Morphotaxonomic study and phytochemical analysis by GCMS method of underestimated medicinal Grass: *Cynodon dactylon* (L.) Pers. (Poaceae)

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Abstract

The family Poaceae is one of the largest and most important families in plant kingdom. No other group of plants is more essential to the nutrition, well-being or even existence of man. Taxonomically grasses are very difficult group of plants and classified and identified on basis of morphological characters. They have great economical and ecological values with multiple uses including medicinal, but still are underestimated and overlooked from studies because of their difficult floral structures which are not showy.

In present attempt, morphotaxonomic study and phytochemical analysis by GCMS method of *Cynodon dactylon* (L.) Pers. which is mentioned for its multiple medicinal uses in ancient literature to determine the phytochemical constituents to ascertain the rationale for its use in traditional medicine has been undertaken. Morphotaxonomic studies which not only found assisting its classification to respective tribe and its justification but also useful for its proper identification.

Through GCMS analyzed results, 3 compounds were identified and reported as phthalic acid di ester, di-isocotyl phthalate, Bis (2-ethyl hexyl) phthalate which are known to possess antimicrobial and antifouling activity. GCMS analysis and the biological activity of each compound were discussed in present attempt.

This was found helpful in proving medicinal importance of this grass also revealing the wisdom of our ancestors in ancient times. Present study suggests that grasses should be given due importance for identification and further studies along with their conservation.

Keywords: Poaceae, *Cynodon dactylon*, morphotaxonomy, GCMS analysis, phthalic acid, Bis phthalate, Di-isocotyl phthalate, grasses

1. Introduction

The family Poaceae (Graminaeae) is one of the largest in the plant kingdom and certainly one of the most important. It is a very distinctive and highly successful group, more than any other. This assemblage of plants feeds man and animals and so covers the earth that soil may be built and held securely from the forces of erosion. No other group of plants is more essential to the nutrition, well-being or even existence of man. The evolution and development of this family is of vital interest to everyone. Taxonomically Poaceae is a very difficult group of plants. Grasses are classified and identified chiefly on the basis of their morphological characters. The characters such as life span, habit, growth form, indumentums type, details regarding leaf sheath, lamina, ligules, etc. are of much help in their identification. However, the inflorescence of grasses forms the chief distinguishing feature for identification and classification.

The characters of panicle, spike, spikelet, pedicels, rachis, rachilla, joints of their rachis, involucral glumes, fertile glumes palea, awn etc. is very useful in classification and identification of grasses. The floral characters such as lodicules, stamens etc. also are of taxonomic value.

The term grass, derived from the same root as "grow" (Dashora and Gosavi, 2013) [3]. Grasses form the climax vegetation in great areas throughout world, approximately with 10,000 to 11,000 species belonging to 700 genera. Their structure and shape demands for very little space and being highly proliferating in nature are main reasons for their survival and spread. They have great adaptive capacity and are of great economic and ecological values having multiple uses from as sources of food, sugar and jiggery, bamboos for construction and paper making, perfumes, thatches for huts, nutritious fodders and even medicines but often these are still underestimated and overlooked from studies because they are always given a cursory look as their floral structures are not showy and difficult to work out and hence being difficult to identify, often neglected from floristic and other studies.

It is need of an hour to identify these important grasses by undertaking their morphoteconomic studies as well as assess their significant properties and develop a strategy for their conservation. Family Poaceae is characterized by annual or perennial herbs, rarely shrubs or trees. Stems erect, ascending or prostrate, usually branched at base, culms cylindrical, rarely flattened, jointed, usually hollow in the internodes, closed at the node. Leaves alternate and two-rowed, sometimes crowded at base, consisting of sheath, ligule and blade; sheaths encircling the stem frequently swollen at the base; ligule placed at the junction of sheath and blade, membranous or reduced to a fringe of hairs, rarely absent; blades usually long and narrow, rarely broad rarely with a petiole like base, flat, convolute, involute, terete, parallel nerved. Flowers usually bisexual, sometimes unisexual, small and inconspicuous, usually consisting of stamens and pistil and of 2 or 3 minute hyaline or fleshy lodicules representing the perianth, subsessile between lemma and palea, the whole forming a floret. Florets one-many, distichous, sessile on a short slender axis and bearing at the base two empty bracts, the florets and glumes forming a spikelet. Spikelets pedicelled in open or contracted panicles or racemes, or sessile in spikes. Stamens hypogynous, 1 to 6, rarely more, usually 3, with delicate filaments and 2-locular anthers; the latter opening by a longitudinal slit; ovary 1-locular with anatropus ovule often adnate to adaxial side of the carpel; styles usually 2, rarely 1 or 3; stigmas generally plumose. Fruit mostly a caryopsis with pericarp and testa fused or rarely a nut or a berry or a utricle. (Ingole, 1979) [5].

