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DILLENIA PENTAGYNA: A COMPREHENSIVE REVIEW OF ITS TRADITIONAL USES, PHYTOCHEMISTRY AND PHARMACOLOGICAL POTENTIAL.

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ABSTRACT

Dillenia pentagyna Roxb is commonly known as 'Mota Karmal' respectively belonging to family Dilleniaceae. These plants are distributed in many Asian countries, in India from Himalaya to south India. Morphologically leaves, bark and fruit are having major differentiating characters of both species. Traditionally the different parts of these plants have curing properties like cancer, wound healing, diabetes, diarrhea, bone fracture, in cut and burns, abdominal pains etc. Based on phytochemical investigations these plants are reported to contain active constituents like betulin, betulinic acid, dillenetin, dipoloic acid, myricetin, quercetin derivatives etc. Different prepared extracts of these plants and their parts has been reported to contain wide range of flavonoids, steroids, triterpenoids, phenolics, saponins, fixed oil which may exert varied pharmacological activities like anticancer, antidiarrheal, antimicrobial, antioxidant and many more. The present review approaches for pharmacognostical description, phytochemical investigations of the species.

Keywords: Butilinic acid, Dillenetin, Dipoloic acid, Myricetin, Quercitin

INTRODUCTION

Dillenia pentagyna roxb is a medicinally important plant belonging to family Dilleniaceae. It is widely distributed in tropical and subtropical regions of South and Southeast asia, including India. India is one of the world's 12 biodiversity centers with the presence of over 45000 different plant species [(1)].

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Volume 16, Number 1, 2026
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The plant is commonly found in deciduous forests and has many traditional uses in ayurveda, folk medicine and indigenous health care practices. Different parts of the plant including bark, leaves, roots, and fruits are traditionally used for the treatment of various ailments. (2)

Dilleniaceae is a family of evergreen shrubs, sub-shrubs, or climbers comprising about 12 genera including Acrotrema, Curatella, Davilla, Didesmandra, Diliocarpus, Hibbertia, Panchynema, Pinzona, Schumacheria, Tetracera and Traxilisa. The leaves are simple, wide, well developed and alternate. The stipules are absent or wing-like. The flowers are mainly bi-sexual or rarely unisexual with colourful petal(white, yellow or red) and visible reproductive component. The fruit is follicle or berry like, sometimes edible, either dehiscent or indehiscent, and enclosed with fleshy calyx. (3)

There are many plant species which has been used by tribal and folk communities of various forest regions of India but their pharmacological as well as phytopharmacological importance is yet unknown as these plants are rarely available. (4) Ethnomedical reports indicate that the plant is used for various disorders such as inflammation, wounds, diarrhoea, fever, diabetes and liver disorders. (5) (6) (7)

There are several methods such as agitation, maceration, percolation and Soxhlet extraction that have already been developed for extraction and preconcentration of bioactive components from D. pentagyna. These techniques require a longer duration of extraction , such as maceration(2-3 days), Soxhlet extraction(20-24hrs) and percolation(4-5 hrs) as well as large amount of organic solvents for extraction of phytochemicals(6)

. These traditional claims have attracted scientific interest leading to several preclinical studies aimed at validating its therapeutic potential. Phytochemical investigations have revealed the presence of bioactive compounds such as flavonoids, triterpenoids, tannins, phenolics, saponins which are believed to contribute to its pharmacological activities. Therefore this review aims to summarize and critically analyze the traditional uses, phytochemical constituents and pharmacological properties of *Dillenia pentagyna* with special emphasis on evidence from preclinical studies.(8)

In developing countries, the prevalence of diabetes is increasing very fast. The World Health Organization (WHO) estimated that around 70 million people suffering from diabetes mellitus. Globally, DM presents enormous and increasingly important public health issues. The prevalence of DM in all age groups are estimated to be 2.8% and the expected rise is up to 4.4% by 2030. The mechanism of alloxan action has been intensively studied, predominantly in vitro. Using isolated islets.(9a) and perfused rat pancreas(10) It was proved that alloxan denominates an emergent elevation in insulin egestion in the presence or absence of glucose. It was validated that alloxan educes a quick rise in insulin secretion in the presence or absence of glucose. This seemed just after alloxan treatment and was not observed after repetitive exposure of islets to this diabetogenic agent(11b) Many researchers recommended that the choosiness of alloxan act is not fairly suitable(12) The diabetogenic reprentative alloxan is having tendency to mix with water and chemically changeable compound. As an outcome there is a urge to quest for compounds with effective anti diabetic activity when contracted orally(13) An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Catalase, peroxidase, superoxide dismutase and the non enzymatic antioxidant compounds

