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Preliminary phytochemical profiling and antifungal activity of the seeds and pericarp of *Putranjiva roxburghii* Wall.

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Abstract
Putranjiva roxburghii Wall. (Putranjivaceae). is a valuable but underutilised medicinal tree. The present study investigates its qualitative analysis of phytochemical constituents and antifungal activity of from seeds and pericarp (Fruit wall). Different extracts of samples were screened for preliminary phytochemical profile. The methanolic extracts were analysed to estimate antifungal activity against five fungal strains. Qualitative analysis showed the presence of Alkaloids, Carbohydrates, Glycosides, Flavonoids, Phenols, Tannins, Fixed oils, Coumarins, Saponins, Sterols and Terpenoids. Antifungal result showed higher value for *Trychorphyton rubrum* for both the samples.

Keywords: *Putranjiva roxburghii* Wall. putranjivaceae, GC-MS, antifungal activity and Chhattisgarh

Introduction
The evaluation based on phytochemical and pharmacological of the natural product leads to the drug discovery. Plant parts such as leaves, bark, roots, flowers, fruits and seeds contain bioactive components. These compounds have numerous activities pertaining to antifungal and antibacterial aspects [1], [2]. Qualitative phytochemical tests helps to understand the role of chemical compounds and their usefulness as pharmaceuticals [3].
To cure various diseases and disorders plant based medicines have been used [4]. *Putranjiva roxburghii* Wall. plays significant role in the traditional Ayurvedic and Unani systems. It has been reported for its effective medicinal values [5]. Leaves and seed were used to cure burning sensation, filarial, inflammatory eye diseases, etc. Seeds are orally consumed in the powder form for various diseases like elephantiasis, constipation, dysuria, ophthalmic, aphrodisiac, semen disorders, infertility and diseases of female genital organs [6]. In Ayurveda, its effective usefulness for antipyretics, anti-inflammatory and anti-rheumatic and also for gynaecological and fertility ailments have been mentioned. Presence of many glycosides, saponins, triterpenes and flavonoids were revealed by pharmacognostic studies. Leaves are very useful for the production of bio-synthesis of nanoparticles [5].

Fig 1: Fruits of *Putranjiva roxburghii* Wall.

Materials and Methods

Collection and Authentication

The plant was collected from Kunkuri in Jashpur district of Chhattisgarh, India and identified by Dr. S.John Britto, Director and Head, The Rapinat Herbarium and Centre for Molecular Systematics St. Joseph's College (Autonomous) Tiruchirappalli, India. The voucher specimen was deposited at the centre with accession number RHT67530. (Fig. 1)

Preparation of plant extract

The plant sample i.e. seed and pericarp were airdried under shade at room temperature, ground with electric grinder into fine powder and stored in air tight container for further use. 10 grams of powdered sample mixed in 150 ml of solvents (i.e. methanol, ethanol, chloroform, acetone, petroleum ether and water) for extraction, was kept in rotary shaker for three days at room temperature. The extracts were filtered by using filter paper then air dried and stored for further usage. The crude extracts were further re-suspended in 1ml of respective solvents for the investigation of phytochemical analysis.

Phytochemical Screening

Test for alkaloids

Wagner's Test: 2 ml of extract was treated with few drops Wager's reagent. Formation of reddish brown precipitate indicated the presence of alkaloids [7].

Hager's Test: 2 ml of extract was treated with few drops of Hager's reagent (saturated solution of picric acid). Formation of yellow color precipitate signified positive result.

Mayer's Test: 2 ml of extract was treated with few drops of Mayer's reagent. Formation of cream precipitate indicated the presence of alkaloids.

HCl Test: 2 ml of extract was treated with 1 ml of 1% HCl and heat gently. Then followed by few drops of Mayer's reagent and Wagner's reagent to the mixture. Turbidity of resulting precipitate was the evidence of the presence of alkaloids.

Test for proteins

Biuret Test: 2 ml of extract was treated with 2 ml 5% NaOH and 2 ml 1% $CuSO_4$ solutions. Violet or purple coloration indicated presence of proteins and free amino acids.

Xanthoprotetic Test: 2 ml of extract was treated with few drops of concentrated HNO_3. Formation of yellow colour indicated the presence of proteins.

Conc. H2SO4 Test: 2 ml extract was treated with few drops of conc. H_2SO_4. Formation of white precipitate indicated the presence of proteins.

Xantho proteins Test: 2 ml of extract was treated with few drops of conc. HNO_3 and NH_3 solution. Formation of reddish orange precipitate indicated the presence of xantho proteins.

