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Tradition to therapeutics: Sacrificial medicinal grasses *Desmostachya* bipinnata and *Imperata cylindrica* of India

[De la tradición a la terapéutica: Hierbas medicinales votivas, Desmostachya bipinnata e Imperata cylindrica de la India]

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Abstract: Desmostachya bipinnata (L.) Stapf. (Fam. Poaceae) and Imperata cylindrica (L.) P. Beauv. (Fam. Poaceae) also traditionally known as Kusha and Dharbha respectively are two grasses that form vital ingredient in various Vedic sacrifices (Yagnas) and rituals. They are found along river beds and plains throughout India and are well known for their medicinal properties as supported by traditional Ayurvedic scriptures and are a vital component in traditional medicinal formulations such as Tripanchamool, Kusadya-ghrita and Kusablecha, etc. to treat many disorders such as dysentery, diuresis, jaundice, skin infections etc. The current review enlightens the bioactive molecules such as flavonoids, glycosides etc. isolated from these grasses so far. Emphasis is laid on the various therapeutic applications of extracts or biomolecules characterized from these medicinal grasses. Reports of antimicrobial, diuretic, anti-ulcerogenic and other activities from both of these plants suggest involvement of various bioactive principles. Also, the future perspectives concerned with medicinal properties of these sacred grasses is discussed.

Keywords: Desmostachya bipinnata, Imperata cylindrical, Sacrificial medicinal grasses, India

Resumen: Desmostachya bipinnata (L.) Stapf. (Fam. Poaceae) e Imperata cylindrica (L.) P. Beauv. (Fam. Poaceae) también conocida tradicionalmente como Kusha y Dharbha respectivamente, son dos plantas medicinales que forman un ingrediente vital en diversos sacrificios védicos (yagnas) y rituales. Se encuentran a lo largo de cauces y llanuras en toda la India y son bien conocidos por sus propiedades medicinales apoyadas por escrituras ayurvédicas tradicionales y son un componente vital en formulaciones medicinales tradicionales como Tripanchamool, Kusadya-ghrita y Kusablecha, etc. para tratar muchos trastornos tales como la disentería, la diuresis, la ictericia, infecciones de la piel, etc. La revisión actual resalta las moléculas bioactivas como los flavonoides, glucósidos etc., aislados de estas plantas hasta ahora. Se hace hincapié en las diversas aplicaciones terapéuticas de los extractos o biomoléculas caracterizadas de estas hierbas medicinales. Informes de actividades antimicrobianas, diuréticas anti-ulcerogénicas y otras de estas dos plantas sugieren la participación de varios principios bioactivos. Además, se discutieron las perspectivas de futuro que se ocupan de las propiedades medicinales de estas hierbas sagradas.

Palabras clave: Desmostachya bipinnata, Imperata cylindrica, plantas medicinales votivas, India

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INTRODUCTION

Natural products are molecules produced by any living organism - plants, animals or microbes (Samuelsson, 1999). Natural products could be primary metabolites- those required for growth of organism, or secondary metabolites- those that are predominantly required for defense and sustenance of the organism (Bhat, 2005). Plants that produce secondary metabolites of medicinal importance are generally regarded as herbs or medicinal plants. The recognition of herbs and their usage for management of diseases could be dated back to antiquity. They are either applied alone or as a formulation in combination of crude/bioactive principles for of these therapy. The applications herbal formulations were written by traditional medicinal practitioners in various scripts and texts which are now preserved and regarded as sacred. Till today, these sacred texts are referred for rediscovery and isolation of pure bioactive molecules for therapy. The current review describes the comprehensive background about the ethnic and modern day importance of two of those plants from these sacred texts. These plants are regarded as sacred because of their medicinal value, and use in ritualistic practices.

Desmostachya bipinnata (L.) Stapf. (Fam. Poaceae) also called as Halfa grass; Big cord grass etc. is culturally an important grass in India called Kusha in Sanskrit. As of now, it contains 3 species under its genus, D. bipinnata, D. cynosuroides Stapf ex Massey, D. pingalaiae Raole & R. J. Desai. Another important Vedic grass, Imperata cylindrica (L.) P. Beauv. (Fam. Poaceae) also called as Cogongrass in English and Dharbha in Sanskrit has 9 species under its genus - I. conferta, I. cylindrica, I. contracta, I. brevifolia, I. brasiliensis, I. tenius, I. cheesemanii, I. condensata, and I. minutiflora. (Gabel, 1982). Both of the grasses are widely used in various religious sacrifices and rituals in India. It has mentioned Rig in and Atharvaveda (Madhihassan, 1987). Both the grasses are perennial green with dense foliage and are always seen spread over vast areas (Fig. 1).

One of the major problems with large scale production of natural products is the availability of source, which usually gets exhausted. The major significance of the described grasses is that they are abundantly available and are therefore sustainable sources. Even though, both the mentioned plants have various biologically active principles and medicinal

value, they have been utilized barely for commercial therapeutic solutions. Thus, this review aims to provide a major leap towards awareness, understanding and utilization of medicinal principles from these plants. Emphasis is given on natural products isolated so far and activity of these bioactive compounds and extracts of these plants.

Desmostachya bipinnata Ethnic prominence

Desmostachya bipinnata belonging to family Poaceae is commonly known as sacrificial grass, as it is being used in Yagnas and religious rites (Sivaranjan and Indira, 1994). Desmostachya bipinnata is a tufted perennial grass with thick scaly root stocks, which sends out creeping rhizomes in all directions. Leaves are many; reach up to 50 cm long and 1 cm broad at the base (Prajapati et al., 2003). It is distributed throughout India in hot and dry places and also found in Nubia, Egypt and Syria (Kirtikar and Basu, 1918). Roots of Desmostachya bipinnata are used in the Indian traditional system of medicine as cooling, sweet, astringent, diuretic and galactagogue and also useful in dysentery, diarrhoea, urinary calculi, dysuria, other diseases of bladder and skin diseases (Joshi, 2003). The culms are said to possess diuretic and stimulant properties. In the Konkan they are prescribed in compound decoctions with more active drugs for the cure of dysentery, menorrhagia, etc. (Kirtikar and Basu, 1918). Almost all Vedic rituals have Kusha as one of their ingredient. The mat or asana to perform vedic rituals is made out of this sacred plant. Also, a ring made of this grass is worn in ring finger during the ritual (Bhalla, 2006).

Bioactive compounds isolated

Although, crude phytochemical screening did reveal various classes of molecules such as alkaloids, flavonoids, terpenoids, glycosides, saponins, etc. (Hegde *et al.*, 2010) only few compounds have been isolated and characterized from *D. bipinnata*. Presence of other phytochemicals such as alkaloids, tannins, flavonoids, steroids, glycosides, and coumarins are also reported recently (Singh *et al.*, 2014). Recent reports of High Performance Liquid chromatography (HPLC) confirmed the presence of phytochemicals (Packialakshmi *et al.*, 2014). Initially, coumarins, scopoletin (1) and umbelliferone (2) were isolated (Hifnawy *et al.*, 1999).

Later, flavonoids like kaempferol (3), quercetin (4), quercetin-3-glucoside (5), trycin (6) and trycin-7-glucoside (7) were isolated from aerial parts of *D. bipinnata*. These compounds were already reported from other plants and are well known for their

bioactivity. However, (5), (6) were reported to be anti-ulcerogenic where they had curative ratio of 79.49 and 80.43% respectively for ethanol induced ulcer in male Wistar rats (Amani *et al.*, 2008).

