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FTIR ANALYSIS AND BIOLOGICAL EVALUATION OF ETHANOL LEAF EXTRACT OF *Ficus carica* (Linn)

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Abstract This study was aimed at using FTIR to analyze the functional groups present in the fraction and to also carry out the antimicrobial analysis of the ethanol leaf extract obtained from Ficus carica (Linn). The FTIR analysis revealed the presence of 3339 cm^{-1} which is typical of –OH (alcohol) while, 2918and 2851 cm^{-1} cm^{-1} can be said to be C-H (stretch) for alkanes. Also, the peak at 1617 cm^{-1} (C=C) is that of conjugated alkenes. Equally observed, is the peak at1438 cm^{-1} (C-H bending) which is equivalent to that of methyl in alkanes. The signal at 1375 cm^{-1} (C-O) is that of alcohol and 1036 cm^{-1} (C-O stretch) is typical of alcohols. While the Mininum Inhibitory Concentration (MIC) of the ethanol leaf extract was obseved at 5mg/ml for Staphylococus aureus and Shigella dysenteriae, and 2.5mg/ml for Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi. The Minimum Bactericidal Concentration (MBC) of the ethanol leaf extract was observed at 10mg/ml for all the microbes. The presence of these group of compounds could be responsible for the sensitivity of the ethanol leaf extract, hence, its role in in traditional medicine practice.

KEY Words: Ficus, FTIR Bacteriocidal, Ethanol, Leaf, Inhibitory

INTRODUCTION

Medicinal plants play important roles in the lives of rural people by curing common ailments especially in developing countries of the world. A large population of the world uses alternative medicines for their daily healthcare needs. More than 80% of the world population in developing countries use plant medicines and about half of the population in industrialized countries also use traditional medicines as first line therapy. About 70% of the world population also practices traditional medicines to meet their healthcare needs (WHO, 2002).

Ficus carica L. is an important member of the genus *Ficus.* It is ordinarily deciduous and commonly referred to as "fig". It is one of the largest genera of medicinal plants. The leaves, fruits, and roots used in native medicinal system in different disorders such as gastrointestinal (colic, indigestion, loss of appetite, and diarrhea), respiratory (sore throats, cough, and bronchial problems), inflammatory, and cardiovascular disorders (Burkil, l935 and Penelope, 1997). Fruits of *F. carica* can be eaten fresh or dried or used as jam. Figs are used as an excellent source of minerals, vitamins, carbohydrates, and dietary fiber because it is fat and cholesterol free and contain high number of amino acids (Slatnar *et al;* 2011; Veberic *et al;* 2008[a]; Veberic *et al;* 2008[b] and Solomon *et al*, 2006). It is also reported that figs have been conventionally used for their therapeutic benefits as laxative, cardiovascular, respiratory, antispasmodic, and anti-inflammatory remedies (Guarrera, 2005). It is then important to revisit this plant for its potency in curing some of the major symptom of current covid-19 pandemic. If from the literature, the plant is effective against some covid-19 symptoms such as respiratory disorder, cough and bronchial problems.

MATERIAL AND METHODS

Sample Collection and Preparation

The Samples was collected from Olamaboro Local Government Area, Kogi State, Nigeria in the month of March, 2019. They were stored in a polythene bag and taken to the Herbarium of Federal University Lokoja for identification and was assigned the voucher number of 0125. Afterwards the stem bark and leaves of *Ficus carica* were collected. They were washed with distilled water and the stem bark was cut into smaller pieces. The leaf and stem bark samples were air-dried in the laboratory for three weeks. The dried samples were pulverized using a wooden mortar and a pestle. The pulverized samples were stored in a tightly covered container until it was required for analysis.

Extraction Procedure

Extraction was done by maceration for 72 hours using 400 Ml of ethanol with 200g of the pulverized leaf samples. The extract obtained were filtered and evaporated in an already weighed evaporating dish.

The dried extracts obtained were further subjected to various analysis.

BIOLOGICAL EVALUATION

The antimicrobial activities of *Ficus carica* leaf extracts was determined using the following microbes; *Staphylococus aureus*, *Salmonella typhi*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Escherichia coli*. These microbes were obtained from the Department of Medical Microbiology, Ahmadu Bello University (ABU) Teaching Hospital, Zaria, Kaduna State, Nigeria.