Though grasses are mostly used as food and fodder, many of them have significant medicinal properties and find their mention in ancient Indian medicine and literature like Atharvaveda and Ayurveda. One of such grass being *Cynodon dactylon* (L.) Pers. which is one of the most commonly occurring weeds in India.

In Hindi it is known as *dhub*, *doob*, or *harialil*; other common names include *durba* (Bengali), *garikoihallu* (Kanarese), *durva* (Marathi), *durva* or *haritali* (Sanskrit), *arugampullu* (Tamil), *garikagoddi* (Telugu) and *dhubkhabbal* (Punjabi) (Sastry and Kavathekar, 1990) [10]. Although it is a potential weed, it is a valuable herbal medicinal and used as first aid for minor injuries (Oudhia, 1999a, b) [6, 7]. It grows in mostly all types of soil. Because of its wide spread roots, the grass does not perish in any adverse climatic condition. It is quite difficult to pull out the grass complete from ground. It is a highly aggressive species, crowding out most other grasses, leading it to be called 'devil's grass' in some areas (Dashora and Gosavi, 2013) [3]. It is often offered to Lord Ganesha in Indian Worship.

Farmers pluck it out from their paddy fields. Farmers raditionally apply crushed leaves to minor wounds (Oudhia and Pal, 2000) [8].

C. dactylon has a renowned position in Indian systems of medicine and many parts of the plans are assumed to have medicinal properties. It is traditionally used for eye disorders and weak vision; the afflicted are advised to walk bare foot on dew drops spread over plant each morning. According to Ayurveda, India's traditional pharmacopoeia, it destroys foulness of breath, useful in leucoderma, bronchitis, piles, asthma, tumors, and enlargement of the spleen. According to Unani system of medicine, *C. dactylon* plant is bitter, sharp hot taste, good odor, laxative, brain and heart tonic, aphrodisiac, alexipharmic, emetic, emmenagogue, expectorant, carminative and useful against grippe in children, and for pains, inflammations, and toothache (Agharkar, 1991) [1]. Virus-affected discolored leaves of *C. dactylon* are used for the treatment of liver complaints. In Homoeopathic system of medicine, it is used to treat all types of bleeding and skin troubles (Oudhia *et al.*, 1998) [9].

Regarding its chemical composition, it is said to be rich in proteins, carbohydrates, fiber, calcium phosphorus and potassium. It provides 11.75 percent ash or burning, which has potassium and sodium salts in it. It contains more than 65% of chlorophyll. This helps in increasing the number of red blood cells in our body. It is an excellent detoxifier. It helps in maintaining the alkalinity of blood. This is one of the reasons it is able to cure diseases (Dashora and Gosavi, 2013) [3].

Siddha and Ayurveda systems use it in preparing various concoctions. It is an excellent supplement for lactating mothers. It boosts prolactin and so increases the quality and quantity of breast milk. Homoeopathy works on the principle of *similia similibus curentur*, or like cures like. Homoeopathic remedy prepared from this grass is used in treating almost all ailments that trouble the urinary tract. The remedy is also useful in treating nosebleed, blood, vomiting, etc. Recent research at University of Allahabad in India has shown its glycemic potential (Dashora and Gosavi, 2013) [3]. It is used to treat urinary tract infection, prostatitis, syphilis and dysentery (Singh *et al.*, 2007) [11].

Hence in present attempt, as a contributoy study, its morphotaxonomical and phytochemical analysis by GCMS method to determine the phytochemical constituents to ascertain the rationale for its use in traditional medicine, because plants have many phytochemicals with various bioactives. Studies have reported that extracts from natural products and medicinal herbs have positive effects against various ailments from cancer to hormonal treatments. Therefore many plants have been examined to identify new and effective antioxidant compounds as well as to elucidate the mechanism of action (Swamy and Tan, 2000) [12].

2. Material and methods

Collection of plant material and morphotaxonomic study

Several specimens of *Cynodon dactylon* were collected from various localities and habitats in and around Amravati (Maharashtra, India) district. The systematic description was made with help of stereozoom microscope and data regarding habitat, phenology, specific association, relative abundance, local names, etc. was recorded. The sketches were made and photographs were taken. Its identification is confirmed by referring standard floras.

Phytochemical Analysis: For the preliminary phytochemical analysis, the method of Chhabra (1984) [2] was adopted and tests given in Harbourne (1976) [4], for qualitative screening of phytochemicals were adopted.

Dry Powder Preparation: The plant leaf sample was dried at room temperature and grind into fine powder.

