EVALUATION OF ANTIBACTERIAL & ANTIOXIDANT ACTIVITIES OF AQUEOUS EXTRACT OF FICUS CARICA LINN

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Abstract: Many species have been reported to present antimicrobial activity. Working on the same line, we have undertaken a study on Ficus Carica fruit antibacterial and antioxidant activities. Preliminary phytochemical analysis of the AEFC showed that the plant has a rich possession of phytochemicals like alkaloids, Proteins, saponins, carbohydrates, tannins, flavonoids and phenols. Phenols, glycosides, gums and mucilage were absent in the extract. The anti-bacterial activities of Ficus Carica Fruit against various microorganisms have been studied. AEFC inhibitory action on different strains of bacteria was compared with standard drug tetracycline. The plant extract was tested for their antibacterial activities. The results of the antibacterial activity revealed that the control (sterile water on filter paper disc) showed no inhibition of growth, while the aqueous extract showed different degrees of growth inhibition, depending on the bacterial strains. In addition, the alcohol soluble extract of the plant shows good inhibitory action on all microbes compared to that of water soluble extract. However the inhibitory effect of the plant is less when compared to that of the standard. In vitro studies using DPPH method, and nitric oxide inhibition assays showed strong antioxidant nature of the aqueous extract. The IC50 values were found to be equal to that of standards ascorbic acid. The results clearly indicated that AEFC was found to be effective in scavenging the DPPH free radical when compared to the nitric radical, since IC50 values obtained were found to be low in DPPH method.

Keywords: Antibacterial, Antioxidant Activities, Aqueous Extract, Ficus Carica Linn

INTRODUCTION:

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immuno compromised patients in developed countries[1]. There is an urgent need to control antimicrobial resistance by improved antibiotic usage and reduction of hospital cross infection[2]; however the development of new antibiotics should be continued as they are of primary importance to maintain the effectiveness of antimicrobial treatment[3]. In developing countries, the World Health Organization[4] estimates that about three quarters of the population relies on plant based preparations used in their traditional medicinal system and as the basic needs for human primary health care. Therefore, several medicinal plants have been evaluated for possible antimicrobial activity and to get remedy for a variety of ailments of microbial origin[5,6].

Oxidative stress is an important contributor to the pathophysiology of a variety of pathological conditions including cardiovascular dysfunction, atherosclerosis, inflammation, carcinogenesis, drug toxicity, reperfusion injury and neurodegenerative diseases[7]. Plants (fruits, vegetables, medicinal herbs) contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites, that are rich in antioxidant activity[8–11]. Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissue[12]. Antioxidants are considered as possible protective agents reducing oxidative damage to the human body[13]. Antioxidants are naturally abundant in fruits and are able to neutralize free radicals donating an electron and converting them to harmless molecules[14].

Natural antioxidants have a wide range of biochemical activities, including inhibition of ROS generation, direct or indirect scavenging of free radicals, and alteration of intra cellular redox potential[15]. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and the concomitant lipid peroxidation, protein damage and DNA strand breakage[16]. An antioxidant, which can quench reactive free
radicals, can prevent the oxidation of other molecules and may, therefore, have health promoting effects in the prevention of degenerative diseases[17]. In addition, it has been reported that there is an inverse relationship between dietary intake of antioxidant rich food and the incidence of human diseases[18].

Ficus carica is an Asian species of flowering plant in the mulberry family, known as the common fig. The fruit, also called the fig, is an important crop in those areas, where it is grown commercially. Native to the Mediterranean and western Asia, it has been sought out and cultivation since ancient time and is now widely grown throughout the world, both for its fruit and as an ornamental plant.

The use of synthetic based Antimicrobial drugs for the control of microbes and poses problem of resistance and serious health hazards to mankind. The scenario therefore inevitably demands the development of newer methods and the usage of natural products derived from plants as they are biodegradable, ecologically safe and have significant efficacy on target organisms and safe for human being. In the present an attempt was also made to screen plant extracts material for their Antimicrobial activity.

METHODOLOGY

The fruit of the plant was collected during the month August to December and was identified pharmacognostically by a botanist. Then it was dried in shade, powdered, weighed and stored in a clean, dry and air tight container. The powder was subjected for successive extraction with distilled water.

EXTRACTION:

Powder was packed in a round bottom flask and extracted with distilled water at 100°C temperature by soxhlet apparatus by condensation method. The above extract was dried on water bath at 100°C to get a solid mass.