Volume 16, Number 1, 2026
ISSN 2249 – 5738
Original Article

such as phenols, ascorbate and glutathione have been viewed as a synergistic antioxidant defensive system, whose combined purpose is to protect cells from active oxygen damage[14] The nutrient rich antioxidant deficiency is one of the causes of numerous chronic and degenerative pathologies[15] But we clearly knew that the use of oral antidiabetics is limited due to their adverse side effects including hematological, hypoglycaemic coma and disturbances of liver and kidney functions[16] About 1.5 billion of the world population are being treated by herbal medicines prepared from medicinal plants which demand new compounds discovery with promising remedial effects and negligible side effects contributing to the treatment of pain, fever, diarrhea and thrombus.[17] Molecular docking is a structure based drug design technology that stimulates the molecular interactions and predicts receptor ligand binding mechanism and affinity[18] Docking may be used to do virtual screening on vast libraries of compounds, grade the findings and provide structural insights into how the ligands interact with target, which are immensely advantageous for lead optimization.[19] Therefore, molecular docking is becoming more popular method for discovering a new drug. Docking against homology modelled targets is now possible for a larger number of proteins as the number of experimentally known structures grows.[20] Thus research should be carried on so that these can lead us to new drug development in the future[21] Dillenia pentagyna Roxb is an ethnomedicinal plant grown in the different district in Bangladesh. The plant is locally known as Banchalta, Hargaza, Ajuli. The tribal people and folk people utilize this species for the treatment of numerous diseases like bone fracture (leaf), pain (root) etc[22,23]

Taxonomical classification.[24]

Kingdom: Plantae

Division: Phanerogamic

Sub-division: Angiospermae

Class: Dicotyledonae

Subclass: polypetalous

Order: Millennials

Family: Dilleniaceae

Genus: *Dillenia*

Species: *pentagyna* Roxb or *Hainanese* Merrill.

PHARMCOGNOSTICAL DESCRIPTION

It is deciduous trees up to 15 m tall and 1 m d.b.h . Bark is grayish in colour, smooth, exfoliating; branchlets glabrous, stout. Leaves are petiolate 2-5 cm, glabrous with narrow wings, shape is oblong to obovate-oblong, 20-60×10-25 cm in size, leathery surface, secondary veins 25-50 on either side, showing parallel margin with shallowly undulate teeth, apex. Flower are 2-7 in number, small fascicled at top of lateral spurs, 2-3cm in diameter, less than 2cm in diameter in bud, pedicels 2-4cm, bractelt is deciduous. Total 5 sepals and petals, yellow coloured and obovate. Stamens are in 2 distinct groups, outer 60-90, 3-4 mm, slightly curved in bud, carpels are 5 0r 6, 3.5-4mm in diameter, stylodia spreading containing 5-20 ovules per

Volume 16, Number 1, 2026
ISSN 2249 – 5738
Original Article

carpel. Pseudocarp is indehiscent yellow/orange/red in colour. Flowering starts in April-May. Fruits are globose in shape, 0.5-1 cm in diameter, indehiscent, greenish in colour.[25- 30]



Fig: Dillenia pentagyna : a) Tree b) Leaf, c) Flower, d) Fruits e) seeds[9]

Qualitative Phytochemical Analysis of DPA

For preliminary phytochemical screening, a stock solution of the sample was prepared by dissolving 500 mg of the aqueous extract of *D. pentagyna*stem bark (DPA) in 100 mL DW. The solution was subsequently filtered through Whatman No. 1 filter paper to obtain a clear filtrate. This filtrate, as well as the dried aqueous extract were used to assess the presence of various phytochemical constituents. Qualitative screening was conducted

following the standard protocols outlined by Trease & Evans (1989)[16] and Rao et al. (2016),[17] briefly summarized as follows:

• Test for alkaloids (Mayer's test): Add 2 mL extract + 2 mL Mayer's reagent.Cream/yellow precipitate indicates presence.

• Test for flavonoids (Shinoda test): Add 2 mL extract + few Mg turnings + followed by dropwise conc HCl. Pink/Crimson-Red colour indicates presence.

• Test for phenols (tannins) (Ferric chloride test): Add 2 mL extract + few drops 5% $FeCl_3$.

Volume 16, Number 1, 2026
ISSN 2249 – 5738
Original Article

Blue-black/green coloration indicates presence.

• Test for saponins (Foam test): Shake 5 mL extract with 20 mL DW in a test tube vigorously for 2 minutes. Persistent foam >10min indicates presence.

• Test for cardiac glycosides (Keller-Kilianitest): Add 2 mL extract + 3 mL glacial aceticacid + 1 drop 5% $FeCl_3$ + 1 mL conc. H_2SO_4.Brown ring at interface, violet ring at acetic acid layer indicates presence.

• Test for steroids (Liebermann-Burchard test):Add 2 mL extract + 2 mL chloroform + 2 mLacetic anhydride + few drops conc. H_2SO_4.Blue-green ring indicates presence.

