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Antimicrobial Potential of Different Plant Parts of Ficus carica against Pathogenic Microbes in Correlation to Phytochemical Analysis

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Abstract: ***In vitro antimicrobial activity of different solvent extracts prepared by using different plant part mainly fruit, leaves and bark of Ficus carica was evaluated by using agar well diffusion assay. Comparative analysis confirmed that the plant possess bioactive compounds having measurable antimicrobial activity against microbial pathogen. Among all the solvent extracts the methanolic leaf extract revealed highest antimicrobial activity against Bacillus, while the fruit extracts were more potent as compared to bark extracts.***
The fruit extracts exhibited significant antimicrobial activity with inhibition zone of diameter 27 mm in methanol, 19 mm in diethyl ether and 25 mm in chloroform.
The present study revealed that the selection of different solvents play important part in altering the results in combination with different tissue excised from plant. The leaves exhibiting highest antimicrobial activity were further analyzed for phytochemical associated and potential correlation was studied for the future aspects to extract them for drug development.
Keywords**: *Ficus carica, Antimicrobial, Zone of Inhibition, Solvent Extracts, Agar Well Diffusion Assay.***

I. INTRODUCTION

The plant family *Moraceae* comprises one of the largest genera of angiosperms having more than 800 species of trees and shrubs along with creepers and climbers. There are six subgenera and 23 species out of which *Ficus carica* belongs to genera Ficus and order Urticales [1].
Commonly known as fig the fruit of the plant is a fleshy receptacle and are considered as good source of carbohydrates, sugars, vitamins, minerals and phenolic compounds. Different plant parts of fig have differential cure of several ailments where majority of them categorized in respiratory, gastrointestinal and cardiovascular disorders. Fig plants have antioxidant, antiviral, antibacterial, anti-inflammation, haemostatic, hypoglycemic, hypocholesterolaemic, cancer suppressive and anthelmintic effects [2]. Figs are among the richest plant sources of calcium and fiber and dried figs are richest in fiber, copper, manganese, magnesium, potassium, calcium, and vitamin K. In traditional Indian medicine, fruit and roots of figs have been used as treatments of leucoderma, ring worms infestations, paralysis, and anti-inflammatory agent [3].
Different studies indicated that figs have antimicrobial effects on various gram positive and gram-negative bacteria as well as drug resistant bacteria, yeasts and mold [4].
Extracts of fig leaves are also used for treatment of gingivitis, cancer, asthma, sneezing and coughing, abscesses, diabetes and constipation [5]). Inspired by the medicinal potential of the fig plant present investigation evaluated the scope of different plant parts of *ficus carica* by using different solvent extracts against pathogenic bacteria.

II. MATERIAL AND METHODS

A. Plant And Culture Collection

The seedling of *Ficus carica* used in the present study was procured from Tau Devi Lal Herbal Park, near Khizrabaad highway, Yamunanagar district, Haryana, for analysis of antimicrobial activity and cross identified from Department of Botany, Kurukshetra University, Kurukshetra, Haryana, India. The seedling was grown to a tree with all recommended agriculture practices. The microorganisms were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, which included Gram-negative bacteria: *Escherichia coli* (MTCC- 5704) and *Pseudomonas aeruginosa* (MTCC-2295) Gram-positive bacteria: *S. aureus* (MTCC-3160) and *Bacillus subtilis* (MTCC - 121).

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B. Preparation of Leaves, bark and Fruit Extracts of Ficus Carica.

The bark of *F. carica* was thoroughly cleaned with brush and allowed for sun drying for seven days and grounded into fine powder. The 5 gm of bark powder was soaked in 20 ml of chloroform, methanol and diethyl ether for 72 hr at room temperature. The extracts were filtered using Whatmann filter paper No-1. The extra solvent from the filtrate was evaporated by using water bath at 45-50°C. The residual powder after solvent extraction was dissolved in DMSO and stored at 4° C. The leaves of *F. carica* were thoroughly washed with water then allowed for shade drying for four days at room temperature being fragile and grounded into fine powder. The 5 gm of leaves powder was taken and was processed similar to bark extract. The fruits of *F. carica* were thoroughly washed with water then allowed for sun drying for seven days and grounded into fine powder. The 5 gm of fruit powder was taken and was processed similar to above procedure.

C. Antimicrobial Activity And Preliminary Phytochemical Analysis

The antimicrobial activities of plant extracts were evaluated by agar well diffusion assay [6]. DMSO was used as a negative control. The antimicrobial spectrum of extracts was determined in terms of inhibition zone diameters. Zones were measured by high media zone scale. The phytochemical analysis was carried out by using standard method of Reference [7].

