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Research Article

ANTIBACTERIAL, ANTI-ALPHA GLUCOSIDASE AND ANTIOXIDANT PROPERTIES OF *DILLENIA PENTAGYNA* ROXB. (DILLENIACEAE)

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ABSTRACT

Objective: The persent investigation is carried out to assess *in vitro* antimicrobial, antioxidant and anti-alphaglucosidase activity of the crude and fraction extracts of both leaf and fruits of the plant *Dillenia pentagyna Roxb.* (Dilleniaceae).
Methods: Extracts prepared from leaf and fruits by drying and fractionation process. Antibacterial activity assessed by disc diffusion and liquid culture method. Antioxidant activity was assayed by superoxide radical scavenging capability and by DPPH scavenging capability. By using the enzyme α-glucosidase and substrate p-nitrophenyl glucopyranoside (pNPG), the inhibitory activity of the extracts was assayed.
Results: Crude extracts of fruits from both butanol fraction and chloroform fraction showed promising antibacterial activity in disc diffusion method through measuring inhibition zone. Both butanol and chloroform fraction showed a significant superoxide radical scavenging activity and using DPPH (1,1-diphenyl-2-picryl hydrazyl), butanol fraction extract of fruit showed a significant scavenging property. α-glucosidase (E.C No. 3.2.1.20) enzyme activity is inhibited significantly by the leaf extract of the plant.
Conclusion: The results suggest that the crude plant extract contain some compounds which have antimicrobial activity, more potent antioxidant activity as well as α-glucosidase inhibitory activity.

Keywords: Dillenia pentagyna, antimicrobial, antioxidant, antidiabetic, superoxide, α-glucosidase, DPPH.

INTRODUCTION

Plants are used medicinally in different countries and are a source of many potent and powerful drugs [1]. A wide range of medicinal plant parts are used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. Many raw drugs are traded in the market as the raw material for many herbal industries [2]. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of plants have not been adequately evaluated [3].

Dillenia pentagyna Roxb. is a plant of Dilliniaceae family (Karmal family), locally known as 'Hargaza', is widely distributed randomly in hilly areas of north east states of India and Bangladesh. The synonyms of the plant are *Dillenia floribunda*, *Dillenia hainanensis*. *Dillenia indica* is another widely used plant of this family. Recent studies have shown that the extract of the bark of this plant had antibacterial and cytotoxic activity [4].

An ethnobotanical study among the various tribal and folk communities of Vindhya Region, Madhya Pradesh, reveal that they use the various parts of it for the treatment of their different ailments and diseases, viz, delivery (bark), bone fracture (leaf), body pain (root), piles(leaf), diabetes (bark), diarrhea and dysentery (bark) [5]. A wide range of pharmacological and biological activities have been shown to be present in the secondary metabolites isolated from plants belonging to family Dilleniaceae. Among those metabolites, terpenoids and flavonoids were the most well known, though, a large amount of alkaloid have also been found. Betulinic acid has been isolated from *Dillenia indica* & *D. return*, which exhibited high antitumor activity [6]. The antibacterial and antifungal activity of the crude extract has been shown in vitro studies [7].

The methanolic extract of *Dillenia indica L.* fruits showed significant anti-leukemic activity in human leukemic cell lines U937, HL60 and K562 along with a major compound, betulinic acid, was isolated from the ethyl acetate fraction by silica gel column chromatography and was identified and characterized [8]. Betulinic acid could explain the anti-leukemic activity of the methanolic extract and the ethyl acetate fraction. This finding led to fractionation of the methanolic extract of the species *Dillenia pentagyna*, on the basis of polarity. In this study, antibacterial, antioxidant and anti-α-glucosidase activities have been tested using the different fractions of fruit and leaf of the plant.

MATERIALS AND METHODS

Plant materials

The plant materials were collected locally as it grows well in hilly areas of North-East India.

