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## Qualitative, quantitative screening and antifungal study of *Pittosporum floribundum* Wight & Arn.

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### ABSTRACT

**Background:** *Pittosporum floribundum* is an ethnomedicinal plant which has a numerous number of medicinal claims and it hasn't been explored thoroughly. Various parts of plant used medicinally such as used in skin disorder, leprosy, etc. **Aims:** To explore different qualitative, quantitative and antifungal aspect of *Pittosporum floribundum*. **Materials and methods:** Different test were carried out to determine qualitative as well as quantitative parameter such as for the presence of protein, glycosides, alkaloids, carbohydrates, terpenoids, etc. **Result & Discussion:** The three extracts were taken for examination such as water, methanolic and hydroalcoholic. The study was carried out on *Pittosporum* bark and leaves and for every sample three extract were prepared. And every extract shows different results. Also, study reveals *Pittosporum floribundum* shows antifungal activity. **Conclusion:** The *Pittosporum floribundum* plant extracts could be used as an antifungal after comprehensive in vitro biological studies.

**Keywords:** *Pittosporum floribundum*, antifungal activity, Phytochemical study.

### INTRODUCTION

Since time immemorial medicinal plants are the primary life supporting system for rural and tribal communities. India has one of the richest plants based traditional system medicine in the world which are not only well documented in many classical texts. There are many plants, these are being use traditionally but not as a part of classical texts of *Ayurveda* or pharmacopoeia.

There are around 20,000 medicinal plants those have been recorded in India; however, traditional practitioners use only 7000- 7500 plants for curing different diseases.

*Pittosporum floribundum* Wight & Arn. Synonym- *Pittosporum napaulense* (DC.) Rehder & E.H. Wilson (Family-*Pittosporaceae*), one among such plant is a small evergreen tree, found along the foot of outer Himalayas from Punjab eastwards to the hills of Assam and in the hills of peninsular India, ascending up to an altitude of 2400 m above mean sea level (msl) and also the Dun in shady places or ravines. It is called *Devasundha*, *debosunda*, *devson*, *purshpashan*, *chachin* (Odia), *Rakamuki* (Telugu), *Kattu sampangi*, *Najundai*, *Tammata* (Tamil), *Tumari*, *Vikharl*, *Vekhali* (Marathi) in their native languages<sup>[1]</sup>.

Because of their medicinal and nutritional value qualitative and quantitative aspect were check for further use also as per traditional claim the plant parts were further analysed for antifungal activity.

### MATERIAL AND METHODS

#### Collection of plant material

*Pittosporum floribundum* was collected from Paikmal. Dist. Bargarh State, Orissa, India, as per standard procedure in the month of January 2018 with assistance of local guide. *Pittosporum floribundum* Herbarium was prepared and Authentication was done from IPGT&RA with letter no. Phm.6274/17-18. Plant parts like leaf and bark materials were collected and thoroughly washed further dried under shade at  $28 \pm 2^\circ\text{C}$  for about 10 days. The dried parts were ground well into a fine powder in a mixer grinder and sieved by 120 sieve size no. The powders were stored in container at room temperature.

## Phytochemical analysis

### Extract preparation

Shade dried leaf and bark powders were subjected to maceration. The above obtained solid extracts were preserved in air tight bottles at 4 °c in a refrigerator for further use.

### Preliminary Phytochemical screening (Qualitative Study)

The extracts of the different parts were subjected to phytochemical screening for the presence of phytoconstituents like Alkaloids, Flavonoids, Phenols, Lignins, Anthroquinones, Steroids, Tannins, Saponins, Fixed Oils and Glycosides by using standard methods. The results obtained from all quantitative studies represented in mean values along with calculated standard deviation through Microsoft excel [2].

### Quantitative study

#### 1. Total Carbohydrate

In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This forms a green coloured product with phenol (Phenol sulphuric acid method) [3].

#### 2. Total Protein

The proteins are first treated with copper ion in alkali solution, and then aromatic amino acids in the treated sample reduce the phosphomolybdate phosphotungstic acid present in the Folin reagent. The end product of this reaction has a blue colour. The amount of proteins in extracts was determined according to the method described by Lowry *et al.* (1951) [4].