III. RESULTS AND DISCUSSION

A. Antimicrobial Analysis Against e. Coli.

Different solvent extracts prepared using different plant parts were found to possess significant antimicrobial activity against gram positive and gram negative bacteria compared to standard. The extracts of different plant parts have been assessed for antimicrobial activity against *E. coli P. aeruginosa, B. subtilis* and *S. aureus*. On comparing the antimicrobial activity of different solvent extract, the methanolic and chloroform extracts of leaves, fruit and bark resulted in greater zone of inhibition against *E. coli* as compared to extracts prepared in diethyl ether solvent as shown in Table 1. The methanolic extract resulted in a comparable zone of 24 mm, 23 mm, 22 mm using leaves, bark and fruit respectively which is almost similar in all three plant parts used. Reference[8] reported broad spectrum antimicrobial activity against both gram positive and gram negative bacteria where alcoholic extracts (methanol and ethanol) were found to be better extractants than other solvents. Methanol has proved to be the best extracting solvent resulting in prominent antimicrobial activity by using fruits as plant part against *S.aureus, S.epidermidis, E.coli* and *K. pnemonia* in *Cassia fistula* [9],whereas all other solvent extract were unable to inhibit the growth of any of the tested strain. While the chloroform extract resulted in a zone of inhibition of size 21 mm, 21 mm and 20 mm using leaves, bark and fruit respectively. The zones were almost similar using methanol and chloroform as solvent system to prepare plant extracts. However the diethyl ether solvent showed a zone of 16mm using leaf extract only while no antibacterial activity was observed using bark and fruit extracts against *E.coli.*

B. Antimicrobial Analysis Against P. Aeruginosa

P aeruginosa has been reported to be primary suspect in a number of pulmonary and urinary tract infections. The leaf extract prepared using methanol as solvent system resulted in a zone of 25 mm (leaf), followed by a zone of 21 mm (fruit) followed by a zone of 18 mm using bark extract as shown in Table 2. Similarly the different plant extracts prepared using chloroform showed a zone of 19 mm using leaves, a zone of 14 mm using fruit extract, while no antimicrobial activity was observed using bark extract. Reference [10] reported similar results exhibiting minimum activity of chloroform extracts against *P. aeruginosa* in *C. auriculata using* fruit. Similarly no activity was observed diethyl ether as solvent in any plant part extract screened against *P. aeruginosa.*

C. Antimicrobial analysis against B. subtilis

Methanolic extract of all three plant parts viz leaves, bark and fruit exhibited prominent antibacterial activity against *B. subtilis* resulting in a larger zone of inhibition of 36 mm using leaf extract followed by 30 mm zone using bark extract and a zone of 26 mm using the fruit extract as shown in Table 3. As compared to methanol the chloroform extracts resulted in significant antibacterial activity although the zone of inhibition was smaller than observed in methanolic extract. The chloroform extract of leaf showed a zone of 25 mm followed by a zone of 24 mm using bark extract while almost comparable zone of inhibition of 23 mm using the fruit extract. All plant parts resulted in almost comparable zone of inhibition against *B. subtilis* and further confirmed that chloroform can be explored as extraction solvent second to methanol in preparing extracts for *Ficus carica*. Our results well corroborate with Sharma and his coworkers [11]. While the third solvent diethyl ether resulted in a zone of 23 mm using leaf extract followed by a zone of 16 mm using fruit extract. No antibacterial activity was observed using bark extracts in diethyl ether solvent. Moreover our result well corroborate with the study as remarkable antimicrobial activity was reported against *B. subtilis* resulting in a zone of 25 mm in aqueous ethanol and 20 mm using chloroform as solvent extract.

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D. Antimicrobial Analysis Against S. AUREUS

The different solvent extracts against *S. aureus* resulted in a comparable zone of inhibition both in methanolic and chloroform extracts showing significant efficacy against the pathogen as shown in Table 5. The methanolic extract of leaves resulted in a zone of 24 mm which was smaller as compared to zone of inhibition observed using bark 26 mm and fruit extract 27 mm. The leaf and fruit extract prepared in chloroform exhibited a similar zone of inhibition of size 25 mm followed by a zone of 19 mm using bark extract. The results showed the sensitivity of *S. aureus* against herbs and spices. The diethyl ether as a solvent against *S. aureus* resulted in a comparable zone of 18 mm and 19 mm which was significant using leaves and fruit extract respectively at 1mg/ml concentration. Similar zones of inhibition (18 mm, 19 mm and 20 mm) were reported by using leaf extracts in *G. Sylvestre* in ether, chloroform and aqueous ethanol solvent extracts respectively at a concentration of 200 µg/ml[12]. The zone observed against pathogen was smaller as compared to methanol and chloroform but still showed efficacy against *S.aureus*. The *S .aureus* pathogen was observed to be most sensitive to fruit extracts may be due to its cell wall structure and outer membrane.