Preparation of plant extracts

The leaves and fruits of *Dillenia pentagyna* were collected separately from a hilly area of South Tripura district. The collected materials were dried in shade and then grinded. The grinded matters (fruits and leaves) were then extracted separately with 10% aqueous methanol by percolation method. The methanolic extract of fruits was then concentrated and defatted with petroleum benzene (60-80) and then fractionated into $CHCl_3$, EtOAc, BuOH. The polar fractions were designated as DPF-C, DPF-E, DPF-B respectively. In a similar manner methanolic extract of leaves was also fractionated and designated as DPL-C, DPL-E, DPL-B. Antimicrobial and antioxidant activity of different fractions of leaves and fruits have studied.

Microbial samples

Eight human pathogenic microbial strains were analyzed viz. *K. pneumonia* (BCH 271*), S. flexneri* 16, *S. dysenteriae* 1, *V. cholerae* non.0139(L4), *V. cholerae* non.0139(CSK6669), *S. pneumoniae, S. aureus* and *E. coli.* These strains were obtained from National Institute of Cholera & Enteric Diseases, Kolkata India and Agartala Government Medical College, Agartala, India.

Anti-bacterial Activity

The anti-bacterial activity of the extracts of *D. pentagyna* was tested by the disc diffusion method [9] and liquid culture method [10] with some modifications on selected microorganisms.

Disc Diffusion Method

Different concentration of the crude extracts (1 mg, 2 mg, 3 mg) were prepared by reconstituting with N-N'-dimethyl formamide (DMF). The test microorganisms (freshly cultured) were seeded into respective medium (Nutrient Agar, SRL) by spreading of 40 µl of freshly prepared each strains after solidification of agar media. The paper disces (5 mm in diameter and 0.4 mm in thickness) were then placed in petridishes (100 mm in diameter) containing different bacterial strains in agar media, then each of the different concentrations of extracts applied on each disc (6 µl for leaf extract and 15 µl for fruit extract). DMF were also applied as control for each extract respectively. The plates were then incubated at 37 ^{0}C for 24 hours. The antimicrobial activities were measured by zone of inhibition expressed in mm. All experiments were repeated three times and the mean of the readings were recorded.

Liquid Culture Method

Each microorganism was primarily cultured in 3ml LB broth medium for 5 to 6 hrs at 37^{0}C. Then 15µl of each extract were added to each test microorganism cultured to make final concentration of 1mg/ml and incubated again at 37^{0}C for overnight. Next day the optical densities (OD) at 600nm were measured in a spectrophotometer. The untreated culture for each microorganism used as control. The experiments were carried out five times (n=5) and the data were expressed as mean ±SD.

Chemicals required for *in vitro* antioxidant activity and anti-diabetic activity

1,1- diphenyl-2-picryl hydrazyl (DPPH) from SIGMA-ALDRICH, USA, Butylated hydroxyl anisole (BHA) and methanol from MERCK Chemicals, Germany, acarbose drug from Buyers Pharmaceuticals, Germany all other chemicals including NBT, NADH, phenazonium methosulphate (PMS), p-nitrophenyl α-D-glucopyranoside (p-NPG), α-glucosidase, Na_2CO_3 and all solvents were obtained from SISCO Research Laboratories Pvt. Ltd, Mumbai, India.

Antioxidant activity

The leaf and fruit extracts were tested for its free radical scavenging property using superoxide radical scavenging activity and all experiments were performed five times and the results averaged.

Superoxide radical scavenging activity

Superoxide scavenging activity of *D. pentagyna* extracts were measured according to the method of *Robak et. al* with some modifications [11]. All the solutions were prepared in 100 mM phosphate buffer (pH 7.4). 0.5ml of NBT (156 µm), 0.5ml of NADH (468 µm), 1.5ml of extracts (to produce final concentrations of 1-200µg/ml) were mixed. The reaction was started by adding 50 µl of PMS (60µm) and the mixture then incubated at 25^{0}C for 5 min followed by measurement of absorbance at 560 nm. Same procedure was used as negative control using distilled water instead of the extracts. Butylated hydroxyl anisole (BHA) were used as a positive control, because it is a synthetic antioxidant [12], dissolved in methanol against the extract concentrations. Now the percent of inhibition was measured by using following formulae.

$$\% \text{ inhibition} = \frac{\text{O.D. of Control} - \text{O.D. of Test}}{\text{O.D. of Control}} \times 100$$

DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method of Cotelle *et al.* with some modification [13]. In brief, 1.5ml reaction mixture containing 100µl of DPPH (100µM in methanol) and 1.4 ml of butanol fraction of fruit extract (at various concentration; 0.5-50µg/ml) in distilled water was incubated at 37ºc for 30 min and absorbance was read at 517 nm using UV-VIS Spectrophotometer. Here, L-ascorbic acid was used as a positive control against the concentration of each extract [14]. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using above formula.