Sample Preparation for GCMS Analysis: About 15g of powdered material of plant was taken in a clean flat-bottomed glass container and soaked in 150ml of methanol. The container with its content was sealed and kept for 24 hours. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whattman filter paper. The filtrate (methanolic extract) obtained for the plant was evaporated under ceiling fan.

GC-MS Analysis: The GCMS analysis was conducted at the Central Instrumentation Laboratory, Punjab University at Chandigarh. 2mL aliquot was injected into a fisons GC8000 series coupled to a TSQ8000 MS (Triplequadrapole) mass analyzer. The chromatography was performed by using the DB5-MS column. Injection temperature was 230 ^{0}C. Helium flow was 1 mL/min. After a 5 min solvent delay time at 70 ^{0}C; the oven temperature was increased at 5 ^{0}C/min to 310 ^{0}C, 1 min isocratic and cooled to 70 ^{0}C, followed by the additional 5 min delay. The ion trace integration was done using the mass lab find target method for the characteristic fragment of assigned peaks.

Identification of Components: Interpretation of mass spectrum GCMS was conducted using data base of the Central Instrumentation Laboratory (CIL) spectra Libraries. Spectrum of the unknown component was compared with the spectrum of known components stored in the CIL. The molecular weight, molecular formula and the number of hints used to identify the name of the compound from CIL spectra Libraries were recorded.

3. Results and discussion

***Cynodon dactylon* (Linn.) Pers. Syn. 1: 85, 1805. (Plate I and II)**

A perennial grass forming dense patches close to the ground and with vertical shoots 7-24 cm. high. Roots adventitious arising from creeping nodes. Stem slender, prostrate, runner type reaching a length of several feet, producing ascending vegetative and flowering shoots, green, smooth, cylindrical, internodes 3-3.5 cm. long. Leaves in two alternating rows, narrow, 2-9 cm. long and 2-3 mm. broad, linear-lanceolate, smooth; leaf sheath about the half the length of internode above, very closely fitted to stem, smooth; ligule as a very fine ciliate rim. Inflorescence of 4 radiating spikes at the top of peduncle. Peduncle slender, purplish-green, 3-3.5 cm. long, each spike 2-4 cm long. Spikelets sessile, 2-seriate, ovate-lanceolate, greenish, one flowered, compressed. Rachilla slender, articulated above lowest glume. Sterile glume I linear, acute, 1-nerved, 1 mm. long, greenish. Glume II longer than I, linear-lanceolate. Lemma larger than sterile glumes, 2-3 mm. long, ovate, acute, 3-nerved, membranous, awnless, keel ciliate. Palea 2 keeled, membranous, shorter than lemma, lanceolate, acute. Lodicules 2, very small, obovate. Stamens 3, exserted, pendulous, filaments 2-3 mm. long, purplish; anthers oblong, dithecous, upto 2 mm. long. Gynoecium monocarpellary; ovary superior, unilocular, with basal placenta, smooth, oblong, 0.5 mm. long; styles 2, lateral; stigmas 2, feathery, 1 mm. long, violet. Caryopsis oblong, free, very small, whitish.

Very common grass species of this place, most abundant forming dense mats and driving away all other grasses in moist places, along roadside, drains, in gardens and cultivated fields. It is a very troublesome weed of heavy soils and irrigated lands and only deep, ploughing almost every year keeps it under check. It is good fodder especially for horses. It is also used as a lawn grass as other soft textured lawn grass varieties do not tolerate the summer heat in a better way. It often replaces other delicate lawn grass varieties.

Flowers: September-April, Chromosome no.: 2n = 18, Local name: Harali.

The characters observed are providing sound base for its classification as this grass belongs to sub family Poideae and under tribe Chlorideae of Poaceae.

Qualitative Screening for Phytoconstituents

Table 1: Phytochemical profile of different parts of *C. dactylon* in different extracts

Plant part	Alkaloids			Anthracene Glycosides		Anthroquinone	Aucubins	Iridoids	Carotenoids
	E	M	W	M	W				E
Whole plant	+	+	-	+	-	-	-	-	-

Plant part	Coumarin			Emodin	Fatty Acid	Flavonoid			Polyuronoid	Tannin		Starch
	E	M	W	E	E	E	M	W	W	M	W	W
Whole Plant	+	-	-	-	-	-	-	-	+	+	+	-

Plant part	Polyoses	Reducing Compounds		Saponin	Steroids			Triterpenoids			Volatile Oil
	W	M	W	W	E	M	W	E	M	W	E
Whole Plant	-	-	-	+	-	-	-	+	+	+	+