The agar disc diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zones against the test microorganism. The result clearly showed that methanol and chloroform extracts of leaves, bark and fruit were found to be effective against all the pathogenic bacteria used in this investigation i.e. *Escherichia coli, Psuedomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis*. The diethyl ether extracts of leaves, bark and fruit were found comparatively lower while the diethyl ether extract of bark did not showed antimicrobial activity against any pathogens used in the present investigation. The results of present investigation clearly indicate that the antimicrobial activity vary with the solvent used for extraction of antimicrobial metabolite. Further research work is needed for identification, isolation and purification of secondary metabolites present in these plant parts (leaves, bark and fruits) of *F. carica* to elucidate the precise molecular mechanism and targets for cell growth inhibition which will allow the rationale design for more effective molecules as therapeutic agents. As a solvent diethyl ether was not found to be effective solvent against all four pathogens tested except *S. aureus*. Even a higher concentration did not resulted in any zone of inhibition. Leaves were reported to be the best plant part to analyze antimicrobial activity in comparison to fruits and bark by exhibiting maximum zone of inhibition and confirming the prominent potential in future. Different plant parts showed differential activity in different solvents as a maximum zone of 36 mm using methanol as solvent against *B. subtilis*, a zone of 27 mm using fruit extract in methanol against *S. aureus*, a zone of 30 mm of bark extract against *B. subtilis*. More solvent system need to explore for maximum activity to be observed using different solvent systems.

E. Phytochemical Analysis

The Ficus leaves resulted in potent antimicrobial activity so were further analyzed for the presence of responsible phytochemicals as shown in Table 5.The leaves revealed the presence of active constituents like saponins, tannins, terpenoids, phlobatinins, leucoanthocyanins, phenols, coumarin, quinines, fatty acids cardiac glycosides and amino acids. The leaves and fruits of the plant have shown that they are rich in phenolics, organic acids, and volatile compounds in the phytochemical analysis. So the need of the hour is to identify bioactive compounds and correlate them to their biological activities for further research to explore the potential of *F.carica* as a source of therapeutic agents. Phytochemical studies on *F. carica* revealed the presence of numerous bioactive compounds such as phenolic compounds, phytosterols, organic acids, anthocyanin composition, triterpenoids, coumarins, and volatile compounds such as hydrocarbons, aliphatic alcohols and few other classes of secondary metabolites from different parts of *F. carica* [13],[14].

IV. CONCLUSION

The Fig plant contains compounds that exhibit measurable antimicrobial activity against tested bacteria. Among the solvents used, methanol extracts of leaves and fruit showed the highest activity in comparison to bark with regard to the inhibition of microbial growth.

All plant parts exhibited antimicrobial activity but the highest was observed in leaves along with fruits. Chloroform extracts of leaves and fruits also showed better antimicrobial activity than the diethyl ether extracts of the plant parts. Fruit extracts in different solvents were found to be highly active against the bacterial strain *S. aureus* and least active against *P. aeruginosa*. Methanolic extracts of all the plant part showed better result with leaves showing highest antimicrobial activity against *B. subtilis*. Diethyl ether extracts of leaves showed the least activity against all pathogens while diethyl ether extract of bark did not showed any antimicrobial activity against pathogens.

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TABLE- I.
Inhibition zone diameters (in mm) of plant extract of *f. Carica* in various solvents and negative control against *e. Coli.*

Solvent	Leaves (L)	Bark (B)	Fruits (F)
Methanol (M)	24	22	23
Diethyl ether (D)	16	-ve	-ve
Chloroform (CH)	21	21	20
Negative control (C)	-ve	-ve	-ve

TABLE -II.
Inhibition zone diameters (in mm) of plant extract of f. Carica in various solvents and negative control against p. Aeruginosa.

Solvent	Leaves (L)	Bark (B)	Fruits (F)
Methanol (M)	25	18	21
Diethyl ether (D)	-ve	-ve	-ve
Chloroform (CH)	19	14	-ve
Negative control (C)	-ve	-ve	-ve

TABLE -III
Inhibition zone diameters (in mm) of plant extract of *f. Carica* in various solvents and negative control against *b. Subtilis*.

