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RESEARCH ARTICLE

ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACTS OF *PUTRANJIVA ROXBURGHII* WALL. (EUPHORBIACEAE)

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ABSTRACT

This plant evaluates the antifungal activity of methanolic extract of *Putranjiva roxburghii* Wall. of Euphorbiaceae. For centuries plants are used as food and medicine. The present study focuses on the antifungal activity of leaves of *P. roxburghii*. Strains of *Aspergillus candidus, Chrysosporium tropicum, Rhizopus stolonifer, Microsporum canis* and *Trychorphyton rubrum* are the fungal strains used to check the mode of action. Methanol leaf extract (sample1) showed highest activity against *M. canis* strain than methanol bark and methanol root extracts. Methanol bark extract (sample 2) showed more activity against *T. rubrum* strain, here *R. stolonifer* and *A. candidus* showed very similar range of activity against fungi. Methanol root extract (sample 3) showed more activity against *T. rubrum* strain. Along with other properties these plant extracts also have antifungal property.

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INTRODUCTION

The Euphorbiaceae are composed of 334 genera and over 8,000 species are in habitat range and variability in morphology. *Putranjiva roxburghii* Wall. belongs to Euphorbiaceae and were known for its medicinal properties. It is reported to be effective for infertility, most effective for fever and liver diseases. *P. roxburghii* has Antibacterial, Anticancer, Anti-inflammatory and Antioxidant properties. Pathogenic fungi are one among the main infectious agents in plants, which alters their development stage (Agrios, 2004). After insects fungi placed in the largest biotic community. In India about 27,000 species of fungi have been recorded (Manoharachary *et al*. 2005). Fungi are heterotrophic in nature, which obtains nutrition from decaying animals and plants and also from other organic matter such a foodstuff, artifacts, clothes, etc. All living beings on the earth are directly or indirectly harmed or benefited by fungi. These can attack building, timbers, stored goods, clothing, animals and even their own bodies, through allergy and diseases. They can also attack objects, specimens, books and paintings even in controlled environment (Singh *et al.* 1999).

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In some cases these produce toxic disorders (allergies) to the consumers and initiate the production of mycotoxins or allergens. For thousands of centuries spices and herbs have been in use to enhance the flavor and aroma and for preservation of foods and also for medicinal purposes (Shelef, 1984). These medicinal plants are documented scientifically that they have antimicrobial, antioxidant and antifungal properties. Phytopathogenic fungi are controlled by synthetic fungicides but usages of these are restricted due to its harmful effect on human health and environment (Harris *et al.* 2001). There is an increasing demand on the production and regulation of agrochemicals with property pathogen resistance (Cowan, 1999). Several laboratory trials have been done on different plant tissues, such as roots, leaves, seeds and flower that showed properties against bacteria, fungi and insects (Davicino *et al.* 2007). The pathogenic control is challenging and still continues to be based on multiple applications of fungicides. Though chemical control shows good resistant against harmful pathogens but remaining residues are phytotoxic in nature, which causes public health problems (Şesan *et al.* 2015). To minimize this factor and for the food safety standard there is an increased interest on the usage of nature compounds of plant extracts to explore new alternatives to synthetic fungicides. Such compounds have less toxicity for humans and environment. Several natural compounds are not yet studied in correlation with their fungicidal action.

Table 1. Antifungal assays of *Putranjiva roxburghii* Wall

Samples	*A. candidus*	*C. tropicum*	*M. canis*	*R. stolonifer*	*T. rubrum*
Sample1	0.98±0.07	0.63±0.05	2.42±0.07	1.28±0.07	1.08±0.07
Sample2	1.07±0.05	0.78±0.12	0	1.07±0.05	1.17±0.05
Sample3	1.23±0.05	1.12±0.12	0	0.75±0.05	1.27±0.05

Sample 1-leaf methanol extract, sample 2 – bark methanol extract and sample 3 –root methanol extract.

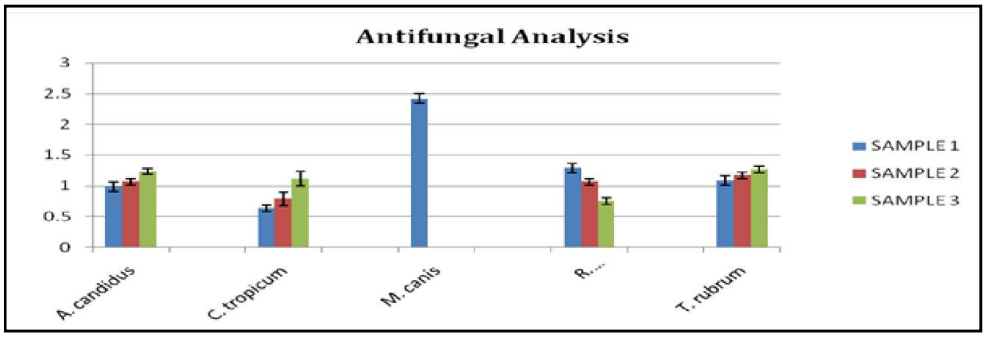

Figure.1. Antifungal activity of *P. roxburghii* extracts (leaf, bark and root)

