



GC-MS Analysis and Antimicrobial Spectrum of Stem Bark Extracts of *Ficus sycomorus*

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Authors' contributions

This work was carried out in collaboration among all authors. Author YS designed the study, performed the collection of isolates, wrote the protocol, did sensitivity and wrote the first draft of the manuscript. Authors MUN and BA supervised GC-MS analysis and extraction procedures. Author RYB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This work investigated the gas chromatography-mass spectroscopy of methanolic extract and antimicrobial spectrum of acetone and aqueous crude extracts of *Ficus sycomorus* stem bark.

Place and Duration of the Study: Department chemistry research laboratory and microbiology laboratory, Yobe State University, Damaturu between April 2019 and August, 2019.

Methodology: Pure isolates of *Staphylococcus aureus*, *Salmonella typhi*, *Shigella dysenteriae*, *proteus* spp, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia* and a fungi, *Candida albicans* were collected from National Veterinary Research Institute (NVRI), Vom,

Plateau State. The plant was collected and the extract was used in preliminary phytochemical screening, GC-MS analysis and reconstituted for antimicrobial activities testing.

Results: The preliminary phytochemical screening revealed presence of saponin, flavonoids, alkaloids, carbohydrates, cardiac glycoside and tannin. GC-MS analysis revealed the presence of heterocyclic members of pyrazole, imidazole, triazole, thiazole and 1, 3-Benzenedicarboxylic acid, bis (2-hethylhexyl) ester. Acetone extract showed the greatest antimicrobial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Shigella* spp while the aqueous extract produced antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Candida albicans*. Both acetone and aqueous extracts of both stem bark extracts did not exhibit any activity against *Escherichia coli*. All the extracts were tested at 100mg/ml concentration. The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) (125 – 250 mg/ml) of both extracts were higher than those of ciprofloxacin (5.0 mg/ml).

Conclusion: There is scientific basis for the uses of *F. sycomorus* in traditional medicine for the treatment of various diseases as prescribed earlier on and stronger basis for recommendation for its cautious use for the presence of 2-ethylhexyl which could be Carcinogenic. This is first study to identify 2-ethylhexyl from *F. sycomorus* stem bark extract and recommend cautious use.

Keywords: Antimicrobial; antibiotics; Klebsiella; shigella; concentration; GC-MS.

1. INTRODUCTION

Directly or indirectly, man depends on plants for life [1]. Breathing, fuel and medicine might have been difficult in a planet devoid of plant [2]. Inquisitive nature of man has led him to test various plant originate in his environment, those that are pleasant or have therapeutic effects have been incorporated into his bio-medical culture like common source of antimicrobial while those that produce toxic effects have been demoted to use in either hunting, warfare or skillfully avoided [3].

According to [4], there is global resurgence in the use of herbal preparations and in some developing countries like Nigeria, it is being gradually integrated into primary and secondary health care systems. Nearly all societies have used herbal materials as a source of medicines, and development of the herbal medicines depend on local botanical flora. Several plants are indicated in folk and other traditional systems of medicines as anti-infectious agents. As a result, different remedies evolved in different region of the world as the communications got improved [5].

Growing misuse of antibiotic and chemotherapeutic agents leading to drug resistance is now pushing a considerable proportion of people in both developed and developing countries to the use of herbal medicine [6]. As a consequence of this, in 1997, the 30th World Assembly adopted a resolution, urging National Government Member Nations to

utilize their traditional system with regulation suited to their national health care system of medicines. In Nigeria, traditional medicine boards were established in all the states to parallel with Hospital Management Boards and Primary Health Care Development Agencies [7], part of which have given birth to Islamic medicine stores in almost every part of the country.

Ficus sycomorus is thick branched, wide spreading tree in Africa and South-West Asia, often buttressed with branches rising from near ground, produce cluster of edible but interior figs with leaflets on short leaflets twigs. The biblical sycamore, *Ficus* is called the sycamore fig or the fig mulberry due to the leave resemblance to those of mulberry. *Ficus sycomorus* belong to the family moraceae, like most other members, it produces latex, grows up to 20m tall, 6m wide, the bark is yellow or orange depending on the age and found mostly along streams. They are widely used in conventional as well as alternative medical practices not only in developing countries like Nigeria but also in the developed countries as a complementary medicine [8].