Method of Preparation of Stock Solution

0.1g was weighed and dissolved in 10 Ml of dimemethyl sulfoxide $(CH_3)_2SO$. This was carried out inorder to obtain a concentration of 10mg/ml. Diffusion method was the method adopted for sreening the extracts.

Microbial Growth Media

The medium used as the growth medium for the microbes was Nueller Hinton agar. The medium was prepared in accordance to the manufacturer's instruction, sterilized at 121^{o}C for 15minutes, poured into sterile petri dishes and was allowed to cool and solidify. The sterilized medium was seeded with 0.1ml of the standard inoculum of the test microbe. The inoculum was spread evenly over the surface of the medium by the use of a sterile swab. By the use of a standard cork borer of 6mm in diameters, a well was cut at the center of each inoculated medium.

Zone of Inhibition

0.1ml of 10mg/ml of the extract solution was introduced into the well on the inoculated medium. Incubation was made at 37^{o}c for 24hrs, after which the plates of the medium were observed for the zone of inhibition of growth, the zone was measured with the help a transparent ruler and the result recorded in millimeters.

Determination of the Minimum Inhibition Concentration (MIC)

The Minimum Inhibition Concentration of the extracts was determined using the broth dilution method. Muller Hinton broth was prepared, 10mls was dispensed into test tubes and the broth was sterilized at 121^{0}c for 15mins, the broth was left to cool. McFarland's turbidity standard scale number 0.5 was prepared to produce turbid solution.

Normal saline was prepared, l0 mls was dispensed into sterile test tube and the test microbe was inoculated and incubated at 37^{0}c for 6hours.Dilution of the test microbe was done in the normal saline until the turbidity marched that of the McFarland's scale by visual comparison at this point the test microbe has a concentration of about $1.5x10^8$ cfu/ml.

Two-fold serial dilution of the extract in the sterile broth was done to obtain the concentrations of 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, and 0.63mg/ml. The initial concentration was obtained by dissolving 0.1g of the extract in 10mls of the sterile broth. Having obtained the different concentrations of the extract in the sterile broth, 0.1ml of the test microbe in the normal saline was then inoculated into the different concentrations, incubation was made at 37^{0}c for 24hrs after which the test tube of the broth was observed for turbidity (growth). The lowest concentration of the extract present in the broth, which shows no turbidity was recorded as the minimum inhibition concentration.

Determination of Minimum Bactericidal Concentration (MBC)

MBC was carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton agar was prepared, sterilized at 121^{o}c for 15minutes, poured into sterile petri dishes and was allowed to cool and solidify.

The contents of the MIC in the serial dilutions were then sub cultured onto the prepared medium, incubation was made at 37^{o}c for 24hrs, after which the plates were observed for colony growth, MBC was the plates with the lowest concentration of the extract without colony growth

Fourier Transform Infrared Spectroscopy (FTIR)

The leaf extract was scanned for functional groups present using Agilent Technologies CARY 630 FT-IR spectroctrophotometer.

RESULTS AND ISCUSSION

Extraction

200g of the pulverized material was extracted using 450ml of ethanol. The ethanol leaf extract yielded 5.7g (2.85%)as shown in table 1.

Table 1: Percentage Yield of the Leaf and Stem Bark of *Ficus carica* (Linn)

Solvent used	Weight of ethanol Leaf extracts (g)	Percentage of ethanol Leaf extract (%)
Ethanol	5.7	2.85

Biological Evaluationb of the Leaf extract of *Ficus Carica*

The crude ethanol leaf extract of Ficus carica was found to be sensitive on all the microbes, with 4 exception to *Shigella dysenteriae.* while the zones of inhibition ranges between 18mm and 21mm, The result is as shown in the table 3 and

Table 2: Antimicrobial Activity of the Leaf and Stem Bark of *Ficus carica*

Test organisms	FCEL
Staphylococus aureus	S
Escherichia coli	S
Pseudomonas aeruginosa	S
Salmonella typhi	S
Shigella dysenteriae	S

KEY ⟶ **R**-Resistance, **S**-Sensitive, **FCEL**- *Ficus carica* ethanol leaf,

Table 3: Zone of Inhibition of the Leaf and Stem Bark Extract of *Ficus carica* (Linn)