W – Water extract; M – Methanol extract; E – Ether extract

GCMS Profile: The spectra of GCMS and analyzed results which include the active principles with their molecular formula are given as below:

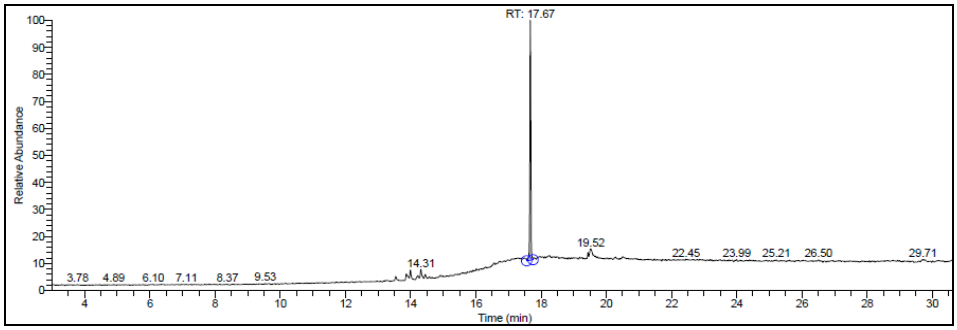

Fig. 1: Spectra of Gas Chromatography of whole plant of C. dactylon in methanol extract

Table 2: List of expected compounds and their activity

S. N.	Expected Compound Name	Molecular Formula	Activity
1	Phthalic acid, Di(2-propyl pentyl) ester	$C_{24}H_{38}O_4$	Antimicrobial
2	Di-isocotyl phthalate	$C_{24}H_{38}O_4$	Antimicrobial and antifouling
3	Bis (2-ethyl hexyl) phthalate	$C_{24}H_{38}O_4$	Antimicrobial and antifouling

GCMS analyzed results which include the active principles with their molecular formula are presented in Table 2. Three components were identified and they are reported as phthalic and most of them possessing antimicrobial and antifouling activity. On further study of each compound, it was found that they individually have their own biological importance and are known to possess antimicrobial and antifouling activity.

Qualitative phytochemical screening shows presence of alkaloids, anthracene glycosides, coumarin, tannin, saponins, triterpenoids and volatile oil. Thus each compound identified from extract of *C. dactylon* has its own biological importance.

Plate I: *Cynodon dactylon* showing radiating spikelets

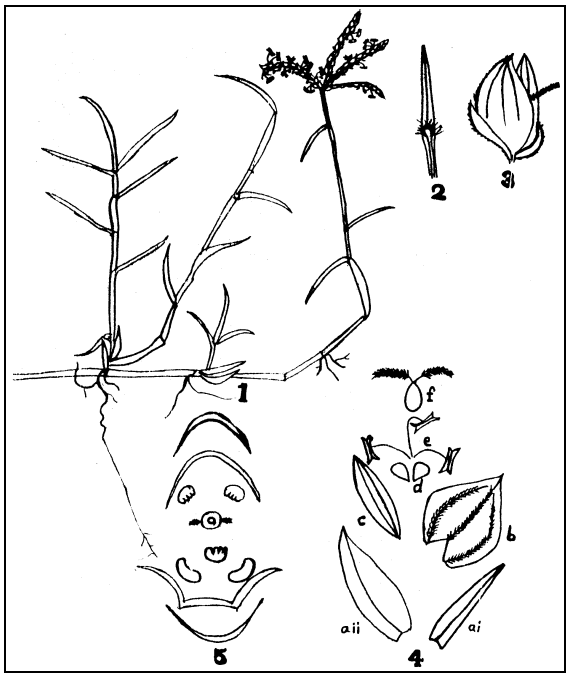

Fig 1-5: *Cynodon dactylon* (Linn.) Pers.

1. Habit
2. Leaf part showing ligule.
3. Single spikelet.
4. Spikelet details: a) Glume i and ii, b) Lemma c) Palea, d) Lodicules, e) Stamens, f) Pistil
5. Floral diagrams

4. Conclusion

GCMS method is a direct and fast analytical approach for identification of phytoconstituents. The importance of the study is due to the biological activity of some of these compounds. The present study which reveals the presence of components in *C. dactylon* suggests that the contribution of these compounds on the pharmacological activity found helpful in knowing the particular action of this medicinal grass and can prove its medicinal importance as well as presence of valuable phytoconstituents in it applicable to various diseases and this fact revealing the wisdom of our ancestors and Rishis even in ancient times as they mentioned this grass in ancient literature.

The present morphotaxonomic studies which not only found assisting its classification to respective sub family Poideae and tribe Chlorideae and its justification but also useful for its proper identification.

The present study also suggests that grasses should be given due importance for identification and further studies along with their conservation.

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