Using GC-MS, 29 active compounds in the leaves and 15 compounds in the fruit of *Ficus Sycomorus* have been identified to possess activities against disease agents [9]. It has been used to treat snake bites, jaundice, chest pains, dysentery, cold, and throat infections [10]. *Ficus sycomorus* stem bark, root and fruits are widely used in Africa for treatment of various diseases such as cough, diarrhea, skin infection, stomach disorder, helminthiasis, infertility, sterility and

diabetes mellitus [11]. The most important bioactive constituent of the plant includes alkaloids, tannins, flavonoids, and phenolic compounds [12,13], because of their antibiotic and antioxidant activities. In-vitro antimicrobial screening of methanolic stem bark extract of *F. sycomorus* revealed that the extract demonstrated activities against *enterococcal faecalis*, *E. coli*, *S. typhi*, *Shigella dysenteriae* and *Candida albicans* [14]. The Plant have been reported to be a potent antimicrobial agent against ciprofloxacin resistant *salmonella typhi* [15]. [16], reported its hepatoprotective capabilities but recommended further study to confirm the hepatocurative potentials.

2. MATERIALS AND METHODS

2.1 Collection and Preparations of Sample

The stem bark was collected based on the report of its wide spread use among the local communities in North-Eastern region of Nigeria in a swamp area in Abbari ward Damaturu. Yobe State located along 12000'N 11030'E The samples were air dried [17], pounded using pestle and mortar and stored at 35°C until used [7].

2.1.1 Extraction procedure

This is done as described in [18], 500 g of powdered plant material was extracted exhaustively with acetone using soxhlet extractor as described by [19]. Similarly, 100 g of the plant material was macerated in distilled water at ambient temperature for 24 hours to obtain aqueous extract. The resulting mixtures was the filtered and concentrated in [20]. The dried extracts were kept in refrigerator for further use.

2.1.2 Preliminary phytochemical screening

Phytochemical Screening of the acetone and aqueous extract of stem bark were carried out using standard methods [21,22].

2.1.3 Gas chromatography-mass spectrum analysis

2 µl of methanol bark extract from *Ficus sycomorus* was used for GC-MS analysis. The extract was dissolved in HPLC grade methanol (1/3 dilution) and subjected to GC-MS analysis performed using an Agilent 7000 Series Triple, Quad Gas Chromatograph interfaced to a Mass

Spectrometer (7890B GC System/5977A MSD) licensed to Yobe State University, Damaturu. The column is fused with silica 30 m x 250 µm x 0.25 µm. Analysis condition was 5min at 110°C, 5 min at 280°C and 0.5 min at 325°C. Injector temperature was 25°C, helium was used as carrier gas. The sample was evaporated in a pulsed split less mode. The runtime was 36min.

2.2 Collection of Test Organisms

The isolates were collected from National Veterinary Research Institute, (NVRI), Vom, Plateau State and were maintained on Nutrient agar Slant. The bacterial isolates include, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella dysenteriae*, *proteus* spp, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia* and a fungi, *Candida albicans*.

2.2.1 Determination of antimicrobial activity using agar well diffusion method

The acetone extracts of the samples were reconstituted in glycerol containing a minimum amount of the extracting solvent to get a final concentration of 100mg/ml for this test. For susceptibility tests, the test organisms were inoculated (using inoculating loop) separately on nutrient broth and after 24 hours, growth of the cultures were standardized to obtain the final concentration of 10⁶ cells/ml.

The susceptibility tests were done using agar diffusion method. Two holes were bored into each plates (nutrient agar plates) previously seeded with 10⁶ cells/ml of test organisms and 0.5ml each of the extracts at 100 mg/ml concentration was aseptically introduced into the holes.