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Later, 4'-methoxy quercetin-7-O-glucoside (8) was isolated which worked against *Helicobacter*

pylori with MIC of 62 μg/ml (Ramadan *et al.*, 2009). Further studies in this plant yielded sterol molecules,

stigmasterol (9), β -sitosterol (10), daucosterol (11), stigmast-5-en-3 β , 7β -diol (12), and stigmast-5-en-3 β , 7 β -diol (13) (Shrestha *et al.*, 2011). Daucosterol or β -sitosterol- D-Glucopyranoside was also reported to act synergistically against common human pathogens

along with other commercial antibiotics especially ciprofloxacin. Time kill analysis showed that it killed most of the pathogens in 5-10 h (Subramaniam *et al.*, 2014).

A new xanthene 2,6-dihydroxy-7-methoxy-3H-xanthen-3-one (14) was recently isolated where the compound exhibited inhibitions of signal transducer and activator of transcription 3-dependent

luciferase activity in HCT-116 colon cancer cell line with IC₅₀ value of 5 μ M and low-density lipoprotein-oxidation with IC₅₀ value of 27.2 μ M (Shrestha *et al.*, 2011).

Bioactivity of extracts

Much of the work has not been done in Desmostachya bipinnata, but reports of various bioactivities substantiate its medicinal properties. Acute toxicity studies of alcoholic and aqueous root extracts of this plant showed it to be safe till 2000 mg/Kg body weight in female albino mice. Studies on aqueous and alcoholic extracts of roots, depicted anti-diarrheal activity with alcoholic extract showing 29.34% and 34.36% inhibition of diarrheal symptoms at 200 and 400 mg/Kg body weight of Albino mice whereas, aqueous extract showed only 24.68 and 27.57% inhibition at 200 and 400 mg/Kg respectively (Hegde et al., 2010) confirming its ethnic use in treating diarrheal indications. Hydro-methanolic extract of whole plant of D. bipinnata was administered to normal Wistar albino rats to find changes in euglycemic index. Experiments revealed that in hypoglycemic state (induced by exercise or work) and hyperglycemic state (induced by food consumption etc.), the extracts maintained the euglycemic index. Thus its use in traditional medicine for diabetic patients along with other herbs was evident (Golla et al., 2013). Further studies with hydro-methanolic extract of whole plant elucidated its use in airway and gut disorders. This extract (100-500 mg/Kg) not only protected mice against castoroil induced diarrhea but also produced an atropinesensitive spasmogenic effect in rabbit jejunum up to 5 mg/mL, followed by a partial relaxation at 10 mg/mL, when assessed on gut preparations. In mice jejunum and pig ileum, both contractions and stimulations were observed. Also in rabbit trachea, these effects were observed. On activity-directed fractionation, inhibitions were found to be due to organic and stimulations due to aqueous fractions. Thus, studies confirmed the presence of calcium antagonist activity, possibly underlying its medicinal outcome in hyperactive gut and respiratory disorders, and cholinergic activity, possibly underlying its digestive effect, provides logic for traditional therapeutic uses of D. bipinnata (Rahman et al., 2012). Antioxidant activities of hydro-methanolic root extracts depicted it to be most potent scavenger of hydrogen peroxide radicals (IC₅₀ -127.07±6.44 μg/ml) against standard Ascorbic acid (IC₅₀ -122.60±2.17 µg/ml). Antioxidant studies with hydro alcoholic extract have also been reported recently (Golla et al., 2014). In addition to above antioxidant studies, cytotoxic studies of the same extract depicted