• Test for triterpenoids (Salkowski test): Add 2mL extract + 2 mL chloroform + 2 mL conc.

H_2SO_4. Red color in lower layer indicates presence.

• Test for coumarins: Add 2 mL extract + 3 mL10% NaOH. Yellow color indicates presence.

• Test for quinones: Add 2 mL extract + 1 mLconc. H_2SO_4. Add 2 mL extract + 1 mL conc.

H_2SO_4. Red color indicates presence.

• Test for proteins and amino acids: Add 2 mLextract + 1 mL 10% NaOH + 2 drops 1%

$CuSO_4$. Violet/pink color indicates presence.

BIOLOGICAL ACTIVITIES

Anti-diabetic activity:

According to reported study, the leaf extract at a concentration of 2µg/ml exhibited the highest inhibitory activity, whereas no such anti-diabetic activity was seen with the fruit extracts. [18] in contrast, another article reported that in induced diabetic rats, administration of the hydroalcoholic fruit extract at a dose of 400mg/kg body weight produced significant antihyperglycemic effects compared to the lower dose 200mg/kg body weight, as evidenced by improvement in various biochemical parameters, including liver enzymes, liver tissue regeneration and favourable metabolic effects. [19]

Anti-microbial activity:

A study revealed that petroleum ether and ethyl acetate extracts of the sundried stemm bark of D. pentagyna exhibited notable antibacterial activity at a conc. of 3mg/disc, producing inhibition zones ranging from 10 to 23m against Bacillus cereus, B. subtilis, B. polymyxin, Shigella sonnei, Staphylococcus aureus, S. flexneri type-1, and Vibrio cholerae, when compared with ampicillin. The same study also reported that the ethanol extract demonstrated antifungal activity Aspergillus fumigatus, A. niger, Candida albicans, C. arriza, Saccharomyces cerevisiae and Trichoderma sp., with inhibition zones ranging from 10 to 17mm, in comparison with griseofulvin. [20] Another report indicated that the methanolic extract of the stem bark exhibited antibacterial activity at 100mg/disc, inhibiting the growth of B. subtilis, B. cereus, S. aureus, Escherichia coli, P. aeruginosa and Salamonella sp., with inhibition zones of 7-10mm, when compared with streptomycin. Additionally, the extract showed antifungal efficacy against Trichophyton verrucosum, penicillin sp., and Aspergillus niger at 1mg/disc, producing zones of 6-10mm, in comparison with albendazole. [21] Furthermore, it was reported that the

chloroform, ethyl acetate and butanol fractions of the aqueous methanolic extract inhibited the growth of municipal sewage microbes, producing inhibition zones of 7-19mm at concentrations ranging from 2 to 3mg/disc, when compared with ciprofloxacin. [22]

Anticancer and antioxidant activity:

D. pentagyna has been reported to shows significant anticancer and antioxidant activities in both in vivo and in vitro studies. The ethanolic extract administered at 50 and 100mg/kg/day demonstrated potent anticancer effects, increasing life span by 55% and 48%, respectively and reducing lipid peroxidation in tumor bearing mice, indicating protection against oxidation stress induced tissue damage. Fruit extracts (20% aqueous methanol and acetone) also showed notable antioxidant activity in FRAP and DPPH assays, with anti-oxidant capacities of 13.19 and 5.03mg ascorbic acid equivalents/g dry fruit and IC_{50} values of 0.51 and 2.21mg/dry fruit, comparable to BHT [23] Stem and bark extracts were reported to be cytotoxic against DL, MCF-7 and HeLa cell lines (IC_{50}: 25.8-76.8µg/mL) and were associated with reduced glutathione levels in treated animals. [24,25] Additionally, treatment lowered sialic acid levels, a tumour marker, in multiple organs and Dalton's lymphoma cells, along with a reduction in ascites tumour volume. [26] The aqueous stem bark extract (90%) exhibited antioxidant activity comparable to ascorbic acid in the DPPH assay. [27] Chloroform and butanol concentration dependent DPPH radical scavenging activity, with inhibition ranging from 6-7% when compared to ascorbic acid. [28]

In Vivo Anti-diarrheal activity

The antidiarrheal activity of methanolic extract of D. pentagyna was evaluated using the castor oil-induced diarrhoea model in mice. In this method, mice weighting 20-25g were fasted for 18hours prior to experimentation. The animals were divided into different groups, each comprising six mice. The control group received normal saline, while the standard group was treated with loperamide. (5mg/kg) The test groups were administered MEDP at doses of 100, 200 and 400mg/kg orally. After one hour of treatment, all animals were given 1ml of castor oil orally to induce diarrhoea. Following castor oil administration, the mice were placed individually in cages lined with absorbent paper and observed for 4 hours to record diarrheal feces. The total number of feces in the control group was considered as 100% and the antidiarrheal effect of the extract was expressed as the percentage inhibition of diarrhoea compared to the control group. [29]