Glycerol containing the minimum amount of extracting solvent was introduced into the holes of another plate to serve as a negative control. The plates were then incubated at 37°C for 24 hours. The same procedure were repeated for aqueous extract for each of the test organisms [23].

2.2.2 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the acetone extract were estimated for selected species *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus* species, *Pseudomonas aeruginosa*,

Salmonella typhi and *Staphylococcus aureus*. To each test tubes containing 2.0 ml of nutrient broth, 0.5 ml of varying concentration of the reconstituted samples were added and inoculated with the test organisms for each selected bacteria species. The procedure was repeated using ciprofloxacin of varying concentration (5.0 – 25.0 mg/ml). After proper incubation, the highest dilution of the extracts and the antibiotics that prevented visible growth of the test strains were taken as MIC's [24].

2.2.3 Determination of Minimum Bactericidal Concentration (MBC)

For each test tubes in the minimum inhibitory concentration, a loop full of broth was collected from those tubes which did not show any visible growth and were inoculated on sterile agar plates, Mac Conkey agar for *Salmonella typhi* and *Klebsiella pneumoniae*. While, nutrient agar was used for *Bacillus subtilis*, *Proteus* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Inoculation was carried out using the streak plate method and incubated at 37°C for 24 hours [25]. The above procedure was also performed using ciprofloxacin for the purpose of comparison.

3. RESULTS AND DISCUSSION

The extracts obtained were dark brownish semi solid and reddish brown crude extracts from aqueous and acetone extracts of *F. sycomorus* stem bark respectively.

Phytochemical constituents identified in the methanol and aqueous stem bark extract of *F. sycomorus* Linn are presented in Table 1. It shows the presence of carbohydrates, flavonoids and tannins present in both extracts, cardiac glycosides and anthraquinolones in acetone extract only, saponins in aqueous extract only.

This result agrees with one's obtained by [26,27,28,29], 2016 from the root bark extract of the same plant, including reducing sugar by [30]. [31], found that the phytochemical analysis of *Ficus sycomorus* revealed the presence of alkaloids, tannins, saponins, flavonoids and steroids in both the aqueous extracts of the leaves and the fruits. These classes of compounds are known to be biologically active and are associated with the antimicrobial activities of *Ficus sycomorus* [32,33,34]. Alkaloids have been associated with medicinal applications in plants, among which is their toxicity against cells of foreign organisms. These bioactivities have been widely studied for their potential use in the inhibitory activities of human cancer cell lines [35,36]. Alkaloids inhibit certain mammalian enzymatic activities like those of phosphodiesterase, prolonging the action of CAMP. They additionally have an effect on glucagon's and thyroid stimulating hormones, while some forms of alkaloids which was extracted from *Rhazya stricta* have been reported to be carcinogenic [37]. Plant phenolic compounds, especially flavonoids are currently of growing interest owing to their supposed properties in promoting health (antioxidants) and antimicrobial activity [38,39]. Some alkaloids are used either as an analgesic, antispasmodic or bactericidal agents [40].

Flavonoids also exhibit a wide range of biological activities such as antimicrobial, anti-inflammatory, analgesic and cystostatic, hypoglycemic and antioxidant properties [41,42,43]. The broad therapeutic effects of flavonoids will be mostly attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages [44].