significant toxicity against Hela (IC₅₀ -109.8 μg/ml), HEp2 (IC₅₀ -166.8 μ g/ml) and NIH 3T3 (IC₅₀ -216 µg/ml) (Rahate et al., 2012). Essential oil of aerial parts of this grass was found to be considerably antimicrobial against Staphylococcus aureus. Staphylococcus epidermis, Escherichia coli and Pseudomonas aeruginosa (Kumar et al., 2010). Hydroalcoholic extract of this plant showed certain toxicity of 17.4 and 42 % death at 500 and 1000 ppm respectively inferring that *Desmostachya bipinnata* variable levels of toxicity concentrations (Golla et al., 2011). Aqueous extract Desmostachya bipinnata administered urolithiasis induced test group rats at a dose of 400 mg/kg for 10 days depicted significant decrease in the quantity of calcium oxalate deposition in the kidneys. It also reversed all the biochemical changes induced by calcium oxalate urolithiasis (Kishore et al., 2014). Reports show that extracts of D. bipinnata possessed good anti-histaminic activity, which was further confirmed with histamine induced lethality test (Singh et al., 2014). Extracts of D. bipinnata have also been used in biogenic synthesis of silver nanoparticle which could be used for medicinal purposes (Garimella et al., 2014). The hydroalcoholic extract of D. bipinnata showed significant diuretic activity and also increased the urinary output at 500 mg/kg compared to furosemide (P<0.01). It also increased urinary electrolyte concentration (Na+, K+, and Cl-). The results for laxative activity showed minimal increase in feces output (500 mg/kg) compared to standard drug sennosides. (Golla et al., 2014). Ethanolic extract of D. bipinnata has also been shown to inhibit various gram negative and gram positive bacteria and unicellular filamentous fungi (Zain et al., 2014).

Imperata cylindrica Ethnic prominence

Imperata cylindrica is a perennial grass, rhizomatous which is endemic to tropical and subtropical regions found throughout the world and often surpasses areas concerned with human activities. (Holm *et al.*, 1977; Brook, 1989; MacDonald, 2004). Extensive rhizome system, tolerance towards drought and saline soils, seed strategies based on wind dissemination, adaptation towards fire and high genetic plasticity make them persistent grass throughout the world (Hubbard *et al.*, 1944; Holm *et al.*, 1977; Brook, 1989; Dozier *et al.*, 1998). It is also termed as a

successful invasive plant (Lucardi et al., 2014; Su et al., 2014). Imperata cylindrica is found in most of the continents. In Europe, it is found south of Mediterranean Sea and also in North Africa to the Middle East. Also, found in Iran, Afghanistan, Pakistan and as vast stretch of Grasslands in India (Garrity et al., 1997). Both Desmostachya bipinnata and Imperata cylindrica have been mentioned in Rig Veda and Atharvaveda. Charaka and Susrutha have also mentioned these grasses as galactagogue and diuretic (Madhihassan, 1987). Both the plants are ingredients in preparation of Tripanchmool and used in urinary calculi, retention of urine, diabetes, cardiac disorders, gout, common cough and cold, anemia (Ayurvedic Formulary of India, 2000). Young inflorescence, young shoots of Imperata cylindrica are edible after cooking. The fibrous roots are rich in starch and sugar and good to eat. Imperata cylindrica is also used medicinally in traditional practices to treat bacterial infections, helminthes. Also used as an astringent, it is found effective in conditions like arthritis, dysentery, diarrhea, cancers, gonorrhea, diuresis etc. Also used as a febrifuge, emollient and as a tonic (Datta S, 1978). Commercially, *Imperata cylindrica* is used for soil stabilization through stuffing, in paper industry (Dalziel, 1948) and weaving (Singh D, 2002). Commercially, this grass is used as fodder for cattle (Holm *et al.*, 1977) and as a material for thatched roofs in India and South East Asia (Potter *et al.*, 2000).

Bioactive compounds isolated

Early in 1960s, the first sets of compounds were isolated from rhizomes of *Imperata cylindrica*, Arundoin (15) and Cylindrene (16), which were methyl ethers of triterpenes (Nishimoto *et al.*, 1965). After a wide gap, molecule Cylindrene (16), a sesquiterpenoid was isolated again from rhizomes of this plant (Matsunaga *et al.*, 1994).

Two novel lignans, Graminone A (17) and Graminone B (18), were isolated later (Matsunaga *et al.*, 1994), where Graminone B at 10⁻⁴ M gave a 50% inhibition of the contractile response of the aorta

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isolated from rabbit to KCI (30 mM) without affecting norepinephrine (10⁻⁷ M) induced contractions.