Test organisms	FCEL
Staphylococus aureus	18
Escherichia coli	20
Pseudomonas aeruginosa	19
Salmonella typhi	21
Shigella dysenteriae	18

Minimum Inhibition Concentration (MIC) of the Extract Against the Test Microorganism

Minimum Inhibition Concentration was conducted at five different levels of concentration; 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml and 0.63mg/ml. Minimum inhibitory concentration was conducted on these sample in order to determine the lowest concentration which prevent the visible growth of the test organism. This experiment was carried out on all the five test organisms. The minimum inhibition concentration for the ethanol leaf crude extract was observed to be 5mg/ml for *Staphylococus aureus* and *Shigella dysenteriae* and 2.5mg/ml for *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* (Table 4).

Table 4: Minimum Inhibition Concentration (MIC) of the Ethanol Leaf Extract of *Ficus carica* against Test Microbes

Test organism	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	0.63mg/ml
Staphylococus aureus	-	0*	+	++	+++
Escherichia coli	-	-	0*	+	++
Pseudomonas aeruginosa	-	-	0*	+	++
Salmonella typhi	-	-	0*	+	++
Shigella dysenteriae	-	0*	+	++	+++

KEY ➡ - ➡ No Turbidity (No growth) 0* MIC, + ➡ Turbid, ++ ➡ Moderate Turbidity, +++ ➡ High Turbidity

Minimum Bactericidal Concentration of the Extract against the Test Microbe

This test was carried out in order to determine the lowest concentration in which the extracts of *Ficus carica* require to kill test bacterium. The results obtained was observed to be higher than the result obtained in minimum inhibitory concentration (MIC). The Minimum Bactericidal Concentration (MBC) of the ethanol leaf extract was observed at 10mg/ml for all the microbes. (Table 5)

Table 5: Minimum Bactericidal Concentration (MBC) of the Ethanol Leaf Extract of *Ficus carica* against Test Microbes

Test organism	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	0.63mg/ml
Staphylococus aureus	0*	+	++	+++	++++
Escherichia coli	0*	+	++	+++	++++
Pseudomonas aeruginosa	0*	+	++	+++	++++
Salmonella typhi	0*	+	++	+++	++++
Shigella dysenteriae	0*	+	++	+++	++++

KEY ➡ - ➡ No colony growth, **0*** ➡ MBC, + ➡ Scanty colonies growth, ++ ➡ Moderate colonies growth +++ ➡ Heavy colonies growth.

The biological evaluation results indicate that the ethanol leaf extract is effective in the inhibition of the growth of these microbes. Among the numerous phytochemicals present in the extracts of *Ficus carica*, flavonoids and tannins are thought to be responsible for the antidiarrhoeal activity caused by *Escherichia coli*. This is made possible by increasing the colonic water and electrolyte readsorption, others act by inhibiting intestinal motility (Palombo, 2006).). This is reflected in the sensitivity of *Escherichia coli* to the leaf extracts. The ethanol leaf extracts was found to contain saponin. Saponin have been found to possess anti-flammatory, immune-boosting properties and antimicrobial activity which guards the body against fungi, bacteria and viruses. In addition, they act as free radicals scavengers (antioxidants) which protect the cells from the damages caused by free radicals. They possess the –OH functional groups which was also detected by the FTIR analysis in the plant extracts (Picincu, 2018). Investigations have shown that the presence of alkaloids, tannins, and flavonoids may be responsible for the antityphoid activity in plants (Bekoe *et al;* 2017) . Thereby justifying the sensitivity of *Salmonella typhi* to the plant extracts. The leaf of *Ficus carica* showed great antimicrobial activity against the test microbes especially in *Salmonella typhi*. The presence of these phytochemicals may contribute to the pharmaceutical and therapeutic properties of *Ficus carica*.

Fourier Transform Infrared (FTIR) Spectroscopy of the Leaf extract of *Ficus carica* (Linn).