Table 1. Phytochemical screening of *F. sycomorus* stem bark

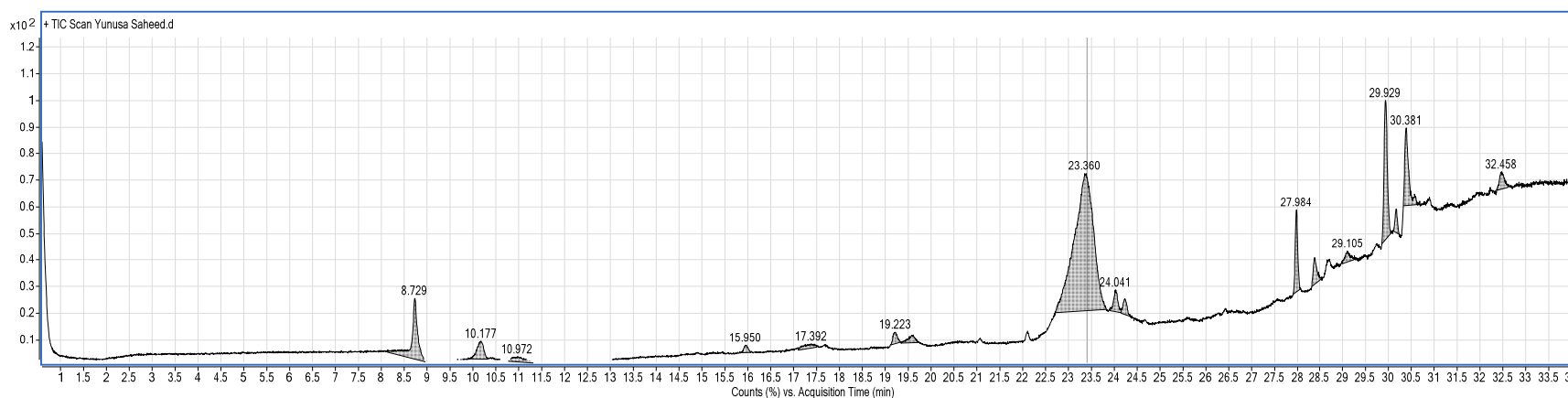
Phytochemicals	<i>F. sycomorus</i> extract and test Inference	
	Water	Acetone
Cardiac Glycosides	-	+
Saponins	+	-
Flavonoids	+	+
Tannins	+	+
Steroids	-	-
Anthraquinones	-	+
Carbohydrate	+	+

Key: (+) Present, (-) Absent

Table 2. Active fractions of *F. sycomorus* Stem Bark extract debated by GC-MS

Derivatives	RT(min)	MF	MW(g/mol)	Peak Area
3-(γ -Methylaminopropyl)-5-(4-bromophenyl)-2-methyl-2H-pyrazole.	8.729	C ₁₄ H ₁₂ BrN ₃	307.00	1104207.29
Ethanethiol, 2-(dimethylamino)	10.177	C ₄ H ₁₁ NS	105.00	359027.02
3-Isopropyl-5-(phenoxyethyl)-2-oxazolidinone.	10.972	C ₁₃ H ₁₇ NO ₃	235.00	187266.56
2-Thiazolamine, 4-(3, 4-dimethoxyphenyl)-5-methyl.	15.95	C ₁₂ H ₁₄ N ₂ O ₂ S	250.00	95493.01
Benzimidazole-5-carboxylic acid, 2-methyl-1-phenyl.	17.392	C ₁₅ H ₁₂ N ₂ O ₂	252.00	143890.00
2-Butanone, (2, 4-dinitrophenyl) hydrazine.	19.223	C ₁₀ H ₁₂ N ₄ O ₄	252.00	149098.17
Benzimidazole-5-carboxylic acid, 2-methyl-1-phenyl.	19.618	C ₁₅ H ₁₂ N ₂ O ₂	252.00	155053.08
1, 3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester.	23.36	C ₂₄ H ₃₈ O ₄	390.00	7787608.07
2-Thiazolamine, 4-(3, 4-dimethoxyphenyl)-5-methyl.	24.041	C ₁₂ H ₁₄ N ₂ O ₂ S	250.00	271592.35
Purine-2, 6-dione, 8-(3-ethoxypropyl amino)-1, 3-dimethyl-3, 9-dihydro.	29.105	C ₁₂ H ₁₉ N ₅ O ₃	281.00	164591.50

Keys: RT-(Retention Time), MW – Molecular Weight, MF=Molecular formula

**Fig. 1. Chromatogram of *F. sycomorus* stem bark extract**

3.1 Phytochemical Constituents of Acetone and Water Extract of *F. sycomorus* Stem Bark

3.1.1 GC-MS analysis

The GC-MS profile of the stem bark extract suggests presence of various compounds, including phthalate, 2-butanone-hydrazone, benzimidazole, oxazolidinone, purine and 1, 3-Benzenedicarboxylic acid.