Anti-diabetic activity

The leaf and fruit extracts were tested for its anti-diabetic activity using α-glucosidase inhibitory assay and all experiments were performed five times and the results averaged.

α-Glucosidase inhibitory assay

The enzyme inhibition assay was done by taking 50 µl of α-glucosidase (0.15 unit/ml) and 50 µl of sample added to start the reaction with 100µl of 3 mM p-nitrophenyl glucopyranoside (pNPG) in 0.2 M sodium phosphate buffer (pH 6.8) as a substrate. The reaction was conducted at 37ºC for 15 min and stopped by the addition of 750 µl of 0.1 M Na_2CO_3. α-Glucosidase activity was assessed by measuring the release of p-nitrophenol as color measurement from pNPG at 405 nm. Acarbose was used as a positive control [15]. All tests were performed in independently five times (n=5) and data were expressed as mean ±SD. Percent of inhibition was calculated using above formulae.

Statistical analysis: All the experimental data were calculated and statistical analysis completed using Microsoft Office Excel 2010.

RESULTS AND DISCUSSION

Antibacterial Activity

Results obtained in the present study revealed that the tested *D. pentagyna Roxb.* extracts posses potential antibacterial activity against , *S. dysenteriae* 1, *V. cholerae* non.0139(L4), *V. cholerae* non.0139(CSK6669) and *E. coli*. The test performed by the disc diffusion method and liquid culture method.

Table1: **Effect of leaf and fruit extracts by disc diffusion method on different pathogenic bacterial strains. NS= Not Significant**

Microorganisms	Zone of inhibition in mm after overnight incubation. (mean of 3 repeats ±SD)								
	Leaf Extract			Butanol Fraction of fruit extract			Chloroform Fraction of fruit extract		
	1 mg/ disc	2 mg/ disc	3 mg/ disc	1 mg/ disc	2 mg/ disc	3 mg/ disc	1 mg/ disc	2 mg/ disc	3 mg/ disc
S. dysenteriae 1	6 ± 0.18	8 ± 0.19	9 ± 0.31	NS	7 ± 0.20	10 ± 0.37	9 ± 0.49	10 ± 0.19	12 ± 0.4
V. cholerae non.0139 (L4)	NS	6 ± 0.19	8 ± 0.4	7 ± 0.2	9 ± 0.2	11 ± 0.3	NS	NS	7 ± 0.4
V. cholerae non.0139 (CSK6669)	NS	6 ± 0.19	7 ± 0.4	8 ± 0.2	9 ± 0.3	12 ± 0.4	NS	6 ± 0.19	7 ± 0.4
E. coli	8 ± 0.19	9 ± 0.49	10 ± 0.33	7 ± 0.3	10 ± 0.23	12 ± 0.4	NS	NS	NS

In disc diffusion method, the crude extracts from the leaf of *D. pentagyna Roxb.* showed (**Table-1**) significant antibacterial activity against E. *coli*, *S. dysentariae* 1 and the inhibition zone is around 10 mm. The lowest activity recorded in *V. cholerae* non.0139 (CCK 6669) where inhibition zone is measured 7 mm. The fruit extract (butanol fraction) exhibit highest activity against *V. cholerae* and E. coli (12 mm) and lowest in *S. dysenteriae* 1 (10mm). The fruit extract (chloroform fraction) posses maximum activity against *S. dysenteriae* (12 mm) and the minimum zone of inhibition observed in both *V. cholerae* strains.

In liquid culture method growth inhibition of bacteria is measured by reading the O.D of the liquid culture medium. In absence of extract, growth of bacteria will be considered as 100% i.e; no inhibition. But if the growth of the microorganism is inhibited by the extracts can be measured comparing the control tube with test according to the formula mentioned above.