#### 3. Glycoside content

Glycosides react with Baljet's reagent and develop an orange-red color with (picric acid in alkaline medium). The glycosidic content of the plant extracts was determined according to the method given by Mosa [5].

#### 4. Total Phenol content

The total phenols were determined by Folin – Ciocalteu reagent method described by Malik and Singh (1980) [6].

#### 5. Total flavonoid content

The total flavonoid content was determined by method of Khatiwora *et al.* (2010) [7].

#### 6. Tannin content

The tannins in the extract react with potassium ferric cyanide ion and oxidized while the Fe (CN) 6<sup>3-</sup> is reduced to ferric cyanide ion Fe (CN) 6<sup>3-</sup>. Then this reacts with ferric ion to form ferric ferric cyanide (Graham, 1992) [8].

## 7. Terpenoid content

Terpenes and terpenoids are primary constituents of essential oils of different type of plants and flowers. Some qualitative estimation methods of terpenoids in plant tissue have been previously described but, there is no protocol of estimating the same quantitatively till date. In the present study, a protocol has been attempted to estimate the total terpenoids concentration of different resin producing plants using a monoterpene, Camphor as standard reagent (Ghorai *et al.*, 2012) [9].

### In-vitro anti-fungal activity of Isolated compounds on *C. albicans*

The antifungal activity was done from Vasu research center Baroda India.

**Culture used:** *Candida albicans* (ATCC 10231)

**Media Used:** Sabroud dextrose agar (SDA) Make: Hi-media

**Reference Standard Used:** Itraconazole capsule – 100 mg

**Culture Preparation:** Freshly prepared slants of *C. albicans* was used and washed the slant by using 10 mL of sterile Normal saline solution. Method: Cylinder Plate Method Method for: 1) Media preparation: Sabroud Dextrose Agar was used for determining the activities of, *Candida albicans*. Media was prepared as per Manufacturer's Instruction. The media was then autoclaved at 121°C temp. & 15lbs pressure for 20 minutes.

#### Method for:

- 1) Media preparation:** Sabroud Dextrose Agar was used for determining the activities of, *Candida albicans*. Media was prepared as per Manufacturer's Instruction. The media was then autoclaved at 121°C temp. & 15lbs pressure for 20 minutes.
- 2) Sample Preparation:** Take approximate 100 mg of sample & dissolved into 1:1 ratio of Methanol: Dimethyl Sulfoxide. Dissolved the samples by cyclomixture. Filter the samples & use filtrate to evaluate anti-fungal activity. 3) Standard preparation of: Itraconazole - 100 mg: Take weight of filled capsule. Active content of capsule i.e. pellets were powdered into mortar-pestle. Took powder equivalent to one capsule weight into 100 ml volumetric flask and make up the volume 100 ml with Dichloromethane. Solution was sonicated and prepared 50 mcg/ml standards solution by dilution method.
- 3) Testing Procedure:** Cool down sterile media up to 55°C then measured 15 ml of SDA media by sterile measuring cylinder and transferred into sterile petri plate. Likewise, prepared 3 plates for evaluation. The plates were allowed to solidify on smooth surface. In rest of the media add 5µl fungal culture and mixed slowly. Then the media was poured on above SDA containing plates. The plates were solidified and then made required wells in SDA plates labeled them as a std. & test, at proper distance by sterile borer. Add std. & test samples in respected labeled well. When samples were diffused completely in well, incubate SDA plates into Biological Oxygen Demand (BOD) incubator at 25°C for 72 hours and observe the zone of inhibition [10].

