing 1 g of plant material was treated with strong hydrochloric acid and 0.5 g of magnesium turnings. Flavonoids were detected by a magenta-red coloring that appeared within 3 minutes.[13]

6.2. Test for tannin and flavonoid

6.2.1. Ferric chloride test

- The presence of tannins and phenols is shown by a blue color that results from dissolving a tiny sample of 50% alcohol extract in water and adding 5% ferric chloride solution [13].

6.2.2. Foam test

- When a tiny bit of extract and water are combined, a foam is created that lasts for ten minutes. It verifies that saponins are present.[14]

6.3. Test for Steroids and triterpenoids

6.3.1. Salkowski test

- The chloroform layer is stirred, a few drops of pure sulfuric acid are added, and the mixture is let to stand. The bottom layer turns yellow and finally deep red. [14]

6.3.2. Liberman- Burchard's Test

- A test tube containing an extract sample was used. Along the test tube's walls, a few drops of acetic anhydride and 1ml of sulfuric acid concentration were applied. When two layers overlap, a brown ring is created.[14]

6.4. Separation of components

6.4.1. Thin layer chromatography

Toluene: ethyl acetate ratio 90:10 is used on a Silica gel 'G' plate for TLC of the drug's alcoholic extract. Its Rs are 0.1 (green), 0.40 (yellow), 0.45 (green), 0.51 (yellow), and 0.57 (green), with five distinct light spots visible. Six spots appear at Rf 0.22, 0.40, 0.45, 0.51, 0.57, and 0.64 (all yellow) when exposed to iodine vapor. Six spots appear at Rf. 0.22, 0.40, 0.45, 0.51 (all grey), 0.57 (green), and 0.64 (grey) using a 5% methanolic-sulfuric acid reagent spray and a 10-minute heating to 105°C.[15].

6.4.2. High Performance Thin Layer Chromatography (HPTLC)

Getting the sample ready 0.1 ml of ghee was combined with 1 ml of hexane. The produced solution was used for chromatography. The next step was pre-chromatographic derivatization. The base, alcoholic KOH, was heated for 10 to 15 minutes in a CAMAG TLC plate heater. The sample application was conducted using the CAMAG linomat. 5. HPTLC was performed on Durvadi Ghrita using the solvent solution petroleum ether: diethyl ether: acetic acid (9:1:0.1v/v). An investigation using HPTLC was carried out for the normal phase. Vanillin sulfuric acid spray reagents were used for POT chromatographic derivatization.[16]

6.4.3. GC MS analysis

The JEOL GCmate II GC/MS system (JEOL USA, Inc.), which has a quadruple double-focusing mass analyzer, was used to analyze the extract from the C. dactylon plant. Colum was HP 5Ms capillary, which is composed of (5%-Phenyl)-methylpolysiloxane and has a film thickness of 0.10 to 1.00 µm. High-purity helium flowing at a rate of 1 milliliter per minute served as the carrier gas. The temperature at the inflow was kept at 250°C. The oven's initial temperature was set to increase by 10 degrees each minute from 50 to 250 degrees Celsius. The temperature of the MS transfer line was maintained at 250°C. Electron impact ionization at 70eV was used to analyze the Mas spectra, and total ion count (TIC) was utilized for interpreting the data in order to identify and quantify the components. A database of known component spectra was compared to the component spectrums.[17]

7. Result

Table 2 Pharmacogenetic Evaluation

Parameter	Observation / Result
Microscopic Feature	Grass with creeping rhizomes, smooth leaves, and nodes; green color, sweetish taste, no specific odor
Microscopy	Transverse section of leaf shows single-layered epidermis with bulliform cells, vascular bundles in parallel rows, and silica crystals; stem shows circular outline with collenchyma Tous hypodermis and sclerenchyma near vascular bundles
Powder Microscopy	Shows fragments of epidermal cells, xylem vessels, spiral thickening, trichomes, and starch grains

Table 3 Phytochemical Evaluation

Test	Result
Alkaloids	Present
Flavonoids	Present
Tannins	Present
Saponin	Present
Saponin	Present
Steroids	Present
Glycosides	Present
Phenolic compound	Present
Carbohydrates	Present
Proteins and amino acids	Present
Terpenoids	Present

8. Conclusion

The pharmacological, chemical, and therapeutic characteristics of cynodont dactylion an herbal remedy that exhibits promise because of its efficacy and safety are examined in this paper. Ethanolic extracts from the plant suggest that C. dactylion L. is a potentially useful natural chemical source. Studies on antimicrobial activity have shown that the aqueous extract has strong antibacterial, analgesic, and antipyretic qualities against conditions like white spot illness. Grass is a better material for experiments that may produce more positive results because it is simpler to grow and isolate. cynodont dactylion is an important part of ethnomedical practices and traditional medical systems. For a variety of diseases and ailments, it is quite beneficial.

Compliance with ethical standards

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