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Study on the Antimicrobial Efficacy of Solvent Extracts From Ficus Carica Latex

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ABSTRACT

Ficus carica, sacred and medicinal plant in India has been endowed in pharmacological properties used alternatively in the traditional therapeutic medicinal practices for the well being of humankind. In this perspective, the latex of this plant was collected and extracted with hexane, chloroform, n-butanol and aqueous solvents which were inturn tests against five different pathogenic bacteria and one fungal strain. The antimicrobial activity was highest shown by aqueous extract against E.coli by 23mm but on an average n-butanol extracts has shown good activity against all the selected micro-organisms. The MIC and MBC values were also in accordance with the antimicrobial zones of inhibition.

Keyword: Ficus Carica Latex, In-Vitro Antimicrobial Activity, Solvent Extracts, Pathogenic Bacteria and Fungi Strains MIC and MBC assay

1. INTRODUCTION

Numerous plants synthesize substances that are useful in the maintenance of health in humans and animals [1]. In recent times, focus on plant research has been increased all over the world and the literature shows that medicinal plants have immense potential which can be used in various traditional treatment systems. Today, scientists are witnessing a great deal of public interest in the use of herbal remedies which endowed a rigorous search and screening of several medicinal plants for their novel compounds with potential antimicrobial activity so as to overcome various diseases caused by these resistant pathogens [2]. The therapeutic utilities of *F. carica* have been indicated in traditional systems of medicine like Ayurveda, Unani, Siddha, etc. [3]. In Ayurveda, *F. carica* belongs to a class of drugs called rasayana which act as rejuvenators, antioxidants and relieve stress in the body [4, 5]. It has got mythological, religious and medicinal importance in Indian culture since ancient times [6], [7] and [8]. Medicinal plants are rich source of a wide variety of secondary metabolites called phyto-constituents like tannins, terpenoids, alkaloids, and flavonoids. These phyto- chemicals are responsible for the numerous pharmacological and antimicrobial activities shown by the plant. The present study deals with the evaluation of in-vitro antimicrobial activity of *F. carica* latex solvent extracts against pathogenic micro-organisms and determining the MIC as well MBC potential.

2.MATERIALS AND METHODS

2.1 Collection of Plant Extract

Ficus carica , latex was collected from wild area of Anatapur District, Andhra Pradesh. Healthy plants were mostly seen in pollution free wild forests from which the latex was collected by making an incision on the tree trunk with a sharp equipment and then brought back to the laboratory for further experimental analysis by storing at 4°C.

2.2 Extraction of Plant Material

The solvents extraction was done by the modified method of dissolving 5 g of dried plant extract in soxhlet apparatus with Hexane, chloroform, dichloromethane, water (200ml) separately for 24 hrs at 65°C. The extracts were concentrated to dryness in rotary pressure evaporator and stored at 40°C for further antimicrobial study [9].

2.3 Antimicrobial Activity

All the bacteria and fungi were grown in the respective growth medium at 32°C (bacteria) and 25°C (fungi) for 24hrs till the pure culture was isolated. The overnight grown cultures were used for the antimicrobial activity. Microbial cultures like *Bacillus subtilis (B. subtilis)* MTCC 511,

Staphylococcus aureus MTCC 7443, *Klebsiella pneumonia* MTCC 3384, *Salmonella typhii* MTCC 98 were obtained from *Microbial Type Culture Collection, Chandigarh and* enteroxigenic *Escherichia coli (E.coli)* isolated from diarrhoeal patients and *Candida albicans were* used for antimicrobial activity and were maintained in the respective medium at 37°C for 24hrs and the assay was carried out using the agar well diffusion method [10]. The plant extracts were resuspended in DMSO (10% v/v) which is further used as negative control and streptomycin (10 µg/mL) for bacteria and nystatin (10 µg/mL) for fungi as positive control. The antimicrobial activity was measured in zone of inhibition (mm) and recorded. The experiments were repeated thrice and the mean values were taken along with standard deviations.

2.4 MIC & MBC Assay

The minimum inhibitory concentration (MIC) was determined by comparing the various concentrations of plant extracts which have different inhibitory effect and selecting the lowest concentration of extract showing inhibition [11]. To determine MBC, the treated Muller Hilton broth culture from well which is not showing any visible growth in MIC assay was cultured on new sterile Muller Hilton Agar plates. The least concentration (highest dilution) of the extract that inhibits colony formation on a solid agar medium afterincubation at 37°C for 24 hr was considered as MBC [12].

3. RESULTS & DISCUSSION

The plant extracts were subjected to solvent extraction using four different solvent systems viz. hexane, chloroform, n-butanol, aqueous. Five different bacterial species and one fungal strain were used to evaluate antimicrobial activity of given plant extract. The results clearly indicate that among the four solvents used for the study, n-butanol extracts were endowed with higher activity. The activity of aqueous and hexane extracts were more or less similar. Streptomycin a broad-spectrum antibiotic and Nystatin were used as positive standard reference, that exhibited good activity towards the entire set of pathogenic microorganisms and DMSO was used as negative standard reference.

3.1 Antimicrobial Activity

Antimicrobial activity was done against five bacterial strains including (enteroxigenic) *E.coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium, Klebsiella pneumonia.* Plant extracts were evaluated for in-vitro antibacterial potential by agar well diffusion method. The results were observed by measuring the zones of inhibition where in the potency of the plant extract is exhibited by inhibiting the growth of test micro-organism. The results are mentioned below in the Table 1.

Table 1: Anti bacterial activity of various concentrations of Ficus carica
*****Each value is the average of three analyses ± standard deviation.**

Solvent	**Concentration**	***E.coli***	***B.subtilis***	***K.pneumonia***	***S.aureus***	***S.typhii***	***C.albicans***
		Diameter of zone of inhibition in mm					
Hexane	100 mg/ml	23 ±0.4	15±0.1	13±0.1	10±0.2	11±0.01	13±0.2
	50 mg/ml	11±0.2	12±0.3	09±0.9	10±0.4	09±0.4	11±0.5
	10 mg/ml	09±0.3	10±0.2	07±0.4	09±0.1	07±0.2	08±0.1
	0.1 mg/ml	09±0.1	10±0.3	07±0.3	08±0.3	05±0.5	05±0.6
Chloroform	100 mg/ml	15±0.5	10±0.1	13±0.2	13±0.4	13±0.1	13±0.2
	50 mg/ml	12±0.1	08±0.2	10±0.0	11±0.1	11±0.2	12±0.7
	10 mg/ml	14±0.2	08±0.4	06±0.4	08±0.0	08±0.5	10±0.6
	0.1 mg/ml	14±0.3	08±0.5	05±0.2	08±0.2	07±0.1	09±0.2
n-Butanol	100 mg/ml	14±0.2	14±0.6	14±1.2	13±0.3	13±0.5	14±0.6
	50 mg/ml	14±0.2	14±0.2	10±0.4	11±0.4	11±0.2	11±0.1
	10 mg/ml	13±0..0	10±0.1	07±0.5	08±0.5	08±0.3	08±0.6
	0.1 mg/ml	13±0.1	08±0.6	07±0.2	07±0.4	07±0.1	08±0.2
Aqueous	100 mg/ml	14±0.7	15±0.3	12±0.5	13±0.2	12±0.9	14±0.6
	50 mg/ml	14±0.4	15±0.5	10±0.6	08±0.4	10±1.2	11±0.2
	10 mg/ml	11±0.2	12±0.2	08±0.1	07±0.1	08±0.2	08±0.5
	0.1 mg/ml	09±0.3	10±0.1	07±0.0	07±0.0	07±0.5	06±0.2
Streptomycin	10 µg/mL	11±0.2	13±0.5	11±0.8	11± 0.4	09± 0.5	---
Nystatin	10 µg/mL	---	---	---	---	---	12 ±0.5
DMSO	10% v/v	---	---	---	---	---	---

In case of *E.coli,* aqueous extract of 100 mg/ml concentration has shown the maximum activity of about 23mm diameter and the minimum diameter shown was 17 mm by hexane extract. In case of *Bacillus subtilis,* hexane extract of 100 mg/ml concentration has shown the maximum activity of about 15mm diameter and the minimum diameter shown was 08mm by chloroform extract. In case of *Klebsiella pneumonia,* n-butanol of 100 mg/ml concentration has shown the maximum activity of about 14mm diameter and the minimum diameter shown was 06mm by chloroform extract of 0.1mg/ml. In case of *Staphylococcus aureus,* aqueous and chloroform extracts of 100 mg/ml concentration have shown the maximum activity of about

13mm diameter and the minimum diameter shown was 06mm. In case of *Salmonella typhimurium*, chloroform and n-butanol extracts of 100 mg/ml concentration has shown the maximum activity of about 13mm diameter and the minimum diameter shown was 05mm.The growth of *Candida albicans* was inhibited at maximum (15mm) by n-butanol and aqueous extracts which implicated that the plant has good antimicrobial properties.

3.2 MIC & MBC

The MIC analysis of plant extracts has shown the most significant bacteriocidal and bacteriostatic concentration for n-butanol and aqueous crude extracts of *F. carica* tested. The Table 2 depicted MIC and MBC of all the plant extracts and the zone of inhibition results reflected the MIC values. The MIC of the extracts were studied from the range of 1.56 to 25.0 mg/mL. The gram negative *K.pneumonia* has least zone of inhibition exhibiting high MIC & MBC values, 6.25 and 25.0 mg/mL respectively.

Table 2: MIC & MBC of F.carica plant extracts against test organisms

Plant/ Micro – organism	*F. religiosa*			
	n- butanol		Aqueous	
	MIC (mg/mL)	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC(mg/mL)**
E.coli	3.125	12.5	1.56	6.25
B. subtilis	3.125	12.5	3.125	12.5
K. pneumonia	3.125	12.5	6.25	25.0
S. aureus	6.25	25.0	3.125	12.5
S. typhii	3.125	12.5	6.25	25.0

4 DISCUSSION

The secondary metabolites present in plants are responsible for the defense mechanisms against predation by many microorganisms in the form of phyto-chemicals. [13, 14]. The present study reveals that the extracts have immense activity against gram positive and gram negative bacteria explaining the strong antimicrobial activity because of the presence of broad spectrum antibiotic compounds [15]. Out of the four solvents used for extraction, the n-butanol extracts showed the highest activity against the test organisms, followed by the aqueous extracts and hexane extracts. Different solvents have been found to have the varied capacity to extract different phyto-constituents depending on their solubility or polarity in the solvent. The aqueous extracts having considerably good activity against all the test organisms provides the scientific basis that the water has high solubility for phyto-constituents, consequently higher activity. the high MIC and MBC values of a micro-organism is an indication that either plant extract are less effective on them or that organism itself has the potential of developing antibiotic resistance. In our study, *Klebsiella* and *Staphylococcus* had high MIC and MBC values.

5 CONCLUSION

The results of the present study in accordance with the earlier reports concluded that n-butanol and aqueous extracts of *F. carica* has potent antimicrobial activity against selected pathogens which is tied to the high concentration of phytochemicals responsible for activity. The present investigation expresses that plants are the potential source of the new antimicrobial compounds used in traditional medicinal practices to ensure valuable therapeutic knowledge with scientific evidence foe their efficiency.

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