Literature review revealed that various heterocyclic members stated above predominantly possess antibacterial and antifungal activity [45]. 1, 3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester, has been extracted from various plants including *Thevetia peruviana* by [46] who proposed it as biomarker and useful in anticancer chemotherapy and *moringa oleifera* by [47] who proposed it as non-invasive, and effective biomarker for commercialization.

Benzimidazoles are remarkably effective compounds, extensive biochemical and pharmacological studies have confirmed that these molecules are effective against various strains of microorganisms [48]. Benzimidazole and their derivatives have shown remarkable Antifungal [49] and antibacterial [50] activities.

[9] In their study found 29 active compounds in the leaves and 15 compounds in the fruit of *Ficus Sycomorus* with good antimicrobial activities which can facilitate within protection against incurable diseases. [51] Confirmed that Triaryl-benzimidazole as having moderate antibiotic activities against antibiotic resistant *E. coli*.

[52] While studying potential of Hydrazone-Hydrazone derivatives discovered that their derivatives play an important role in development of various pharmacological activities such as anticonvulsant, antimalarial, analgesic, anti-inflammatory, antiplatelet, antimicrobial, antihypertensive, antiviral, anti-tubercular, anti-proliferative and antitumor activities.

Oxazolidinone is a five-member heterocyclic ring exhibiting potential medicinal properties by inhibiting protein synthesis by binding at the P site at the ribosomal 50S sub unit. It represent a new class of synthetic antibacterial agents active against multiple-resistant gram positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant streptococci, and vancomycin-resistant enterococci [16]. [53] Synthesized Hydrazone-

Hydrazone from benzocaine with very good activities against both Gram's Positive and Gram's positive bacteria. Resistance to other protein synthesis inhibitors does not affect Oxazolidinone activity; however rare development of oxazolidinone resistance cases, associated with 23S r-RNA alterations during treatment, has been reported [54].

3.2 Antimicrobial Activity

Antimicrobial activity was expressed as an average diameter of the zones of inhibition calculated as the difference in diameter of the observed zone and the diameter of the well. Zones of inhibition greater than the control disc were regarded as a measure of antimicrobial activity [55].

All the extracts were tested against *Salmonella typhi*, *Shigella* species, *Proteus mirabilis*, *Candida albicans*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*.

As shown in the Table 3, acetone extract of stem bark produce the highest antimicrobial activity against *Bacillus subtilis* (32 mm), *Pseudomonas aeruginosa* (26mm), *Salmonella typhi*.(25 mm), *Shigella* sp. (21 mm) and *Proteus mirabilis* (18 mm) and. On the other hand, the aqueous extract of the stem bark exhibited highest antimicrobial activity against *Bacillus subtilis* (20 mm), moderate activities against *Klebsiella Pneumoniae* and *Staphylococcus aureus* (15 mm) and *Candida albicans* (12 mm). Both extracts did not exhibit any antibacterial activity against *Escherichia coli*.

This work agreed with the study of [56], in which ethanol and n-butanol extracts of leaves of *Ficus sycomorus* showed antimicrobial activity against *Bacillus subtilis*, *Candida albicans*, *Staphylococcus aureus*, *Escherichia Coli* and *Pseudomonas aeruginosa* in the ranges of inhibition between 10 mm and 25 mm. On the contrary, the aqueous and acetone extracts of leaf of *Ficus sycomorus*, in this study did not show antimicrobial activity against *staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa*. [24], revealed that the acetone and petroleum ether extract of *Azadirachta indica*'s seed showed significant antibacterial activity against Ps. Aeruginosa, E. coli, Staphylococcus aureus, S. typhi, Klebsiella pneumonia, Proteus sp., and Bacillus cereus because of their specific property of extracting tannins and other phytochemical compound.