Further studies in this plant yielded 4 chromones from methanolic extract of rhizomes, 5-hydroxy-2-(2-phenylethyl) chromone (19), 5-hydroxy-2-[2-(2-hydroxyphenyl)ethyl]chromone (20)

flidersiachromone (21), 5-hydroxy-2-styrylchromone (22) (Yoon et al., 2006). Compounds 19 and 20 showed significant neuroprotective activity against

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glutamate-induced neurotoxicity at 10.0 concentration in primary cultures of rat cortical cells.

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Impecyloside (23) or 6-acetyl-1-[1, 3-(4, 40dihydroxy-3, 30-dimethoxy-b-truxinyl)-b-Dfructofuranosyl]-a-D-glucopyranoside, a novel lignin

glycoside was later isolated from rhizomes (Lee et al., 2008).

Hydroalcoholic extract of aerial parts of this grass yielded several compounds Tricin (6), Jaceidin (24), Quercetagetin-3, 5, 6, 3-tetramethyl ether (25), 3, 5-Di-O-methyl-kaempferol (26), β-Sitosterol-3-0-

β-D-glucopyranosy1-6"tetradecanoate (27),Hydroxy-4-methoxy benzaldehyde (28),daucosterol (11), β-sitosterol (10) and a-amyrin (29) (Mohamed et al., 2009).

For the first time from essential oil extract of aerial parts of *Imperata cylindrica*, a phytotoxic compound called tabanone (30) was isolated (Cerdeira *et al.*, 2012). Tabanone inhibited growth of frond area of duckweed, root growth of garden onion, and fresh weight gain of garden lettuce with 50%

inhibition values of 0.094, 3.6, and 6.5 mM, respectively. The target site of tabanone remains unknown, but its mode of action results in rapid loss of membrane integrity and subsequent decrease in the rate of photosynthetic electron flow.

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Chemical investigation of Imperata cylindrica led to the isolation of new compounds using various chromatographic techniques. Three phenylpropanoids, 1-(3, 4, 5-trimethoxyphenyl)-1, 2, 3-propanetriol (31), 1-O-p-coumaroylglycerol (32), 4-methoxy-5-methyl coumarin-7-O-beta-D-gluco-

pyranoside (33); four organic acids, 4-hydroxybenzene carboxylic acid (34), 3,4-dihydroxybenzoic acid (35), vanillic acid (36), 3, 4-dihydroxybutyric acid (37); one phenolic compound, salicin (38) and triterpenes, fernenol (39), simiarenol (40), glutinone (41) (Liu *et al.*, 2010).

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Advent of 2013 witness many new compounds being reported from this sacred grass with several phenolic compounds reported 4, 7-dimethoxy-5-methylcoumarin (42), 7-hydroxy-4-methoxy-5-methylcoumarin (43), $7-O-\beta-D$ -glucopyranosyl-4-methoxy-5-methylcoumarin (33),

6-hydroxy-5-methoxyflavone (44), 5-methoxyflavone (45), 5, 7-dihydroxy-8-methoxyflavone (46), 4-hydroxybenzaldehyde (47), 4-hydroxy-cinnamic acid (48), 4-hydroxy-3-methoxybenzoic acid (49), 3,4-dimethoxyphenyl-O- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (50) (Rong-hua *et al.*, 2013).

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Also, two new chromones, 8-hydroxy-2-(2-phenylethyl)chromone (51) and 2-(2-phenylethyl) chromone-8-O- β -D-glucopyranoside (52) along with

a novel flavone glycoside, 4'-methoxyflavone-6-O- β -D- glucopyranoside (53) were reported (Xuan *et al.*, 2013).

Recently 4 more new compounds were isolated, impecylone (54), deacetylimpecyloside (55), seguinoside K 4-methylether (56) and impecylenolide (57) along with Impecyloside (23) and seguinoside K (58) (Liu *et al.*, 2013).

