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STUDIES ON ANTIFUNGAL AND ANTIOXIDANT ACTIVITY OF PLANT EXTRACTS AGAINST HUMAN PATHOGENS

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ABSTRACT

Total of six extracts of three plant samples viz., *Punica granatum* (peels) *Swertia chirata* (leaves) and *Citrullus lanatus* (seeds) in two solvents were evaluated for their antifungal activity. Aqueous and ethanolic extracts of the samples were prepared by Hot Extraction Method. The antifungal activity was measured by Agar Well Diffusion method and concentration was 100 mg/ 10 µl. Minimum Inhibitory Concentration was found out using Broth Dilution Method. The results of the study showed the presence of wide spectrum of antifungal activity against human pathogens such as *Saccharomyces cereveciae* and *Candida krusei.* The ethanolic extracts of *Punica granatum* showed the best results. The efficiency of the peel extracts was compared to the most commonly used commercially available drugs. *Punica granatum* showed the highest antioxidant property when compared to *Swertia chirata* and *Citrullus lanatus.* The extracts showed the presence of phytochemicals such as alkaloids, flavonoids, steroids, tannin, saponin which are responsible for the antifungal activity.

KEYWORDS: Antifungal, Antioxidant, *Citrullus lanatus, Punica granatum, Swertia chirata.*

INTRODUCTION

India is very rich in resources from the perspective of culture, ecological climate and floral diversity and nature has been a source of medicinal agent for thousands of years and a number of modern drugs have been isolated from natural sources.[1] Many people still use traditional herbs to treat a variety of diseases and infections. In the recent years the pathogens have developed resistance to many of the commonly used antibiotics and thus attempts have

been made to search new microbial agents.[2] In this study three plant samples were randomly sampled to evaluate their Antifungal activity against Human Pathogens. *Punica granatum* commonly called as pomegranate is said to be a cure for all illness.[3] The peel of this fruit is valuable in the treatment of diarrhea and acidity. It has also gained importance in beauty industry and is used in the manufacture of beauty creams and oils.[4] *Swertia chirata* is a popular medicinal herb used in traditional medicine to treat a number of disorders and infections.[5] They have a wide spectrum of pharmacological properties. On the other hand, *Citrullus lanatus* commonly called as watermelon is easily available throughout India. The seeds are demulcent, diuretic and used as a tonic to treat urinary infections. Fatty oil in the seed, as well as aqueous or alcoholic extracts, paralyzes tapeworm and roundworms.[6] Due to the wide applications of these plants an attempt was made to analyze the antifungal properties of these against human fungal pathogens viz., *Saccharomyces cereveciae* and *Candida krusei.*

Saccharomyces cereveciae commonly called as Baker's yeast is widely used in baking, brewing and wine preparation in many food industries. Recently many evidences indicate the involvement of S. *cereveciae* in a range of superficial and systemic diseases, numerous cases of *S. cereveciae* induced vaginitis have been documented in case of oropharyngeal infection.[7] *Candida krusei*, budding yeast is involved in chocolate production and is an emerging fungal nosocomial pathogen primarily found in immunocompromised individuals and those with hematological malignancies.[8] These organisms have become resistant to antibiotics over a period of time which becomes difficult for recovery among patients suffering from the disease. The antibiotics also cause certain side effects such as headache, nausea and hypersensitivity reaction and the over dosage of antibiotics can even lead to death.[9] Hence there is a need to find natural remedies for these pathogens which is effective and has no side effects.

MATERIALS AND METHOD

Sample Collection

The samples were purchased from the market and brought to the laboratory. It was thoroughly washed under running tap water and rinsed with distilled water. Peels (*Punica granatum),* leaves *(Swertia chirata)* and seeds (*Citrullus lanatus*) were separated from the samples and were shade dried for 24 hours and placed in a hot air oven at 40ºC for 3 days. After complete removal of moisture, the samples were powdered using a mixer grinder. The powdered sample was stored in an air tight container for further use.

Preparation of the Extract

10 g of the powdered material was dissolved in 40 ml of the respective solvent and left undisturbed for 24 hours. Aqueous and ethanolic extract was obtained by Hot Extraction Method and the extracts prepared were immediately evaluated for their antifungal property.[10,11] Minimum Inhibitory Concentration of the two extracts was found out using Broth Dilution Method.[12]

Phytochemical screening

Phytochemical screening was made to detect the presence of essential phytochemicals such as alkaloids, flavonoids, steroids, terpenoids and steroids, tannin and saponin.[13,14]

Test for Alkaloids: To the extract 6 drops of 1% HCl, Dragendoffs reagent and Mayer's reagent were added. An orange precipitate indicates the presence of Alkaloids.

Test for Flavonoids: To the extract 5 ml of dilute ammonia and 3 drops of conc. Sulfuric acid were added. A yellow precipitate indicates the presence of Flavonoids.

Test for Steroids and Terpenoids: To the extract 1ml of chloroform was added and filtered. 1ml of acetic acid was added and mixed. Few drops of conc. Sulfuric acid were added slowly. Appearance of pink or pinkish brown ring indicates the presence of terpenoids. Blue or bluish green indicates the presence of steroids. Amalgamation of pink and blue color indicates the presence of both terpenoids.

Test for Tannin: To the extract 2 ml of ferric chloride was added. Dark green indicates positive test for Tannin.

Test for Saponin: 2 ml of the extract was diluted with distilled water, mixed well and kept aside for five minutes. Development of foam on the surface of the mixture indicates the presence of saponin.

Antifungal Assay

S. cereveciae (YJM789) and *C. krusei* (ATCC6258) were used as test organisms to assess the antifungal potential. The test organisms were acquired from Department of Biosciences, Mangalore University. The efficiency of the extracts of *P. granatum*, *S. chirata* and *C. lanatus* were evaluated against *S. cereveciae* and *C. krusei* using disc diffusion method. Potato Dextrose Agar plates were inoculated with different fungal strains. The sterile

Whatman filter paper disc (3 mm) containing extracts of the sample with different concentrations were placed on the plates and incubated at 28ºC for 48 hours in an incubator to observe the zone of inhibition. Distilled water and alcohol was used as control. The results were expressed in mean ± Standard Deviation. The statistical analysis was done using Graph Pad Prism 6.0. All the assays were performed in triplicates.

Total Antioxidant Activity

The total antioxidant activity of plant samples was determined using standard protocol.[12] The minimum inhibitory concentration was determined using aqueous and ethanolic extracts of samples which inhibit the visible growth of microorganisms. MIC is usually considered as the most basic laboratory measurement of the activity of antimicrobial agent against microorganisms.[15]

RESULTS AND DISCUSSION

Aqueous and ethanolic extracts of the samples were tested for the presence of phytochemical constituents such as alkaloids, flavonoids, terpenoids, steroids, saponin and tannin. And the results are shown in Table 1. These phytochemical compounds are produced by plants through primary or secondary metabolism and help the plants to fight against competitors, predators and pathogens.[16] These compounds are known to be biologically active and therefore aid in antimicrobial activity.

Tannin has been found to form irreversible complexes with proline-rich protein resulting in the inhibition of cell protein synthesis. Herbs that have tannin as their main component, act as astringent and are used for treating intestinal disorders such as diarrhea and dysentery.[17] Alkaloids possess toxicity against cells of foreign organisms and used to treat a number of infections caused by bacteria and fungi.[18] Saponin is useful in managing inflammation.[19] Terpenoids have gained importance in the beauty industry in the manufacture of soaps and perfumes.[20] Flavonoids are polyphenolic compounds found in plants that have high antioxidant capacities that provide important health benefits.[21]

Table 1: Phytochemical screening of plant sample extracts.

Samples	Phytochemicals					
	Alkaloids	**Flavonoids**	**Steroids**	**Tannin**	**Saponin**	**Terpenoids**
***C. lanatus* (Aqueous)**	+	-	+	-	-	+
***C. lanatus* (Ethanolic)**	+	-	-	+	-	-
***P. granatum* (Aqueous)**	-	-	-	-	-	+
***P. granatum* (Ethanolic)**	-	-	-	+	-	+
***S. chirata* (Aqueous)**	+	-	-	+	+	+
***S. chirata* (Ethanolic)**	+	-	+	+	-	+

+: Present **-:** Absent

Antifungal Activity

The extracts of *C. lanatus, P. granatum,* and *S. chirata* were tested for their antifungal property using Agar Well Diffusion Method. Results of the study showed the presence of wide spectrum of antifungal activity against Human Pathogens in ethanolic extracts. Zone of Inhibition was measured in mm and the results are summerized in Table 2. Zone of inhibition is shown in fig. 1. The extracts of *C. lanatus, P. granatum,* and *S. chirata* were screened for their antifungal activity. It was found that the extract of *P. granatum* showed a wide range of antibiotic spectrum when compared to the extracts of of *C. lanatus* and *S. chirata.*

C. lanatus ***P. granatum*** ***S. chirata,***

Fig. 1. Zone of inhibition of antifungal activity of plant samples.

Table 2: Statistical analysis expressed in mean ± standard deviation.

Solvents	Samples	DIAMETER OF INHIBITION ZONE (mm)							
		10µl		15 µl		20 µl		25 µl	
		S. cereveciae	*C. krusei*	*S. cereveciae*	*C. krusei*	*S. cereveciae*	*C. krusei*	*S. cereveciae*	*C. krusei*
Aqueous	*P. granatum*	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
	S.chirata	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
	C.lanatus	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Ethanolic	*P.granatum*	15±0.125	16±0.45	25±0.354	18±0.24	27±0.354	21±0.17	32±0.452	29±0.45
	S.chirata	11±0.145	11±0.47	12±0.175	11±0.42	14±0.419	12±0.34	16±0.412	16±0.35
	C.lanatus	0±0	3±0.12	0±0	4±0.256	0±0	6±0.421	0±0	7±0.423

The antifungal activity of *P. granatum* was compared to the standard drug Flucanazole. Ethanolic extract of *P. granatum* was found to be much more efficient against *S. cereveciae* than the standard drug used. However in case of *C. krusei* the standard drug was proved to be much more efficient. The Minimum Inhibitory Concentration (MIC) of ethanolic extract of *P. granatum* was determined by Broth Dilution Method. It was found to be 9 µl for *C. krusei* and 7 µl for *S. cereveciae.*

Total Antioxidant Property

The extracts of *C. lanatus*, *P. granatum*, and *S. chirata* were tested for their total antioxidant property and the results are shown in the fig. 2.

Antioxidants have already been found in plant materials and supplements. Due to their natural origin, the antioxidants obtained from plants are of greater benefit in comparison to synthetic ones. The use of natural antioxidants from plants does not induce side effects, while synthetic antioxidants were found to have genotoxic effect.

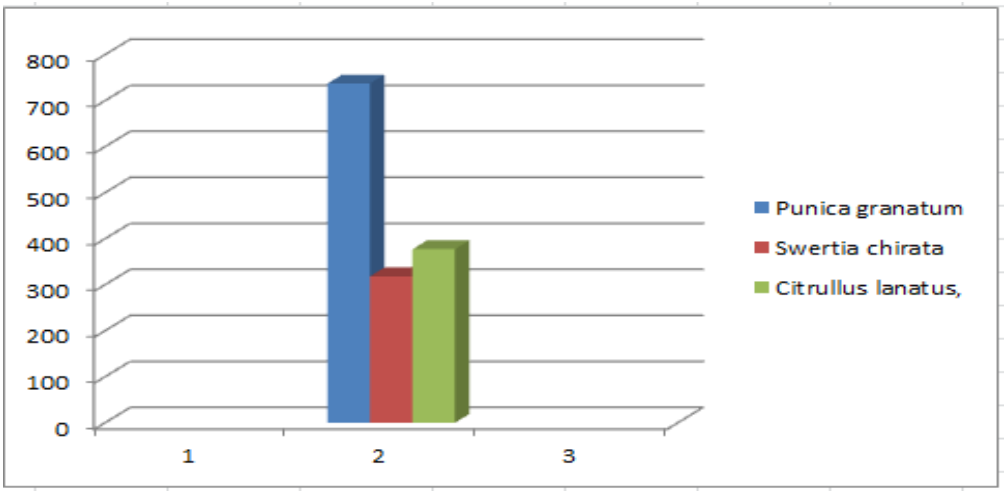

Fig. 2: Antioxidant activity of samples (µg/g).

The total antioxidant activity was expressed as (µg) equivalents of ascorbic acid/mg of the extract.

Antioxidant activity was found to be higher (700 mg/g) in *P. granatum* when compared to *C. lanatus* (345.25 mg/g) and in *S. chirata* (298.75 mg/g).

CONCLUSION

Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. Due to the presence of different phytochemicals and high antioxidant activity, the selected samples were able to inhibit the activity of tested human fungal pathogens. Hence the study showed that *P. granatum* peel extracts have the highest antifungal and antioxidant properties.

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