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Minimum Inhibitory Concentration (MIC) of *Fiscus capensis* Extract against test Organisms using Broth Dilution Method

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ABSTRACT

This research evaluated the minimum inhibitory concentration (MIC) of *Fiscus capensis* extract against test organisms using broth dilution. Fresh plant parts (leaf and stem-bark) of *Fiscus capensis* were collected from Emene Community in Enugu East Local Government Area of Enugu State, Nigeria. The plant parts were identified dried under room temperature. The dried leaves and stem-bark were pulverized using pestle and mortar to obtain a powdered form, then stored in air tight container until when needed for analysis. A total of 50g of the ground plant parts were weighed out and dispensed into sterile conical flask and mix with 500ml of distilled water, ethanol and methanol and covered with sterile foil paper. The mixture was agitated intermittently and left to soak for 48h. The mixture was filtered out using whatman filter paper, covered with perforated sterile foil paper. The filterate was concentrated using rotary evaporator and stored in the refrigerator under standard condition. The samples were cultured using nutrient agar, potato dextrose agar and chromogenic agar respectively under standard condition. The clinical isolates were subjected to biochemical and molecular analysis for confirmation. The results obtained showed that the ethanol and methanol extracts were sensitive to the test organisms with the zone diameter inhibition of 16.00mm-20.00mm for *Streptococcus mutans*, 11.00mm-16.00mm for *Streptococcus sobrinus* and 20-00mm-24.00mm for *Candida albicans* respectively where as the organism was resistant to aqueous extract after 24-72h. The statistical comparison of various concentrates of extracts was made using one way analyses of variance (ANOVA) followed by dunnet post hoc test at $P<0.05$ was established to be statistically significant. The results indicated that different plant parts of *Fiscus capensis* have therapeutic potentials for the organisms tested and are readily available in Nigeria. The findings indicated that infections caused by *Streptococcus mutans, Streptococcus sobrinus and Candida albicans* could be potentially managed by using plant based mono-or combination therapies.
Keywords: Minimum Inhibitory Concentration, *Fiscus capensis* and medicinal plants.

INTRODUCTION

[1,2], describe medicinal plants as important in modern medicine due to their clinical constituents. Furthermore, [3] describe medicinal plants as plants whose roots, leaves, bark and any other tissues possess therapeutic properties. The use of medicinal plants for treatment of microbial disease is well known and has been documented since ancient's times. [4,5], reported that more than 80°%o of the world population relied on traditional medicines for their primary health care needs. [6,7] presented a range of 70 – 80% of world population, mostly in developing countries, using herbal drugs. Medicinal plants have pharmaceuticals and antimicrobial properties [8]. Plants synthesize many components, which act as defensive agents, helping to protect them from microbial infection and other diseases [9]. Those compounds are bioactive and can be medicinal, intoxicating or toxic depending on circumstance. Several plants species have been tested for antimicrobial properties but vast majorities have not yet been adequately evaluated [10]. Various studies have been published, investigating the antifungal and antibacterial activities of plant derived compounds against a range of pathogens [11,12]. Antimicrobial compounds derived from plants might inhibit bacterial through different mechanisms and provide clinical values for the treatment of infection caused by resistant microbes

[13]. Different substances have been identified in medicinal plants which are believed to be antimicrobial agent and these include; different forms of alkaloids, diterpens, saponins, flavonoids, sterols, quinine, different forms of other proteins as well as lipids [14]. Synthetic antibiotics accumulate in the body causing liver damage and other tissue problems. Such problems are not seen, when natural antibiotics extracted from plants are used, they are safe and potentially effective [15]. Ethnobotanical survey shows that several plant species have been used in the treatment of different forms of disease conditions and in general have medicinal properties. The increasing prevalence of multidrug resistant strain of bacteria and fungi and recent appearance of strains with reduced susceptibility to antifungal and antibacterial drugs raises the specter of untreated bacterial and fungal infections. *F. capensis* has been shown to be locally effective in treatment of fever [16]. It has also been used as antianemic and antisickling agents in Nigeria [8].

Aim of the Study

The aim of this study was to determine the minimum inhibitory concentration (MIC) of *Fiscus capensis* extract on the test organisms.

MATERIALS AND METHODS

Plant Material Collection and Identification

The plant leaves and the stem of *fiscus capensis* (wild fig) were collected from Emene in Enugu East local Government Area of Enugu State. The leaves and the stem were identified and authenticated by Dr. I.F. Ugwuanyi of the Department of Plant Science, University of Nigeria Nsukka. The identified plant materials were kept in herbarium until when needed for analysis.

Sample Preparation

The leaves and stem of the plant were picked in the morning, properly washed with 10% of saline water and rinsed in sterile distilled water (Eneh *et. al.,* 2017). The stems were cut and air dried at room temperature for one month (Eneh *et. al.,* 2017). The dried leaves and stem were pulverized using sterile pestle and mortar to obtain a powdered form and then were stored in air tight sterile plastic containers under room temperature until needed for the analysis.

Preparation of Aqueous Extracts

Total of 50g of different powdered *Fiscus capensis* were weighed out using mechanical weighing balance and dispensed into sterile conical flask. Then, 500ml of distilled water was measured out and dispensed into the flask with the ground, *Ficus capensis* then covered with sterile foil paper. The mixture was agitated intermittently and left to soak for 48h at room temperature. Thereafter, filtered using whatman filter paper into sterile beaker. It was concentrated using water bath at 37ºc for 6h and then allowed to cool and stored in refrigerator until when needed [5].