In Vitro Thrombolytic Activity

The in vitro thrombolytic activity was evaluated using the method described by Ahmed et al [30] In this assay, 5ml of venous blood was collected from healthy volunteers and distributed into preweighed sterile Eppendorf tubes (0.5ml per tube). The tubes were incubated at 37^0C for 45minutes to allow clot formation. After clot formation, the serum was carefully removed without distributing the clot, and the tubes were weighed again to determine the clot weight. Subsequently, 100µl of the test extract solution was added separately to each tube. Streptokinase(100µl) was used as a positive control, while 100µl of distilled water served as a negative control. All tubes were incubated at 37^0C for 90 minutes. Following incubation, the released fluid was carefully removed, and the tubes were weighed again to assess clot lysis.

Volume 16, Number 1, 2026
ISSN 2249 – 5738
Original Article

The percentage of clot lysis was determined by comparing the clot weight before and after treatment.

In Vivo antipyretic activity

The antipyretic activity of the test sample was evaluated using the Brewer's yeast-induced pyrexia model with slight modifications, as described in previous studies. [31,32] Initially, the basal rectal temperature of each mouse was recorded using a digital thermometer. Pyrexia was induced by subcutaneous administration of a 15% w/v suspension of Brewer's yeast in distilled water at a dose of 10ml/kg body weight. After 18hrs of yeast injection, rectal temperature was measured again, and only animals showing an elevation of at least 0.6^0F (0^0C) were slected for further experimentation. [33] The selected animals were randomly divided into five groups, each comprising six mice. Group I served as the control and received 1% Tween-80 in normal saline orally. Group II was treated with standard antipyeric drug, paracetamol (10mg/kg) Groups III, IV and V were adminsterd the methanolic extract at doses of 100, 200 and 400mg/kg, respectively. Rectal temperature of all animals were recorded at 0, 1, 2, 3 and 4 hrs after treatment to assess the antipyretic effect.

Others

In addition to its established biological properties, Dillenia pentagyna has been reported to exhibit several other therapeutic benefits. The fruit extract has demonstrated the ability to inhibit angiotensin converting enzyme (ACE), [34] suggesting potential antihypertensive activity. Furthermore, bioactive extracts and fractions derived from bark of D.pentagyna have shown protective effects against doxorubicin (Dox)-induced cardiotoxicity. LC-QTOF-ESI-MS analysis of *D. pentagyna* and its phenolic-rich fraction (F1) identified polyphenolic antioxidant compounds such as gallic acid, syringic acid, and sinapic acid, which are believed to contribute to its significant cardioprotective effects. These findings highlight the broader therapeutic potential of *D. pentagyna* in managing cardiovascular and related disorders. [35]

Conclusion

The extensive literature survey as well as reports on research revealed that Dillenia pentagyna are highly regarded to have good potential in the herbal medicine. Detailed pharmacognostical description will give an overview about the identification of these plants in the different forest regions throughout India. Betulin and betulinic acid are the major constituents found to be present in almost all the parts of the plants which can serve good potential in curing various ailments and diseases.

Phytoconstituents like flavonoids, steroids, triterpenoids, phenolics, saponins, fixed oil which may be responsible for good pharmacological activities performed on animal models. From available reports it has been observed that different parts of D. pentagyna have curing properties like wound healing, diabetes, bone fracture, in cut and burns, abdominal pains and many more but scientific evidence of these reports is yet not much developed. Few pharmacological investigations has been done using different parts like leaves are having various activities like antioxidant, cytotoxic, antimicrobial, antidiarrheal and anxiolytic. Others parts like seeds are hepatoprotective and antimicrobial, fruit used as antileukemic. Bark of D. pentagyna is having good antitumour potential against Dalton's lymphoma. Other parts are also having good therapeutic potential; therefore large-scale, controlled pharmacological study is

Volume 16, Number 1, 2026
ISSN 2249 – 5738
Original Article

needed to validate these results. Secondly, there is still little evidence for quantification of different active phytoconstituents which may be responsible to have good pharmacological activity. It is evident from the available reports and literatures that this plant belonging to family Dilleniaceae possesses adequate therapeutic potential and could be explored further for chemical and pharmacological investigations. Additionally, employing new modern techniques, which are more economical for better extraction of the required active phytochemicals needs to be applied. D. pentagyna is available only in forest regions and rarely cultivated but found to have good therapeutic potential so further evaluation needs to be carried out on these plants in order to explore the concealed areas and their practical pharmacological as well as clinical applications, which can be used for the welfare of the mankind.

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Original Article

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