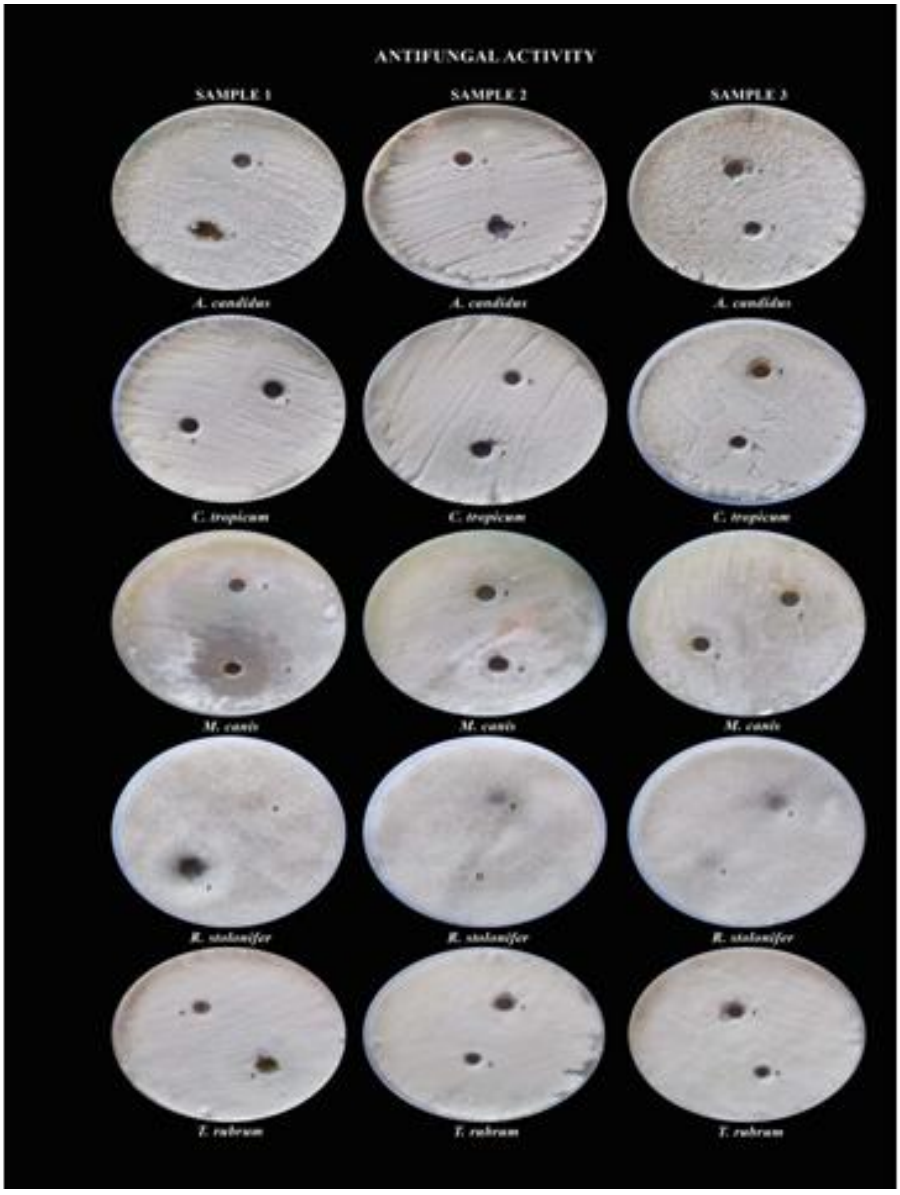

Figure 2. Antifungal activity of *P. roxburghii* extracts (leaf, bark and root)

Very few reports have reported the mode of action of antifungal acitivity in *P. roxburghii* Wall. The phytochemical screening has revealed the presence of flavonoids, phenolic compounds, steroids etc. Flavonoids and steroids show remarkable anti inflammatory activity (Nijveldt *et al.,* 2001). The *In-vitro* antioxidant and anti-inflammatory activity has been established from of the leaf extract of *P. roxburghii* along with preliminary phytochemical investigation. Leaf extract showed potent antioxidant activity (Rajagopal PL. *et al.* 2014). The best antibacterial activity was shown by the methanol extracts. The presence of compounds such as phenols, alkaloids, saponins (Okwu and Josiah, 2006). Similar reports have been published for pharmacological applications except antifungal activity.

MATERIALS AND METHODS

Collection and Authentication

The plant sample was collected from kunkuri, Jashpur district, Chhattisgarh, India, during August 2015 and was identified by Dr. S. John Britto, Director and Head, The Rapinat Herbarium and Center for Molecular Systematics St. Joseph's College (*Autonomous*) Tiruchirappalli, India. The voucher specimen was deposited at the centre with accession number RHT67164.

Extraction and plant material

The plant sample i.e. leaves, bark and root were air dried 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 methanol (solvents) for extraction, was kept in rotary shaker for three days at room temperature. The extracts were filtered by using Whatman No.1 filter paper then air dried and stored for further usage. The resulting methanolic extract solution was used for the antifungal assay.

Fungal strains

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

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

Sample 1 showed highest activity against *M. canis* than sample 2 and sample 3, followed by *R. stolonifer, T. rubrum, A. candidus* and *C. tropicum.* Sample 2 showed more activity against *T. rubrum* strain followed by *R. stolonifer, A. candidus* and *C. tropicum. R. stolonifer* and *A. candidus* showed very similar range of activity against fungi. Sample 3 showed more activity against *T. rubrum* followed by *R. stolonifer, A. candidus* and *C. tropicum.* For *M. canis* sample 2 and 3 did not show any activity (Table 2, Fig. 1 and Fig. 2).

Conclusion

The results clearly establish that the methanolic extracts of (leaf, bark and root) of *P. roxburghii* exhibit antifungal effects against *M. canis* strain. Among all extracts leaf extract showed the highest activity range of 2.42±0.07 than bark and root extracts. But for the same strain bark and root extracts showed zero activity. Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity and checked for other harmful fungal strains. Natural plant-derived fungicides may be a source of new alternative active compounds, in particular with antifungal activity.

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