Solvent	Leaves (L)	Bark (B)	Fruits (F)
Methanol (M)	36	30	26
Diethyl ether (D)	23	-ve	16
Chloroform (CH)	25	24	23
Negative control (C)	-ve	-ve	-ve

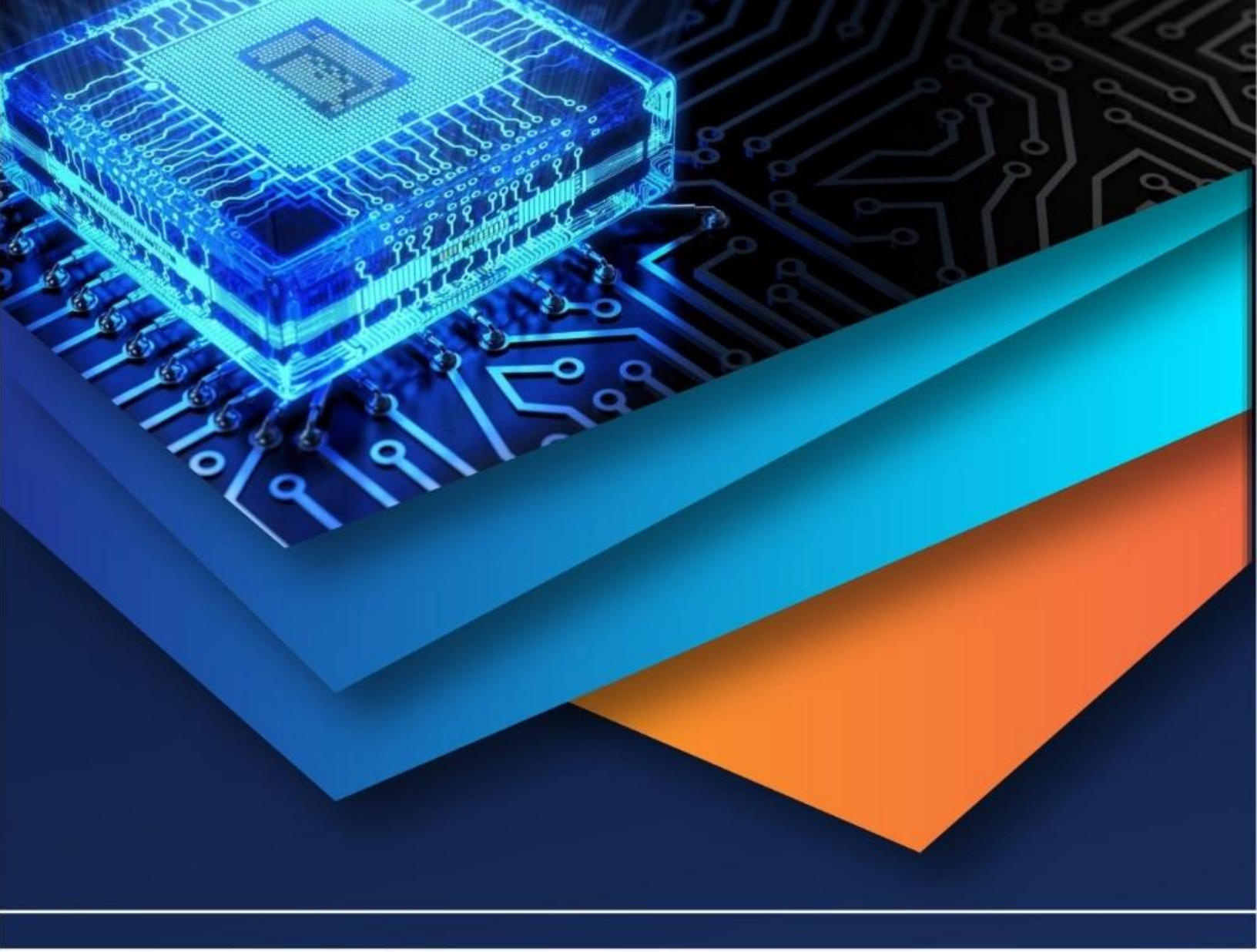
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TABLE -IV.

Inhibition zone diameters (in mm) of plant extract of *f. Carica* in various solvents and negative control against *s. Aureus.*

Solvent	Leaves (L)	Bark (B)	Fruits (F)
Methanol (M)	24	26	27
Diethyl Ether (D)	18	-Ve	19
Chloroform (CH)	25	19	25
Negative Control (C)	-Ve	-Ve	-Ve

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