The peak observed at 3339 cm^{-1} is typical of –OH (alcohol) while, 2918 and 2851 cm^{-1} can be said to be C-H (stretch) for alkanes. Also, the peak at 1617 cm^{-1} (C=C) is that of conjugated alkenes. Equally observed is the peak at 1438 cm^{-1} (C-H bending) which is equivalent to that of methyl in alkanes. The signal at 1375 (C-O) is that of alcohol and 1036 cm^{-1} (C-O stretch) is typical of alcohols also. (Table 6 and Fig. 1)

Table 6: FT-IR Profile of the Ethanol Leaf Extract of *Ficus carica* (Linn)

Peak value (cm^{-1})	Functional group	Class
3339	-OH	Alcohol
2918	-C-H (Strech)	Alkane
2851	C-H	Alkane
1617	C=C	Alkene(conjugated)
1438	C-H (bending)	Alkane (methyl)
1375	C-O	Alcohol
1036	C-O (strech)	Alcohols

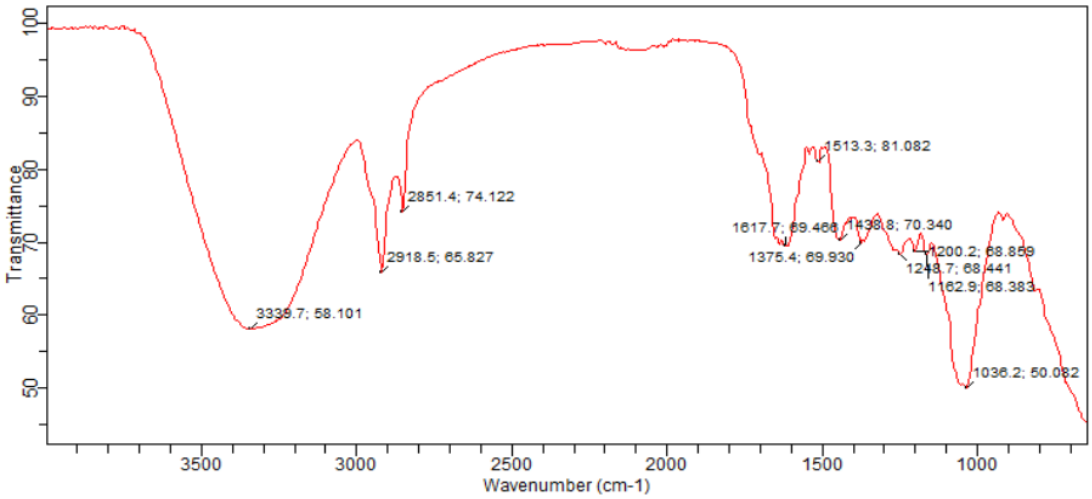

Figure 1: FT-IR Spectrum of Ethanol Leaf Extract of *Ficus carica* (Linn)

The FTIR analysis revealed the presence of two basic functional group; C=O and O-H which indicate the presence of alcohol, phenol or esters. The O-H (Alcohol/ Phenol) group was observed in the ethanol leaf extract with wavelengths of 3339cm^{-1} . The peaks between the range of 1000-1500cm^{-1} are present in the extract is due to the presence of the C-O bond. This band may either be an alcohol, ester, ether or

anhydride. Bands representing the presesnce of Alkanes were also detected at 2918cm^{-1} and 2851cm^{-1} in the ethanol leaf extract. The presence of secondary amine was also detected at 1617cm^{-1} for the extract. Lignins, tannins and flavonoid are simple phenolic compounds that serve as defense against pathogens and herbivores. Phenols when associated with vitamins such as carotenoids and vitamins E and C, acts as reducing agents that protect specific tissues in the human body against oxidative stress.The antioxidant property of phenolic compounds is attributed to the free radical scavanging capacity, donating hydrogen atom, electrons or chelate metal cations. (Minatel et al; 2017). Amines which are derivatives of Ammonia are largely used in the pharmaceutical industries as analgesics, anesthetic, disinfectants of water. Amino acid are the major building blocks of proteins in humans. In addition, many vitamins are also made from amino acids. (Joseph, 2014).

Conclusion

The leaf extract have displayed great antimicrobial activity against the test microbes. The FTIR analysis also revealed the presence of the major functional groups as C=O and OH thereby indicating the presence of phenolic, alcohol and esters which are of great importance in plants. The use of different solvents with different levels of polarity for extraction to further explore the antimicrobial activity of this plant and for possible iolation of the secondary metabolite is recommended.

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