Bioactivity of extracts

Many previous reports demonstrate the medicinal properties of Imperata cylindrica. In vitro antihelmintic activity of methanolic extract of roots of Imperata cylindrica against Indian earthworms Pheretima posthuma tested at various concentrations exhibited (10-80)mg/50ml) a maximum antihelmintic activity comparable to standard drug albendazole (1000 mg/50 ml). The extract presented antihelmintic activity in dose dependent manner giving shortest time of paralysis (3.3 min) and death (6 min) with 80 mg/ml concentration (Parvathy et al., 2012). Cytotoxic studies of this plant extracts depicted IC₅₀ values of 12.11 µg/ml (Mia PaCa2 cells), 8.4, 7.18 µg/ml in leukemia cells (CCRF-CEM, CEM/ADR5000). It also showed only 54.84% inhibition of normal HUVEC cells at 80 µg/ml, with EC50 value of 47.73 µg/ml (Kuetea et al., 2010). Further studies in cytotoxicity were done with extracts of this grass which depicted cytotoxicity against cell lines with IC₅₀ values of 3.28 mg/mL [against HCT116 (p53 -/-) cells] to 33.43 mg/mL (against HepG2 cells). Also, studies on cell cycle distribution of CCRF-CEM cells showed extract of I.

cylindrica induced arrest between Go/G1 and S phases. Further, CCRF-CEM cells treated with above IC₅₀ concentrations depicted progressive apoptosis, with percentages in sub-G0/G1 phase ranging from 40.5% (24h) to 80.4% (72h) (Kuete et al., 2013). Also, reports emerged that extracts from rhizomes, inhibited Ouorum-sensing metabolites of CV026 Chromobacterium violaceum with antibacterial zone of 12mm and Quorum-sensing inhibition zone of 20 mm; however, it showed no inhibition on Pseudomonas aeruginosa PA01 (Koh et al., 2011). Antioxidant studies of hydroalcoholic root extract of this plant showed activity of 14.33 ± 0.045 $(10 \mu g/ml)$ to 36.56 ± 0.053 (50 $\mu g/ml)$ by DPPH assay. Antioxidant activity was also confirmed with reduction by FRAP assay (Jayalakshmi et al., 2011). Hexane, ethyl acetate and methanol extracts of this plant worked against Trypanosoma brucei rhodesiense STIB 900 (a parasite) with Mean IC₅₀ of 12.56 ± 0.09 , 42.49 ± 0.99 and 30.37 ± 4.40 respectively (Abiodun, 2012). Aqueous exracts of Imperata cylindrica was also reported to have antiinflammatory activity by relieving the auricular edema in mice induced by dimethyl benzane, ameliorate the paw-swelling in rats by carrageenan, significantly suppressing the increased permeability of celiac blood capillary by glacial acetic acid and was able to remarkably resist paw swelling in rats induced by zymosan A (Yue et al., 2006).

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Conclusions and future perspective

The present review comprises detail description of two medicinal grasses used in India in various Traditional rituals. These plants belong to the same family and are more or less used for similar kind of rituals. The medicinal value of these plants is also documented in traditional Medicinal texts.

Although, many molecules are discussed in this review, it is evident that only few molecules have been isolated so far from *Desmostachya bipinnata*, where most of the molecules are already known to be reported from other plants. Thus, it becomes a matter of thought that more research work has to be done in

this sacred plant, especially with more emphasis on isolation of new unreported molecules. The rich sugar content of this plant, further yields anticipation of glycosidic molecules, which are known for their specialized bioactivities.

Detailed review concerned with *Imperata cylindrica*, depicts that even though plenty of molecules are isolated and characterized, much work has not been done in finding out the best bioactivity of these molecules. Thus, further research should be done in determination of bioactivity of these new molecules which might yield a way in cure and

management of uncontrollable diseases therapeutically.

Since, both these plants have been reported to survive under harsh environmental conditions and are potentially unaffected by pests, insects and diseases, more pesticidal and anti-microbial molecules against phytopathogens could be screened such as Tabanone from *I. cylindrica*.

Taking into account all these aspects, it seems that through more research work in similar or allied domains of therapeutic realms, it is possible to find more bioactive molecules which could be utilized for expanding our understanding of management of challenging diseases.

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