Research Article

Synergistic Evaluation of Anti-Candidal property of *Rosa floribunda*, *Butea monosperma* and *Aloe vera* plant extracts

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ABSTRACT

The aim of study was to evaluate the anticandidal activity of the aqueous extract of *Rosa floribunda, Butea monosperma* and *Aloe vera*. The extract contains large amounts of phenolic compounds and flavonoids. The aqueous extracts and the combinations of *Rosa floribunda, Butea monosperma and Aloe vera* were screened for anticandidal activity by using disc diffusion method. The *Rosa floribunda, Butea monosperma* against *c. albicans* material can be used as a potential source of antifungal agent against the human pathogens.

Keywords: Anti-fungal, Anti-candidal, *Candida albicans, Rosa floribunda, Butea monosperma, Aloe vera.*

INTRODUCTION

The uses of medicinal plant as a source for the treatment of various diseases have been used over five millennia to civilization of India, China and east of Asian.

Medicinal plants which are used for various traditional, complementary and alternate systems of medicine are rich in broad categories of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, and glycosides etc, which have been found *in vitro* to have antimicrobial properties. *Rosa floribunda* belonging to family Rosaceae, is used for soothing, antioxidation, skin care and anti-inflammatory properties[1]. *Aloe vera* belonging to family Liliaceae is used for the treatment of skin disease, gastrointestinal disorder, itchy spash etc.[2] *Butea monosperma* from the family of Fabaceae is used for the treatment of inflammation, dysentery, ring worm, snake bite etc.

Recently on the Earth, it was estimated that out of 8.7 million numbers of eukaryotic species more than 1.5 million species of fungi are pathogenic to humans.[3] Candida spp. is the fourth most common cause of hospital-acquired systemic infections in the United States, mortality rate being 50%.[4] Candida albicans is a dimorphic fungus which has commensalism with warm-blooded animals including humans. It colonizes on the mucosal surfaces of the oral, vaginal cavities and the digestive tract thereby causing a variety of infections which depends on the nature of the underlying host defect[5]. Therefore, *C. albicans* infections (candidiasis) are infrequent in healthy individuals. Immunosuppressive treatments, long-term catheterization, use of broad-spectrum antibiotics and longer survival of immunologically compromised individuals, have increased dramatically over the last two decades.[6] The present study therefore envisages with the synergetic evaluation of Anti-Candidal activity in flower extract of *Rosa floribunda, Butea monosperma and Aloe vera* plant extracts and their combinations.[7]

MATERIALS AND METHODS

Collection of samples

Flowers of *Rosa floribunda Aloe vera, and Butea monosperma* were collected, rinsed with distilled water and then kept for shade dried. After homogenizing them, they were stored in air tight bottles.

Aqueous Extraction

Table 1: Mixing of crude extracts

S. No.	Extract 1 (ml)	Extract 2 (ml)	Final extract (ml)
1.	5 ml Rose extract	5 ml Palash extract	10
2.	5 ml *Aloe vera* extract	5 ml Palash extract	10
3.	5 ml *Aloe vera* extract	5 ml Rose extract	10

4g dried powder of *Rosa floribunda* was boiled in 100 ml distilled water, filtered using Whatman filter paper no. 1. The filtrate was collected and stored at 5°C. Similar procedure was followed for preparation of extracts of *Aloe vera* and *Butea monosperma.* All the extracts were prepared in different volumes of distilled water (150 ml, 50 ml, 10 ml, and 5 ml) for getting varying concentrations of bioactive molecules in crude extracts. Synergistic / additive effect was the study objective and the extracts were mixed following proportion:

1) 5 ml Rose extract + 5 ml Palash extract (RP)

2) 5 ml *Aloe vera* extract + 5 ml Palash extract (AP)

3)5 ml *Aloe vera* extract + 5 ml Rose extract (AR)

The details are shown in Table 1.