Table2: Effect of leaf and fruit extracts by liquid culture method on pathogenic bacterial strains.

Microorganisms	Growth inhibition in liquid culture (mean of 5 repeats % inhibition ±SD) at 1mg/ml concentration of extract		
	Leaf extract (crude)	Butanol fraction of Fruit Extract	Chloroform fraction of Fruit Extract
K.pneumoniae (BCH27)	19.382±2.292	13.998±1.247	16.928±1.914
S. dysenteriae 1	64.998±7.979	33.772±3.868	35.836±3.399
V. cholerae non.0139 (L4)	47.812±3.631	64.294±5.573	60.434±6.146
V. cholerae non.0139 (CSK6669)	7.06±0.979	13.812±1.832	20.074±1.265
E.coli	25.8±2.708	37.172±4.96	17.53±1.512
S. aureus	27.666±2.816	44.284±3.506	24.978±4.934
S .pneumoniae	15.6±2.563	36.994±3.348	32.428±3.38

In this study leaf extract showed (**Table-2**) significant inhibition (64.998%) on *S. dysenteriae* 1. All the three extracts of the plant showed a significant growth inhibition to *V. cholerae non 0319(CSK6669) strain.* Other strains also inhibited by the extracts modestly. Whether these extracts are bactericidal or bacteriostatic is not known. Plants are rich in antibacterial compound including tannin, terpenoids, alkaloids and flavonoids [19]. But exactly which compound is present in this extract is yet to be analyzed.

Superoxide radical scavenging activity

Free radicals are associated with various physiological and pathological events such as inflammation, aging, mutagenicity and carcinogenicity. Reactive oxygen species (ROS) capable of damaging DNA, proteins, carbohydrates and lipids are generated in aerobic organisms. These ROS include superoxide anion radical (O_{2^-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and singlet molecular oxygen [11]. The different portions of the plant extracts are used if the ROS can be scavenged by these extracts and can save the host cell.

Table3: Effect of leaf and fruit extracts to assess superoxide radical scavenging activity

Concentration of extracts and BHA	Percentage of inhibition (Mean ± SD) each of 5 experiments.			
	Leaf extract	Fruit extract		BHA as positive control
		Butanol fraction	Chloroform fraction	
1 µg/ml	5.911 ±2.676	15.561 ±0.710	11.648 ±3.544	10.146 ±4.170
25 µg/ml	54.178 ±4.210	30.987 ±1.191	28.489 ±3.125	15.345 ±3.888
50 µg/ml	67.980 ±1.572	52.774 ±3.828	52.398 ±1.190	17.902 ±0.946
75 µg/ml	70.443 ±0.827	66.508 ±0.234	64.911 8±1.574	23.020 ±3.472
100 µg/ml	67.980 ±0.837	73.410 ±0.700	72.953 ±1.695	27.115 ±7.446
200 µg/ml	37.438 ±2.020	70.568 ±1.086	60.836 ±1.426	37.042 ±9.575
Control (Negative with water without extract)	0	0	0	0

Calculating percentage of inhibition of the extract on superoxide radicals *in vitro* condition, scavenging activity is measured. Superoxide anion is also very harmful to cellular components. It has been reported that flavonoids are effective antioxidants mainly because they scavenge superoxide anions [16]. As shown in **Table-3**, the superoxide radical scavenging activities of this plant extract and the reference compound are increased markedly with increasing concentrations. Highest percentage of inhibition is found in case of leaf extract (70.4%) for the concentrations of 75µg/ml. In case of fruit extract of butanol fraction shows 73.4% inhibition for the concentrations 100 µg/ml. Fruit extract of chloroform fraction shows 72.953% inhibition for the concentrations of 100µg/ml. Several laboratories used BHA as a good ROS scavenger [21] and in this study maximum inhibition with BHA is 37.042 % in its 200 µg/ml concentration where this plant extract shows higher inhibition in lower concentration than the positive control.