Table 3. Antimicrobial activities of *Ficus sycomorus* stem bark extract

Microorganisms	Aq. Ext.	Acetone Ext.		Ciprofloxacin		
	ZI(mm)	ZI(mm)	MIC(mg/ml)	MBC(mg/ml)	MIC(mg/ml)	MBC(mg/ml)
<i>Bacillus subtilis</i>	20.00	32.00	125.00	200.00	5.00	5.00
<i>C. albicans</i>	12.00	-	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-	-	-
<i>K. pneumonia</i>	15.00	-	-	-	-	-
<i>Pseud. Mirabilis</i>	-	18.00	150.00	200.00	5.00	5.00
<i>Ps. Aeruginosa</i>	-	26.00	200.00	200.00	5.00	5.00
<i>S. typhi</i>	-	25.00	175.00	200.00	5.00	5.00
<i>Shigella spp.</i>	-	21.00	175.00	200.00	5.00	5.00
<i>Staph. Aureus</i>	15.00	-	-	-	-	-

Keys: - - No incubation, ZI – zone of inhibition, MIC – Minimum Inhibitory Concentration, MBC – Minimum Bactericidal Concentration, Aq. – aqueous

Thus, the significant antimicrobial activity exhibited by the acetone extract of both leaf and stem bark of *Ficus sycomorus* in this study may be attributed to the presence tannins and other phytochemical compounds presence in both leaves and stem bark of *Ficus sycomorus* as reported by [17] and [56]. The spectrum of activity for both extracts revealed that both Gram positive and Gram negative bacterial are susceptible to the extracts in varying proportions and the variation of the antimicrobial activity for these extracts might be due to the extracting solvents. The size of zones of inhibition is influenced by a complex of factors such as rate of diffusion of the extracts through the agar, the size of the inoculum, the rate of growth of the organisms and the organisms' susceptibility to the antimicrobial agents [57].

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of acetone stem bark extract were generally higher (125 – 200 mg/ml) than those of ciprofloxacin which were the same at 5 mg/ml. The crude form of the extract may have been responsible for that. For *Ps. Aeruginosa* both MIC and MBC are the same (200 mg/ml), *Bacillus subtilis* MBC (200 mg/ml) was higher than MIC (125 mg/ml), *Salmonella typhi* and *Shigella sp.* share the same MIC (175 mg/ml) and MBC (200 mg/ml) and *Proteus mirabilis* with higher MBC (200 mg/ml) than MIC (150 mg/ml).

Higher MIC of various plant extracts against some bacteria species have been reported by [21,24,58]. The differences may be due to the susceptibility testing conditions, physicochemical characteristics of the extracts and even strain to

strain differences [59]. [24] Which revealed that the MIC and MBC of Ciprofloxacin were the same at 5.0 mg/ml concentration.

4. CONCLUSION

In this investigation, acetone extract of *Ficus sycomorus* stem bark was found to be more effective than the aqueous extract. This work has also shown that the extracts from stem bark of *Ficus sycomorus* which suggest its use as antimicrobial against Nosocomial, skin infection, urinary tract infection, bacteremia, Pneumonia [60,61] typhoid fever, bacillary dysentery [62] and fungal infections.

The result obtained from this study have proven scientifically, that *Ficus sycomorus* poses antimicrobial activities, as such, there is scientific basis for its uses in traditional medicine for the treatment of various diseases as prescribed earlier on and stronger basis for recommendation for its cautious use for the presence of 2-ethylhexyl which could be Carcinogenic.

Finally, in this modern days of multiple drug resistance syndrome, aid of natural bioactive plant agents like *F. sycomorus* is a brilliant move.

5. RECOMMENDATION

The following suggestions for further investigation are recommended:

1. To isolate active agent present in the leaves and stem bark responsible for inhibiting the growth of the said microbial species.

2. The need to educate more people about its importance as pharmaceutical agents.
3. To carry out stability studies of the antimicrobial constituent to determine the practical usability and toxicity as a chemotherapeutic agents.
4. To explore the stem bark for isolation of 2-hethylhexyl for its anti-cancer potentiality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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