Table 2

S. No.	Ingredients	Quantity (g/l)
1.	Agar	20.0
2.	Glucose	10.0
3.	Peptone	2.5
4.	Yeast extracts	1.5
5.	Malt extracts	1.5
6.	Aniline blue	0.05

Table 3: Inhibition zones on *Candida albicans* plates by different samples of plant extracts

	Diameter of inhibition zones (in mm)			
Samples	**150 ml**	**50 ml**	**10 ml**	**5 ml**
Rose	0.0	0.0 cm	1.0 cm	1.5 cm
Aloe	0.0	0.0 cm	0.0 cm	0.0 cm
Palash	0.0	0.0 cm	0.8 cm	0.0 cm
Rose – Aloe	0.0	0.0 cm	1.5 cm	0.8 cm
Aloe – Palash	0.0	0.0 cm	1.7 cm	1.7 cm
Palash – Rose	0.0	0.0 cm	0.0 cm	1.9 cm

Test microorganism for antifungal assay

Test microbe for antifungal assay was *Candida albicans* was isolated from its primary culture and subsequently sub-cultured onto agar plates forming pure colonies.

Preparation of Culture Media

For making Candida isolation agar media, accurate quantities of ingredients were weighed and transferred to volumetric flask. The chemical ingredients were dissolved in triple distilled water and final volume was made up with distilled water. Then store in refrigerator. Quantities were withdrawn using composition as per Table 2.

All the ingredients were accurately weighed, mixed in 500 ml of distilled water (DW) and volume was then made up to 1000 ml with distilled water.

The mixture was heated up to boiling and dispensed in desired volume in 150 ml flasks and autoclaved for 15 minutes.

Addition of Candida suspension to molten media

Loop full of *Candida albicans* was suspended in 10 ml sterile distilled water. It was then vortexed to get a homogenous suspension. The suspension was added to molten and cooled previously prepared candida agar media. After gentle mixing, the media containing test organism was poured on to petri-plates.

Culture preparation for antifungal assay

The glass wares were sterilized and then used. The floral samples were washed in 0.5% of NaCl solution. Extracts were prepared by the procedure described earlier. *Candida albicans* was then spread on to candida isolation agar plate. Two separate culture media were prepared for testing, one acted as control and the other for mixed extracts. Separate holes were made with the help of sterile cork-borer for each extract. Extracts were tested separately as well as in combination on culture plate of *Candia* albicans. The plates were incubated at 37°C observed at an interval of 24 hours for zone of inhibition around the wells.

Statistical Analysis

The results were analyzed by using standard deviation (SD) statistical method**.**

RESULTS

The present study was carried for the evaluation the anti-fungal properties of *Rosa floribunda*, *Butea monosperma* and *Aloe vera* against C*andida albicans* using cork borer method. Hot water extraction method was used for the preparation of extract and was screened for its antifungal activity (fig.1.). Zone of inhibition of 2 mm was observed in 10 ml of total volume *Rosa floribunda* and *Butea monosperma* against *c. albicans.* Mixture of *Rosa floribunda* and *Aloe vera*, *Aloe vera* and *Butea monosperma* showed synergistic effect and inhibited the growth of *C. albicans*. In 5 ml of total volume, *Rosa floribunda* formed inhibition zone against *c. albican* was reduced inhibition in mixed of *Rosa floribunda* and *Aloe vera*. Mixture of *Aloe vera* and *Butea monosperma* shown synergistic property and inhibit the growth of *c. albicans*. Mixture of *Rosa floribunda* and *Butea monosperma* shown synergistic properties and inhibit the growth of *c. albicans*. This inhibition range and correlated graph has been shown in the table 1.

Inhibition property was high in *Rosa floribunda* in 5 ml as compared to other samples in a total volume of 10 ml. Mixture of *Rosa floribunda* and *Butea monosperma* had highest synergistic properties as compared to other samples in 5 ml of volume. The overall findings suggest that a suitable formulation of 10 ml aqueous extracts of petals of *Rosa floribunda* and *Butea monosperma* may be developed to treat candidiasis. No inhibition zone was observed in sample prepared in a total volume of 150ml.