Preparation of Ethanolic Extract

Total of 50g of different powdered *Ficus capensis* were weighed out using mechanical weighing balance and dispensed into sterile conical flask. Then 500ml of ethanol was measured out and dispensed into the flask with the grounded *Ficus capensis*. Then it was covered with sterile foil paper to soak for 48h. Thereafter, the mixture was filtered through whatman filter paper into sterile beaker and covered with perforated sterile foil paper. The filtrate was concentrated using rotary evaporator and stored in refrigerator until when needed.

Preparation of Methanolic Extract

Total of 50g of different powdered *Fiscus capensis* was weighed out using mechanical weighing balance and dispensed into sterile conical flask. Then 500ml of methanol was measured out and dispensed into the flask with the ground *Fiscus capensis.* Then it was covered with sterile foil paper. The mixture was agitated intermittently and left to soak for 48h. Thereafter, the mixture was filtered using sterile whatman filter paper into sterile foil paper. The filtrate was concentrated using rotary evaporator and store in the refrigerator until when needed.

Preparation of Sensitivity Disc

The sterilized filter disc were introduced with 20ml of each extract, left for 24h then harvested and dried in a hot water air oven at 37^{0}C for few minutes.

Preparation of Broth Culture of the Test organism for Sensitivity Testing.

Fungus (*Candida albicans*)

Potato Dextrose Broth

A total of 0.72g of Potato Dextrose Broth powder was weighed out and dispersed into 30ml of sterile conical flask. It was allowed to dissolve completely for at least 10 minutes with agitation and then 10ml each was dispense into labeled test tube and autoclaved at 151b/sqm at 121^{0}C for 15minutes, then, allowed to cool at 45-50^{0}C using sterile inoculating loop, the test organism was inoculated into the test tube and incubated at 37^{0}C for 48h. Thereafter, 0.5ml of the preparation was inoculated into a freshly prepared nutrient broth using sterile 1ml syringe and incubated for 48h. The contents of the test tubes were standardized to 0.5 MC farland by means of dilution with sterile nutrient broth and measuring the optical density at 625mm wavelength. Absorbance reading was fixed within the range of 0.08-0.15 (equivalent to approximately 1.5x10^{8}cfu/ml) Thereafter it was adjusted to 10^{6}cfu/ml before used for antimicrobial activities.

Sensitivity Testing

The antibacterial testing of *Fiscus capensis* were carried out using two different methods:

AGAR–WELL METHOD

Serially diluted broth culture (10^{-4}) of the test organisms were inoculated into sterile chromagenic agar and spread using a sterile swab stick. Sterile cork borer was used to make three wells on each plate. Thereafter the wells were filled with each extract carefully into the wells. The preparations were incubated for 24 to 48h at 37^{0}C. The preparation was observed for zones of inhibition and measured and recorded.

DISC DIFFUSION METHOD

Serially diluted broth culture (10^{-4}) of the test organisms were inoculated into ready prepared chromagenic agar plate, sterile swab stick was used in spreading. The already prepared sensitivity disc were placed at different locations on the plate and incubated for 24h at 37^{0}C. The zones of inhibition were observed and measure and recorded.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

The Minimum Inhibitory Concentration (MIC) was determined using agar diffusion technique, two fold dilution was done. Total of 0.3ml of diluted methanolic and ethanolic extracts were incorporated at varying concentration into chromogenic gar plate containing the test organisms. The plates were incubated at 37^{0}C for 24 and 48h respectively. The lowest concentration of the extract that did not allow the growth of the test organism within the incubation period was taken to be the minimum inhibitory concentrations (MIC), and diameter of zones of inhibitions were read and recorded accordingly.

RESULTS

Table 1 shows the results of the minimum inhibition concentration (MIC) of *Fiscus capensis* extract against the organisms tested. The methanol and ethanol extract had a minimum inhibition concentration of 0.78 mg/ml respectively. This is the concentration at which the extracts was able to inhibit the growing microorganisms.

Table 1:Minimum Inhibitory Concentration (MIC) of *Fiscus capensis* Extract Against Test Organisms Using Broth Dilution Method.

Test Organisms	Extracts /Average MIC (mg/ml)						Remark
	LEE	LME	SBEE	SBME	LSBEE	SBME	
S. mutans	14	18	18	20	22	26	Sensitive
S. sobrinus	13	17	19	24	20	28	Sensitive
C. albicans	17	20	22	22	24	26	Sensitive

Key: LEE (Lead Ethanol Extract), LME (Leaf Methanol Extract), SBME (Stem-bark Methanol Extract), SBEE (Leaf +Stem Ethanol Extract), LSBEE (Leaf + Stem bark Ethanol Extract), LSBME (Leaf + Stem-bark Methanol Extract)

DISCUSSION

Plants can contribute to the advancement of novel chemo-preventive agents as they have been proven essential in forming potentially useful structures. The initial steps to this achievement is performing antibacterial activities (Tona *et al.*, 2004). In this study, minimum inhibitory concentration (MIC) of *Fiscus capensis* extract against test organisms using broth dilution method. Methanol and ethanol extracts had the highest inhibitory activities against test organisms while the aqueous extract had little effect on the test organisms. This is probably because the plant bioactive compounds are more soluble in organic solvents. The growth of all the pathogens tested in this was inhibited by the plant extracts.

CONCLUSION

The result obtained in this present study deduced that the methanol, aqueous and ethanol derived fractions of *F. capensis* stem and leaf extract have antibacterial and antifungal potential against the organisms tested.

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