Table No. 1 Nature, Percentage and Yield of the extracts

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of the Extract</th>
<th>Nature</th>
<th>Colour</th>
<th>% yield of Extract (%w/w) in gms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aqueous extract of Ficus Carica</td>
<td>Sticky</td>
<td>Dark Green</td>
<td>10 gms</td>
</tr>
</tbody>
</table>

PRELIMINARY PHYTOCHEMICAL INVESTIGATION:

The aqueous extract of Ficus carica (Linn) was subjected for the qualitative preliminary phytochemical identifications by the standard methods described in practical pharmacognosy by Dr. C.K. Kokate and Khandelwal K.R.

IN-VITRO METHODS OF ANTIOXIDANT ACTIVITY

FREE RADICAL SCAVENGING ACTIVITY (DPPH Method)

The free radical scavenging activity of methanolic extract of H.radicata was measured by using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) method of Blois (1958). 0.2mM solution of DPPH in methanol was prepared and 100µl of this solution was added to various concentrations of methanolic leaf and root extracts at the concentrations of 200, 400, 600, 800 and 1000µg/ml. After 30 minutes, absorbance was measured at 517nm. Butylated hydroxytoluene (BHT) was used as the reference material. All the tests were performed in triplicate and percentage of inhibition was calculated by comparing the absorbance values of the control and test samples.

HYDROGEN PEROXIDE SCAVENGING ASSAY

The ability of plant extract to scavenge hydrogen peroxide was estimated according to the method reported by Ruch et al. with minor modification. A solution of hydrogen peroxide (43mM) is prepared in phosphate buffer (1M pH 7.4). Different concentration of sample (2-10mg/ml) was added to a hydrogen peroxide solution (0.6ml, 43mM). Absorbance of hydrogen peroxide at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard. The free radical scavenging activity was determined by evaluating % inhibition as above.
NITRIC OXIDE RADICAL SCAVENGING ASSAY

Nitric oxide generated from sodium nitroprusside interacts with oxygen to produce nitrite ions which was measured by the Griess reaction. This assay was done by the procedure described by Green et al. the reaction mixture containing 3.0ml of 10mM sodium nitroprusside in phosphate buffered saline (pH7.4) and various concentrations of (50,100,150,200and250mg/ml) extracts. The resulting solution was then incubated at25°C for 60minutes. To the incubated sample 5.0ml of Griess reagent (1%sulphanilamide, 0.1% NEDD in 2% H3PO4) was added and the absorbance of the chromophore formed was measured at 546nm against a reagent blank. Percentage inhibition of the nitrite ions generated is observed. The standard ascorbic acid and BHT was used for comparison. The free radical scavenging activity was determined by evaluating % inhibition as above.

ANTIMICROBIAL ACTIVITY
Screening techniques for anti-microbial activity:
The screening techniques which measure only the growth inhibition (bacterio-static) includes.
1. Cup plate technique
2. Serial dilution method
3. Ditch plate technique
4. Solid dilution method
5. Gradient plate technique
6. Agar diffusion method.

Cup plate technique:
In this technique the test solution is placed in contact with agar, which is already inoculated with test organism. After incubation, zones of inhibition is observed. The test solution may be placed in a small cup sealed to the agar surface in a well cut from theagar with a sterile cork borer, or applied in the form of an impregnated disc of filter paper. Serial dilution method:
In serial dilution technique the graded doses of test substances are incorporated into broth and the tubes inoculated with the test organism are incubated. The concentration at which no growth occurs is taken as minimum inhibitory concentration (MIC).

Ditch plate technique:
In this technique the test solution placed in a ditch cut in nutrient agar contained in a petri dish, or it may be mixed with a little agar before pouring into the ditch. The test organisms (as many as six may be tested) are streaked upon the ditch. The plate is then incubated and observed for anti-bacterial activity.

Solid dilution method:
In this method the dilution of the substance under test are made in agar instead of broth. The agar containing the substance under test is subsequently poured into a petri dish then incubated and observed for any failure of growth. It has the advantage for any one concentration of the test substance, several organisms may be tested.

Gradient plate technique:
In this technique the concentration of drug in a agar plate may be varied infinitely between zero and a given maximum number. To perform the test, nutrient agar is melted, the solution under test added, and the mixture poured into a sterile petri dish and allowed to set in the form of a wedge. A second amount of agar is then poured onto the wedge and allowed to set with the petri dish flat on the bench. The plates are incubated overnight to allow diffusion of drug and to dry the surface. The test organisms must be streaked in a direction running from the highest to the lowest concentration. Unto six organisms maybe tested in this way.