## RESULT

## Preliminary Phytochemical Screening

Table 1: Qualitative parameter of parts of *Pittosporum floribundum*

<i>Pittosporum floribundum</i>							
Sr No.	Parameter	Water extract		Methanol Extract		Hydroalcoholic Extract	
		Leaves	Bark	Leaves	Bark	Leaves	Bark
1	<b>Alkaloids</b>						
	Mayer's reagent	-	+	+	+	-	+
	Wagner's reagent	-	+	-	+	-	+
2	<b>Flavonoids</b>						
	Shinoda test	-	+	+	+	-	+
	Lead Acetate test	-	+	+	+	-	+
3	<b>Phenols</b>						
	FeCl <sub>3</sub> test	+	-	+	-	+	-
4	<b>Lignins</b>	-	-	-	-	-	-
5	<b>Anthraquinone</b>	-	+	-	+	-	+
6	<b>Steroids</b>						
	Salkowski test	-	-	+	-	+	-
7	<b>Tannins</b>						
	Lead Acetate test	-	+	-	+	-	+
	FeCl <sub>3</sub> test	-	+	+	+	-	+
8	<b>Saponin</b>	-	-	-	-	-	-
9	<b>Fixed Oils</b>						
10	<b>Glycosides</b>	+	+	+	+	+	+
11	<b>Proteins</b>						
	Biuret Test	-	-	-	-	-	-
11	<b>Amino Acid</b>						
	Ninhydrin Test	-	-	-	-	-	-
12	<b>Carbohydrates</b>						
	Molisch test	-	-	-	-	-	-

+ Present; - Absent.

## Quantitative Study

Table 2: Quantitative study of Parts of *Pittosporum floribundum*

Name of the test	Quantitative (in µg/ml) (n=3) (Mean±SD)	
	PB	PL
Total Carbohydrates content	2227.80 ± 540.69	835.30 ± 120.35
Total Protein content	N.D.	N.D.
Total Phenolic content	45.61 ± 4.653	201.97 ± 21.378
Total Flavonoid content	300.45 ± 12.45	126.64 ± 25.45
Total Tannin content	845.40 ± 45.55	579.00 ± 55.55
Total Glycoside content	N.D.	N.D.
Total Terpenoid content	1952 ± 25.45	1453 ± 12.207

Where

PB stands for *Pittosporum Bark*PL stands for *Pittosporum Leaf*

## Antifungal Activity

Table 3: Antifungal activity of *Pittosporum floribundum*

Sr No.	Name of Sample	Sample Concentration	Zone of Inhibition in mm
1	Blank	Methanol: DMSO	NZ
2	Reference Standard - Itraconazole	50 mcg/ml	24 mm
4	PBM	100 mg/mL	14 mm
6	PLM	100 mg/mL	12 mm

## DISCUSSION

The qualitative phytochemical test of water extract of *Pittosporum floribundum* revealed the presence of phenols, glycosides in leaves extract. Alkaloids, Flavonoids, anthraquinone, tannin, glycosides in bark extract. The qualitative phytochemical test of methanol extract of *Pittosporum floribundum* revealed the presence of Alkaloids, flavonoids, phenols, steroids, tannin, glycosides in leaves extract. Alkaloids, Flavonoids, anthraquinone, tannin, glycosides in bark extract. The qualitative phytochemical test of hydroalcoholic extract of *Pittosporum floribundum* revealed the presence of phenols, steroids, glycosides in leaves extract. Alkaloids, Flavonoids, anthraquinone, tannin, glycosides in bark extract. The given data shown in table no 1.

Quantitative estimation of carbohydrates, protein, phenolic, flavonoid, tannin, glycosides, terpenoids were done in which *Pittosporum* bark shows high percentage carbohydrates than *pittosporum* leaves. Total protein and total glycosides were in negligible amount which is not detectable by method. In total phenolic content *pittosporum* leaves show more percentage than *pittosporum* bark. In total flavonoids content *pittosporum* bark shows high content than *pittosporum* leaves. In total tannin content *pittosporum* bark shows high content than *pittosporum* leaves. In total terpenoidal content *pittosporum* bark shows high content than *pittosporum* leaves. The given data shown in table no 2.

The antifungal study was done on total 4 sample. Blank is taken as one of the samples for checking strain. Reference sample were taken as itraconazole showing zone of inhibition as 24mm. PBM i.e *Pittosporum* bark methanolic extract showing zone of inhibition 14mm. PLM i.e *Pittosporum* leaves methanolic extract showing zone of inhibition 12mm. The given data shown in table no 3.

## CONCLUSION

In methanolic extract study reveals the presence of Alkaloids, flavonoids, phenols, steroids, tannin, glycosides in leaves extract. Alkaloids, Flavonoids, anthraquinone, tannin, glycosides in bark extract. Methanolic extract against *C.albicans* shows the effective antifungal activity in both parts i.e., bark, leaf of *Pittosporum floribundum*.

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