CONCLUSION

In present work, flower extracts of *Hibiscus rosasinensis* were screened for antibacterial activity against human pathogenic bacterial strains. Therefore the result justifies the use of the flower extract in treatment against pathogenic strains for the production of new antibiotics. It is essential to isolate and purify of the active components of these plants therefore suggested to research out further about *in vivo* studies and use in experimental animals.

Figure 2(A)

Figure 2(B)

Figure 2(C)

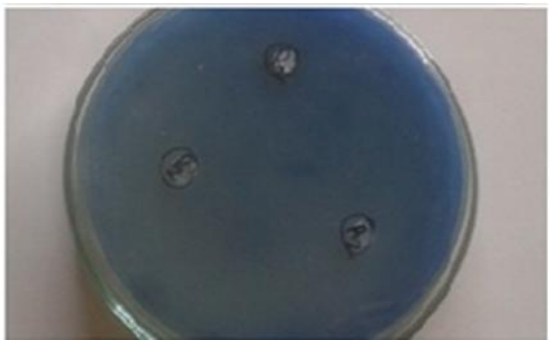

Figure 2(D)

Figure 2(E)

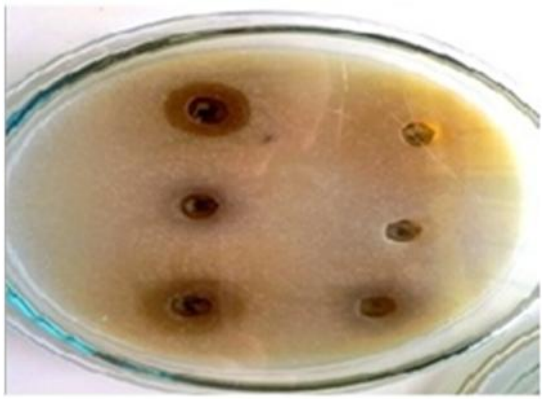

Figure 2(F)

Figure 2(A–F): Antifungal effect of aqueous extract of *Rosa floribunda*, *Butea monosperma* and *Aloe vera* and their mixture against *Candida albicans.*

2 (A): No inhibition zone was observed in sample prepared in a total volume of 150 ml in which R1, P1 and A1 for Rose extract, Palash extract and Aloe extract respectively.

2 (B): No inhibition zone was observed in sample prepared in a total volume of 150 ml in which RA, PR and AP for Rose-Aloe extract, Palash-Rose extract and Aloe-Palash extract respectively.

2 (C): No inhibition zone was observed in sample prepared in a total volume of 50 ml in which R1, P1and A1 for Rose extract, Palash extract and Aloe extract respectively.

2 (D): No inhibition zone was observed in sample prepared in a total volume of 50 ml in which RA, PR and AP for Rose-Aloe extract, Palash-Rose extract and Aloe-Palash extract respectively.

2 (E): Small inhibition zones (0.0–1.7 cm) were observed in sample prepared in total volume of 10 ml in which R1, P1and A1 for Rose extract, Palash extract and Aloe extract respectively.

2 (F): Small inhibition zones (0.0–1.7 cm) were observed in sample prepared in total volume of 10 ml in which RA, PR and AP for Rose-Aloe extract, Palash-Rose extract and Aloe-Palash extract respectively.

2 (G): Significantly large inhibition zones (0.0-1.9 cm) were observed in sample prepared in total volume of 5 ml in which R1, P1and A1 for Rose extract, Palash extract and Aloe extract respectively.

2 (H): Significantly large inhibition zones (0.0-1.9 cm) were observed in sample prepared in total volume of 5 ml in which RA, PR and AP for Rose-Aloe extract, Palash-Rose extract and Aloe-Palash extract respectively.

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