Agar diffusion method:
Standard preparation of rapidly growing bacteria is inoculated on the surface of Muller Hinton agar plates. Filter paper discs containing specific concentration of anti-
Microbial agents are pressed on to the surface and incubated at 350°C over night (18-24 hours) zone of inhibition of growth around each disc is measured and the susceptibility determined.

A. **Preparation of test solutions:** Pet. ether and ethanol extracts were dissolved in minimum amount of distilled water, whereas aqueous extract was prepared using distilled water to get 25 and 50 mg/ml concentrations.

B. **Preparation of standard solutions:**
Tetracycline, streptomycin were the reference standard drugs prepared in distilled water to get 20 μg/ml.

C. **Test organisms used were**
1. Escherichia coli (MTCC 46)
2. Pseudomonas aeruginosa (MTCC 442)

D. **Preparation of sub culturing media**
Peptone water media was prepared using following ingredients.
1. Beef extract – 10 g
2. Peptone – 10 g
3. Sodium chloride – 5 g
4. Distilled water – Q.S. to 1000 ml

E. **Preparation of media:**
1. Peptone - 6 g
2. Casein hydrolysate of soyabean - 4 g
3. Yeast extract - 3 g
4. Beef extract - 1.5 g
5. Dextrose (dehydrated) - 1 g
6. Agar - 15 g
7. Distilled water sufficient to make 1000ml pH at 25°C adjusted to 6.6 ± 0.1

The above shown quantities of different ingredients were accurately weighed and dissolved in 1 liter of distilled water and 250ml of each of the media was distributed in to 4 conical flasks. Media so prepared was sterilized by autoclaving at 15 Lbs/Sq. inch for 15 minutes.

F. **Preparation of inoculum:**
The peptone water medium was sterilized by autoclaving at 15 Lbs/Sq/inch for 15minutes. Loop full organisms were transferred from a laboratory-maintained culture in to a conical flask (250 ml) containing sterilized peptone water medium. The flask was incubated for 24 hours at 37°C.

G. **Sterilization of apparatus required:**
Petridishes, cork borer (8mm), glass syringes and test tubes were sterilized by autoclaving at 15 Lbs/Sq. inch for 15 minutes.

**Procedure for performing the disc diffusion test:**
The required amount of Petri plates is prepared and autoclaved at 121°C for 15 minutes. And they were allowed to cool under laminar air flow. Aseptically transfer about 20 ml of media into each sterile Petri dishes and allowed to solidify. 1 ml inoculums suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. The readily prepared sterile discs were loaded with 100mg/ml of plant extract and antibiotic ampicillin into each separate disc of about 100μl. The paper diffuse discs were placed on the medium suitably apart...
and the plate were incubated at 5°C for 1 hour to permit good diffusion and then transferred to an incubator at 37°C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm).

RESULTS

Preliminary phytochemical evaluation:
The phytochemical studies when brought to little existence, revealed the presence of phytoconstituents like alkaloids, saponins, steroids, flavonoids, carbohydrates and absence of proteins. The ethanolic extract of whole plant was subjected to different chemical tests separately for the identification of various active constituents was tabulated in the table.

Anti-microbial activity

The anti-microbial activities of aqueous extract of Ficus Carica (AEFC) against various microorganisms have been studied. The results of the antibacterial and antifungal activities revealed that the control (sterile water on filter paper disc) showed no inhibition of growth, while the AEFC showed different degrees of growth inhibition, depending on the various bacterial and fungal strains. The results are depicted in figures and tables.

Antioxidant activity

1-diphenyl-2-picryl hydrazyl [DPPH], superoxide [O₂•], hydrogen peroxide [H₂O] and nitric oxide [NO] scavenging activity

The ability of EEPO to scavenge DPPH, superoxide and nitric oxide were measured in vitro. The IC₅₀ values of the samples are mentioned respectively. AEFC significantly reduced DPPH levels. In similar manner, Superoxide and nitric oxide scavenging activity for AEFC was found to be high as witnessed by their low IC₅₀ values.