DPPH radical scavenging assay

DPPH is a relatively stable free radical and the assay determines the ability of the extract to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electrons to paired ones. Antioxidants exert their activity by converting the unpaired electrons to paired ones. The yellow coloured diphenylpicrylhydrazine is formed due to reduction of stable redical DPPH by extract. The antioxidants property of the extracts reduce the absorbance of the tests and this might be due to the hydrogen donating ability resulted in the scavenging of the radical [17].

Table4: Effect of leaf and fruit extracts to assess DPPH radical scavenging activity

Concentration of extracts and L-Ascorbic acid	Percentage of inhibition (Mean ± SD) each of 5 experiments.			
	Leaf extract	Fruit extract		L-Ascorbic acid as positive control
		Butanol fraction	Chloroform fraction	
500 ng/ml	7.124 ±1.225	12.089 ±3.680	6.415 ±2.066	35.714 ±6.705
1 µg/ml	13.524 ±2.396	22.959 ±3.410	12.622 ±1.550	55.535 ±1.801
5 µg/ml	45.688 ±3.723	40.625 ±4.194	20.569 ±3.165	64.643 ±3.232
10 µg/ml	63.098 ±3.422	65.522 ±6.374	26.193 ±1.731	74.560 ±4.477
25 µg/ml	70.614 ±0.938	72.761 ±1.395	34.721 ±3.035	84.365 ±1.155
50 µg/ml	67.072 ±2.455	65.646 ±1.831	61.886 ±3.384	90.555 ±2.915
Control (Negative with water without extract)	0	0	0	0

In this study the butanol fraction shows DPPH reduction significantly at different concentrations (50µg/ml-65.6%, 25µg/ml-72.7% and 10µg/ml-65.5% of inhibition as shown in **Table-4**) where as other fractions also can reduce DPPH significantly at the concentration of 50µg/ml though none of these can reach up to the control's level.

α –glucosidase inhibitory assay

Diabetes is still not completely curable by the present anti-diabetic agents. Insulin therapy is the only satisfactory approach in diabetic mellitus, even though it has several drawbacks and the major one is insulin resistance [18]. Herbal drugs are gaining popularity in the treatment of diabetic mellitus [19] as the side effect is none or very negligible. The major advantages of herbal medicine seem to be their efficacy, low cost, and low incidence of side effects.

Table5: Effect of leaf extract to assess the α-glucosidase inhibition compared to acarbose

Concentration of leaf extract and Acarbose	Percentage of inhibition (Mean of 5 repeats ± SD)	
	Leaf extract	Acarbose as positive control
250 ng/ml	3.441 ±7.761	47.310±8.499
500 ng/ml	29.240 ±11.54	50.160±11.822
750 ng/ml	45.518 ±16.493	58.250 ±6.477
1000 ng/ml	46.440 ±15.054	63.620 ±3.832
2000 ng/ml	74.694 ±11.540	65.440±3.931
5000 ng/ml	70.327 ±4.303	79.640±3.394
Control (Negative with water without extract)	0	0

α–glucosidase inhibition is another *in vitro* experiment to assess the indirect anti-diabetic activity [20] of the plant extract. As shown in **Table-5**, the leaf extract shows highest 74.6 % inhibition for the concentration of 2 µg/ml. But with fruit extracts is no such activity. In this experiment positive control acarbose shows the maximum activity 79.6 % inhibition at the dose of 5 µg/ml.

CONCLUSION

In this study, butanol fraction from the fruit of *Dillenia pentagyna Roxb.* shows significant antibacterial, antioxidant as well as indirect antidiabetic activity compared to other fractions of fruit and leaves. But exactly which compound of this fraction is active is not yet explored. Based on these results, *Dillenia pentagyna* may be considered as a pharmaceutical agent. Specific compound from the crude extract may have better activity as antibacterial, antioxidant as well as anti-diabetic but need to be explored. Finally *in vivo* experiment may show some direction to use this fruit as supplementary with food or as therapeutic agent.

ABBREVIATIONS

DMF: Dimethyl formamidel; DPPH: 1,1-diphenyl-2-picryl hydrazyl; NBT: Nitroblue tetrazolium; PMS Phenazonium Methosulphate; p-NPG: p-Nitrophenyl α-D-Glucopyranoside

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CONFLICT OF INTEREST

The authors declare that there is no conflict of Interest.

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