Test for amino acids

Ninhydrin Test: 2 ml of extract was treated with 1ml of freshly prepared 0.25% ninhydrin reagent and boiled for few minutes. Formation of blue color indicated the presence of amino acids.

Test for flavonoids

Alkaline Test: 2-3 ml of extract was treated with few drops of NaOH solution. Formation of intense yellow color which turned colorless on addition of few drops of dilute HCl.

Pew's Tests: 2-3 ml of extract was treated with zinc powder in a test tube, followed by drop wise addition of conc. HCl. Formation of purple, red or cherry color indicates the presence of flavonoids [8].

Lead acetate test: 1 ml extract was treated with 1 ml 10% lead acetate ($Pb(OAc)_4$) solution. Formation of yellow color precipitate indicated the presence of flavonoids

Conc.H2SO4 test: 5ml of dilute ammonia solution was added to the extract followed by conc.H2SO4. Yellow color indicated the presence of flavonoids.

Test for fixed oils

CuSO4 Test: 2 ml of extract was treated with 1 ml of 1% CuSO4 solution and 10% NaOH solution. Blue coloration indicated the presence of fixed oils.

Test for phenols and tannins

Ferric chloride test: 2 ml of extract was treated 2-3 drops of 5% ferric chloride solution. Formation of bluish-black color showed presence of phenols and black color shows tannins.

Potassium dichromate test: 2 ml of extract was treated with 5% potassium dichromate solution. Positive result was confirmed by a formation of brown precipitate (for phenol).

Braymer's Test: 2 ml of extract was treated with 2 ml H_2O and followed with 2-3 drops of $FeCl_3$ (5%). Green precipitate proved presence of tannins.

Test for coumarins: 2 ml of extract was treated with 3 ml of 10% NaOH solution. Yellow coloration indicated the presence of coumarins.

Test for saponins

Foam Test: 2 ml extract was diluted with 10 ml of distilled water and warmed gently. It was shaken for 5 minutes. Persistent froth indicated the presence of saponins. The same extract was added with few drops of olive oil. Formation of a soluble emulsion, confirmed the presence of saponins [7].

Test for Glycosides

Keller kiliani Test (Test for cardiac glycoside): 2 ml extract was treated with 1 ml glacial acetic acid, one drop 5% $FeCl_3$ and 1 ml conc. H_2SO_4. A brown ring of the interface indicated the presence of cardiac glycosides [8].

Glycoside Test: Small amount of extract was treated with 1 ml water and shake well. Then aqueous NaOH was added. Yellow color appeared that indicated the presence of glycosides [9].

Test for sterols

Salkowski's Test: 2 ml of extract was treated with 2 ml chloroform and 2 ml conc. H_2SO_4. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence which indicated the presence of sterols.

Keller killiani Test: (Test for cardiac glycoside): 2 ml extract was treated with 1 ml glacial acetic acid, one drop 5% $FeCl_3$ and 1 ml conc. H_2SO_4. A brown ring of the interface indicated the presence of cardiac glycosides [7].

Test for terpenoids

Salkowski's Test: 2 ml of chloroform and 1 ml of conc.H_2SO_4 was added to 1 ml of extract and observed for reddish brown color that indicated the presence of terpenoids [6].

Antifungal Activity

Fungal Strains

Aspergillus candidus, *Chrysosporium tropicum*, *Rhizopus stolonifer*, *Microsporum canis* and *Trychorphyton rubrum* are the fungal strains used for the antifungal analysis. These are commonly crop infecting strains.

Determination of antifungal activity

Petri plates containing 20ml PDA were seeded with mature culture of fungal strains. Wells were cut using a sterile Cork Borer and 100µl (200µg/well) of extracts were added into the well. For the negative control, 100µl of the distilled water was added into the wells. The plates were then incubated at room temperature for about a week. The antifungal activity was assayed by measuring the diameter of the inhibition zone formed around the well.

Results and Discussion

The results of qualitative screening of phytochemicals of *P. roxburghii* seed and pericarp showed the presence of Alkaloids, Carbohydrates, Glycosides, Flavonoids, Phenols, Tannins, Fixed oils, Coumarins, Sponins, Sterols and Terpenoids. High concentrations of phytochemicals were found in methanolic, followed by ethanolic, acetone and aqueous extracts while a very low concentration in chloroform and petroleum ether extracts (Table No.1). *Aspergillus candidus*, *Chrysosporium tropicum*, *Rhizopus stolonifer*, *Microsporum canis* and *Trychorphyton rubrum* are the fungal strains for which methanolic extracts were checked. *T. rubrum* showed highest result for both samples and seed sample showed no results for *C. tropicum* and *R. stolonifer*. Pericarp showed no result for *A. candidus* (Table No.2 and Fig. 2).

Table 1: Phytochemical analysis of *Putranjiva roxburghii* Seed and pericarp

S. No.	Phytochemical Constituents	Extract									
		Acetone		Aqueous		Ethanol		Methanol		Petroleum Ether	
		S	P	S	P	S	P	S	P	S	P
1	Test for Alkaloids										
	Hager's Test	+	+	+	+	+	+	+	+	-	-
	Mayer's Test	+	+	+	+	+	+	+	+	-	-
	Wagner's Test	+	+	+	+	+	+	+	+	-	-
2	Test for Carbohydrates										
	Molisch's Test	+	+	+	+	+	+	+	+	-	-
	Fehling test	-	-	-	-	+	+	+	+	-	-
	Benedict's Test	-	+	+	+	-	+	+	+	-	-
3	Test for Flavonoids										
	Conc.H_2SO_4 Test	+	+	-	+	-	-	-	-	-	-
	Pew's Test	-	-	-	-	-	-	-	-	-	-
	Lead acetate	-	+	-	+	-	+	-	+	-	-
4	Test for fixed oils										
	$CuSO_4$ Test	+	+	+	-	+	-	+	+	+	-
5	Test for Phenols										
	Ferric chloride Test	-	-	-	+	-	-	-	-	-	-
	Potassium Dichromate Test	-	-	-	+	-	-	-	-	-	-
6	Test for Tannins										
	Ferric chloride Test	-	-	-	+	-	-	-	-	-	-
	Braymer's Test	-	-	-	+	-	+	-	+	-	-
	Lead acetate	-	-	-	+	-	-	-	-	-	-
7	Test for saponins										
	Foam Test	-	-	+	+	+	-	+	-	-	-
8	Test for Glycosides										
	Keller kiliani Test	-	+	-	+	-	+	-	+	-	-
	Glycoside Test	-	+	-	+	-	+	-	+	-	-
9	Test for coumarins										
	10%NaOH Test	+	+	+	+	+	+	+	+	-	-
10	Test for Sterols										
	Salkowshi's Test	-	+	+	-	+	+	+	+	-	-
	Keller killiani Test	-	+	+	-	-	+	-	+	-	-
11	Test for Proteins										
	Biuret Test	-	-	+	-	-	-	-	-	-	-
	Xanthoproteic Test	-	-	+	-	-	-	-	-	-	-
	Conc.H_2SO_4 Test	-	-	-	-	-	-	-	-	-	-
12	Test for Amino acids										
	Ninhydrin Test	-	-	-	-	+	-	+	-	-	-
13	Test for Terpenoids										
	Salkowshi's Test	-	+	-	-	+	+	+	+	-	-

S=Seed, P=Pericarp, Present= + and Absent= -

Table 2: Antifungal assay of *Putranjiva roxburghii* seed and pericarp

A. candidus	*C. tropicum*	*R. stolonifer*	*M. canis*	*T. rubrum*
8.2±0.41	0	0	13.3±0.52	14.2±1
0	9.0±0	12.5±0.55	20.5±0.84	22.7±0.52

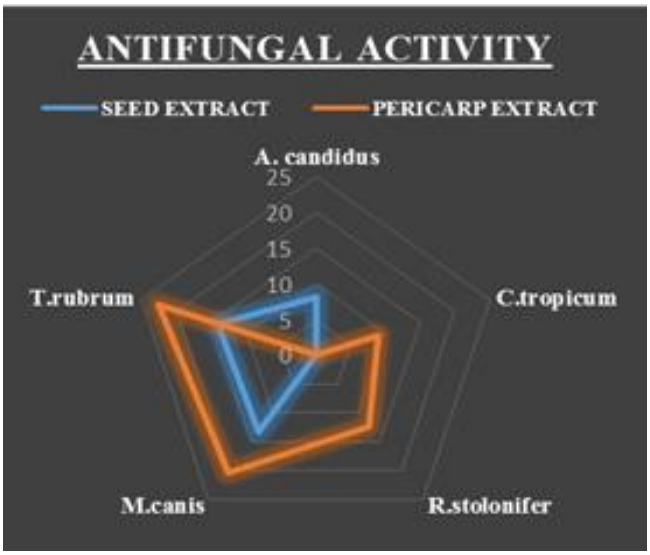

Fig 2: Antifungal activity of seed and pericarp extract.

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