Table no. 2: Percentage yield of the extract

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of solvent</th>
<th>Colour</th>
<th>Consistency</th>
<th>Yield (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aqueous</td>
<td>Brown</td>
<td>Sticky</td>
<td>10 gr.</td>
</tr>
</tbody>
</table>

Table no. 3: Qualitative Phytochemical analysis of Ficus Carica Fruit

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Constituents</th>
<th>Test</th>
<th>Ethanolic Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>a) Mayer's reagent</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Dragendorff's reagent</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) Hagner's reagent</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d) Wagner's reagent</td>
<td>+ve</td>
</tr>
<tr>
<td>2.</td>
<td>Carbohydrates</td>
<td>a) Molisch's reagent</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Fehling's solution A and B</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) Benedict's reagent</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d) Barfoed's reagent</td>
<td>-ve</td>
</tr>
<tr>
<td>3.</td>
<td>Protein</td>
<td>a) Biuret test</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Millon's reagent</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Tannins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---------</td>
<td>-----------------------------------------------------------------</td>
<td>---</td>
</tr>
<tr>
<td>4.</td>
<td>a) 10% Lead acetate solution</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) Acetic acid solution</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c) Aqueous bromine solution</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Flavanoid</td>
<td>a) Alkaline reagent test</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Con. H₂SO₄</td>
<td>+ve</td>
</tr>
<tr>
<td>6.</td>
<td>Cardiac Glycosides</td>
<td>Glacial acetic acid + Ferric chloride</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ Con. Sulphuric acid</td>
<td>-ve</td>
</tr>
<tr>
<td>7.</td>
<td>Resins</td>
<td>Acetic anhydride test</td>
<td>+ve</td>
</tr>
<tr>
<td>8.</td>
<td>Terpinoids</td>
<td>Terpinoid test</td>
<td>+ve</td>
</tr>
<tr>
<td>9.</td>
<td>Triterpinoids</td>
<td>Salkowski’s test</td>
<td>+ve</td>
</tr>
<tr>
<td>10.</td>
<td>Oil and fat</td>
<td>Baudouin test</td>
<td>-ve</td>
</tr>
<tr>
<td>11.</td>
<td>Reducing sugars</td>
<td>Glucose test</td>
<td>-ve</td>
</tr>
</tbody>
</table>

**Fig 1: Anti-bacterial activity of alcohol soluble AEFC on Escherichia coli**

S: Sandard(Tetracycline), T: Test(AEFC), Control
Fig 2: Anti-bacterial activity of alcohol soluble AEFC on pseudomonas aeruginosa

![Image of bacterial activity](image1)

S: Standard(Tetracycline), T: Test(AEFC), Control

Fig 3: Antibacterial activity of alcohol soluble AEFC on staphylococcus aureus

![Image of bacterial activity](image2)

S: Standard (Tetracycline), T: Test(AEFC), Control

Table no 4: Antibacterial activity of EEPS on various strains of bacteria

<table>
<thead>
<tr>
<th>S .No.</th>
<th>Test organism</th>
<th>Zone of inhibition in mm</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Standard (Tetracycline)</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Escherichia coli</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>2.</td>
<td>Pseudomonas aeruginosa</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>
Plants have been used as source of drugs for the treatment of microbial infections in developing countries where the cost of conventional medicines represents a burden to the population. Many species have been reported to present antimicrobial activity. Working on the same line, we have undertaken a study on Ficus Carica fruit antibacterial and antioxidant activities.

Preliminary phytochemical analysis of the AEFC showed that the plant has a rich possession of phytochemicals like alkaloids, Proteins, phenols, carbohydrates, tannins, flavonoids and phenols. Phenols, glycosides, gums and mucilage were absent in the extract.

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In addition, the alcohol soluble extract of the plant shows good inhibitory action on all microbes compared to that of water soluble extract. However the inhibitory effect of the plant is less when compared to that of the standard.

In vitro studies using DPPH method, and nitric oxide inhibition assays showed strong antioxidant nature of the aqueous extract. The IC50 values were found to be equal to that of standards ascorbic acid. The results clearly indicated that AEFC was found to be effective in scavenging the DPPH free radical when compared to the nitric radical, since IC50 values obtained were found to be low in DPPH method.

CONCLUSION

Finally, It was concluded that Ficus Carica fruit was traditionally used to treat various diseases. In this study fruit has shown the presence of Antioxidant constituents. The anti-bacterial action of the fruit may be because of the antioxidant property only. It is further research is needed to find the particular constituent responsible for antibacterial and antioxidant action. Hence plant possess different pharmacological actions and used for different ailments. Further research is to be carried out to find its various therapeutic activities.

<table>
<thead>
<tr>
<th>Test material</th>
<th>IC 50 (µg/ml) ± SEM&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPPH</td>
</tr>
<tr>
<td>AEFR</td>
<td>164.5±40.1</td>
</tr>
<tr>
<td>ASCORBIC ACID</td>
<td>203.70±32.70</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average of 3 determ
REFERENCES: