

# Antifungal activity and phytochemical analysis of *Ficus sycomorus* leaf extract on *malassezia glubosa*

## Abstract

The Antifungal activity of the aqueous extract of *Ficus sycomorus* on *Malassezia glubosa* was carried out at different concentrations to determine its effect on the test isolate. *Ficus sycomorus* leaves were collected from the farmland at Gidan yamma village in Usmanu Danfodiyo University, Sokoto, Nigeria. The leaves of the plant were air dried under shed and at room temperature for 7days. The plant (leaves) was grinded into fine powdered form using sterilized mortar and pestle. 100g of the powder was weighed and dissolved in 1000ml of distilled water and allowed to soak for 24hrs. It was sieved and the aqueous extract was put in a drying cabinet machine to evaporate the moisture content for 72hrs. The extract was prepared into different concentrations. The concentrations were (10, 20, 40, 60, and 100) mg/ml which was all tested against the test isolates (*Malassezia glubosa*). The antifungal activity of the aqueous extract of *Ficus sycomorus* on *Malassezia glubosa* increases with higher concentration and the highest mean radial growth was (7mm) at 100mg/ml. The phytochemical analysis carried out on the extract showed the presence of Tannins (+++), Saponins (++), Alkaloids (+++), Glycoside (+++), Flavonoids (++). The presences of these compounds have effect on the altering growth of the fungi at increasing concentration.

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## Introduction

Medicinal plants have been used extensively as a source for numerous active constituents for treating human diseases and they, as well, have high contain of therapeutic value.<sup>1</sup> The *in vitro* antibacterial or antifungal assay is the first aim to evaluate the importance of these plants since the antibiotic resistance has become a global concern.<sup>2</sup> Phytochemical investigations of some *Ficus* species revealed that phenolic compounds as their major components considering the enormous potentially of plants as sources for antimicrobial drugs with reference to antifungal agents, a systematic investigation was undertaken to screen the antifungal activity of different *Ficus* species.<sup>3</sup> In developing countries, 80% of the population continues to use medicinal plants and plant products in handling primary medical problems due to their accessibility, availability and affordability. In these countries, a variety of plants are claimed to have fertility regulating properties and a few have been tested for such effect.<sup>4</sup>

Herbal plants produce and contain a variety of chemical substances derived from plants that acts upon the body. Substances derived from plants remain the basis for a large proportion of the commercial medication used for the treatment of diseases, and represent a huge storehouse of drugs.<sup>5</sup> Medicinal plants are now being given serious attention as is evidenced by the recommendations given by the World Health Organization (WHO) in 1970.<sup>6</sup> The proven traditional remedies should be incorporated within national drug policies, by recent moves towards a greater professionalism within African medicine<sup>7</sup> and also by the increased commercialization of pharmaceutical production using traditional medicinal plants with known efficacy. Plants with potent bioactive are regarded as components of phyto medicine. plant based natural constituents can be derived from any part of the plant like leaves, bark, flowers, roots, fruits, seeds, etc.<sup>8</sup> The antimicrobial activities of plants are attributed to the variety of chemical substances synthesized by plants. These bioactive agents of plant include alkaloids, saponins, tannins, flavonoids, glycosides, anthraquinones

among others.<sup>9</sup> The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories.<sup>10</sup>

*Ficus sycomorus* belongs to the family *Moraceae*, it is commonly known as sand paper tree (“Baure in Hausa”) and it is widely spread in West Africa. It is a scrambling shrub or small tree of approximately 4-8m high viable on dumpsite, riverbanks, river rine forest or closed Secondary jungle. It is also found in Nigeria, Senegal, Uganda, Tanzania, Natal (South Africa), Madagascar and Cameroon. The leaves are enormous and are displayed spirally, the limb is largely oval or has a form of ellipse and roots are most often fibrous.<sup>11</sup> The tree has light bark with rough and hairy leaves, which are used in ancient Africa as sand paper for polishing woods where it derived the name “sand paper” tree. The bark of this plant is used in the treatment of liver diseases, pain killer, and parasitic infection. The leaves are also used for the treatment of wounds and coughs. In many traditional medicines of Africa, the leaves extract of *Ficus sycomorus* is used as an anthelmintic and a purgative. The common practice of taking crude extract orally is often associated with the hazards of as a result of other toxic constituents that may be present in them. Also the increasing need to limit toxicities of most drugs today is the drive towards developing less toxic clinical drugs. It is therefore, highly important to investigate each medicinal or herbal plant through phytochemical screening.<sup>11</sup> *Ficus sycomorus* (Sand paper tree) is widely distributed across Africa. Nkafamiya et al.,<sup>12</sup> have reported its presence in Senegal, Cameroun, Sudan, Central and East Africa. It is also found in Toro Local Government Area in Bauchi State, Michika, Hong and Song Local Government Areas in Adamawa State and Omala Local Government Area in Kogi State all in Nigeria Nkafamiya et al.<sup>12</sup> The bark of *Ficus sycomorus* is used in the treatment of liver disease, pain killer, and parasitic infections; a decoction of leaves is used in the treatment and management of high blood pressure.<sup>13</sup> Given the alarming incidence of antibiotic resistance in fungi of medicinal importance, there is constant need for new and effective therapeutic agents. Therefore,

there is need to develop alternative antifungal drugs for the treatment of infectious diseases from medicinal plants.<sup>14</sup> Nkafamiya et al.,<sup>12</sup> also published that the leaves of *Ficus sycomorus* has a higher protein, crude fibre and mineral contents than some Nigerian vegetables. *Malassezia* (formerly known as Pityriasis) is a genus of related fungi. These yeasts are naturally found on skin surfaces of many animals including man. In occasional opportunistic infections, some species can cause hypopigmentation of the trunk and other locations in humans. *Malassezia* belongs to the division of fungi Basidiomycota in the class Exobasidiomycetes (those found outside the host body) and in the order *Malasseziales*. The lipid-dependent yeasts of the genus *Malassezia* are part of the normal cutaneous microflora and are commonly found on human skin. Certain conditions, such as high humidity, greasy skin and immunodeficiency, can cause these yeasts to become pathogenic, resulting in several skin diseases such as dandruff, pityriasis versicolor, atopic dermatitis and seborrhoeic dermatitis.<sup>15</sup>

It has been observed that some parts of the human body such as scalp, shoulders, chest and back, etc, are affected by *malassezia* infections, which is mostly caused by *Malassezia globosa*. As a result, these diseases are constantly increasing among population. The research in the field of natural and synthetic antifungal is still at high interest. The study will guide and help to determine the antifungal activity of *Ficus sycomorus* (leaves) extracts on *Malassezia globosa* which is locally sourced and can be found in abundance. Nowadays medicinal plants are considered as a source for numerous active constituents for treating human diseases and infections. Therefore, the study will help to investigate the potentials of *Ficus sycomorus* act as an antifungicidal or anti fungistatic on *Malassezia globosa*. The main aim of this study is to evaluate the activity of *Ficus sycomorus* leaves extract acting as antifungal agent on *Malassezia globosa* after phytochemical screening of the extracts.

## Materials and methods

### Collection of plant material

Samples of *Ficus sycomorus* (leaves) were obtained from its live tree at Gidan Yamma village farm, Usmanu Danfodiyo University Sokoto using sterilized polythene bag and were brought to the herbarium of the Department of Biological Sciences, Usmanu Danfodio University, Sokoto for authentication and identification. The leaves were authenticated and identified with Butcher number (UDUSH/ANS/0079).<sup>16</sup>

### Collection of test samples

A sharp and sterile epilating force was used to detach the hair from the scalps of the infected persons. The infected region was first washed with 70% ethanol followed by scraping with the sterile epilating forceps which was held at an angle of 90° with the head. The specimen was then transferred into a dark sampling paper to prevent exposure to sunlight. Each sample was labeled with the patient's name. The samples were then taken to the laboratory for further analysis.<sup>17</sup>

### Sample processing

#### Processing of plant sample

The leaves of *Ficus sycomorus* were washed thoroughly using distilled water, then was air dried at room temperature (25°C) for 7days. The sample was pounded into powder form using a sterilized

mortar and pestle. The powdered sample was poured into a sterilized polythene bag for further analysis.<sup>16</sup>

#### Aqueous extraction of the plant

100g of the powdered leaves was measured using digital weighing balance and poured into 1000ml conical flask containing 1000ml of distilled water and the mouth was cupped with cotton wool and wrapped with aluminum foil, the mixture was shaken carefully to dissolve and allowed for 24hrs. The solution was sieved and heated using drying cabinet 50°C for 72hours until the water content evaporated totally as described by Bajwu et al.<sup>18</sup>

#### Preparation of plant extracts concentration

Five milliliters (5ml) of distilled water was poured into test tubes. A total number of 15 test tubes were provided and were cupped with cotton wool and wrapped with aluminum foil. The test tubes were sterilized using autoclave. The stock solution was prepared using 10g of the solid plant extract dissolved in 100mls of normal saline making a stock of 100mg/ml. The concentration was prepared from the stock solution using dilution formula as shown below:

$$C_1V_1 = C_2V_2$$

Where:  $C_1$ =present concentration

$V_1$ =volume to use

$C_2$ =Required concentration

$V_2$ =Required volume.

The test tubes were arranged serially and label as 10mg/ml, 20mg/ml, 40mg/ml, 60mg/ml, and 100mg/ml accordingly. To prepare these concentrations a given amount of 0.1g, 0.2g, 0.4g, 0.6g, 1.0g of the stock solution of *Ficus sycomorus* was dissolved in three different test tubes each containing 5mls of sterilized distilled water. Sabouraud dextrose agar (SDA) media was prepared according to the manufacturer's instructions and sterilized. Then the media and prepared concentrations of *Ficus sycomorus* were mixed together by agar incorporation method. Then the mixture was poured into sterilized Petri dishes and left to solidify. The control plate contained 20ml of the sterilized media. The organism was inoculated and incubated at 25°C.<sup>17</sup>

#### Inoculation of samples

The collected scrapings from the scalp from three different places were inoculated in nutrient broth using sterile forceps. The test tubes were labeled and incubated at room temperature (25°C) for 14days to revive some of the fungi that might be denatured in the cause of taking the sample to the laboratory. The appearance of white suspension confirms the revival of fungi.<sup>17</sup>

#### Incubation

Each of the scalp infection suspension obtained from Nutrient Broth Agar (NBA) was introduced directly on sterilised Sabouraud Dextrose Agar (SDA) in Petri dishes using sterilized inoculation needle. It was then incubated in the incubator at 20°C for fourteen (14) days to obtain different growth of fungal colonies.<sup>17</sup>

#### Purification of isolates

The resultant fungal colonies were purified by cutting the advancing edge of visibly seen isolates in the colonies and introduced each in

fresh Sabouraud Dextrose Agar (SDA) media plates containing olive oil. The oil was used to enhance the growth of organism. This was continued until pure cultures were obtained. The pure culture of the isolates was maintained on Sabouraud Dextrose Agar (SDA) slope in McCartney bottles at 4°C in the dark.<sup>19</sup>

#### Identification of isolated organism

According to the method described by De Hoog et al.,<sup>20</sup> slide of the mycelium observed from different fungal isolates was prepared as follows: A drop of Chloramphenicol solution was placed in the center of a grease free clear slide. A small portion of the unidentified fungi culture was cut out with inoculation needle; the portions was placed in the Chloramphenicol droplet on the slide and spread out with another needle. A cover slip was lowered over teased portion and was mounted on the stage of a binocular microscope for viewing and examination. The characteristics such as texture, structure of mycelia, fruiting bodies, colour and shape of the upper talus as well as the production of pigment on the underside spore structure were noted and identified with the help of standard mycology atlas.<sup>20</sup>

#### Determination of antifungal activity (Sensitivity test)

The isolated organism (*Malassezia globosa*) was inoculated on SDA using spread method. Five millimeter (5mm) of test organism was punched using cork borer and poured at the centre of the solidified plates that contains agar and the extract using sterilised inoculating needle which was incubated at temperature of 20°C. The Zone of inhibition was measured using a meter ruler and the mean recorded in millimeter as described by Mukherjee et al.<sup>21</sup>

#### Phytochemical Analysis of Aqueous extracts of the plants

10g of the powder leaves will be weighed using weighing balance and will be suspended into 300ml beaker containing 100ml of distilled water, it will then be shaken carefully to dissolve. The solution will be sieved to determine the presence of phytochemicals.<sup>22</sup>

#### Test for alkaloids

2ml of the extract was stirred with 2ml of 10% hydrochloric acid. 1ml was treated with few drops of Wagner's reagent and the precipitation formed indicates the presence of Alkaloid.<sup>23</sup>

#### Test for saponins

5ml of the extract was collected into a test tube and distilled water was added and shaken strongly. When the whole tube becomes filled with the front and lasted for some minutes, it indicates the presence of saponin.<sup>24</sup>

#### Test for tannins

5% of ferric chloride solution was added in drops in (2-3ml) to form a dark green coloration which indicates the presence of Tanins.<sup>25</sup>

#### Test for glycosides

To one of the herb extract 2ml of 3.5% of ferric chloride solution will be added and allow to stand for one minute, then 1ml of concentrated H<sub>2</sub>SO<sub>4</sub> will be carefully poured down the wall of the tube so as to form a lower layer. A reddish brown colour indicates the presence of glycoside.<sup>23</sup>

#### Test for anthraquinone

5ml of plant extract will be shaken with 10ml of benzene, and 5ml of 10% ammonia solution will be added and the mixture will be

shaken, the appearance of pink, red, or violet indicates the presence of Anthraquinone.<sup>23</sup>

#### Test for flavonoids

3ml of the liquid extract will be added to 1ml of NaOH and a yellow coloration will be formed, which indicates the presence of flavonoids.<sup>25</sup>

## Results

The Antifungal activity of the aqueous extract of *Ficus sycomorus* leaves at different concentrations of 10,20,40,60 and 100mg/ml on *Malassezia globosa* is shown in Table 1. The results indicated that, *Malassezia globosa* was more susceptible to aqueous extract of *Ficus sycomorus* at higher concentrations of 40, 60 and 100mg/ml and at lower concentrations of 10 and 20mg/ml, there was no activity. The results of phytochemical analysis of the aqueous extract of *Ficus sycomorus* is presented in Table 2. The results revealed the presences of different phytochemical components such as Tannins (+++), Flavonoid (++), Saponin (++), Glycoside (++) while Anthraquinine was found negative (-) (Plate I).



Plate I *Ficus sycomorus* tree (Source [www.google](http://www.google.com))

Table 1 Antifungal activity of *Ficus sycomorus* on *Malassezia globosa* at different concentration

Test organism	Concentrations in mg/ml	Mean radial growth(mm)			Mean (X×X) radial growth(mm)
Malassezia globosa	10	40	42	39	40
	20	35	31	34	33
	40	25	20	21	22
	60	18	15	19	17
	100	10	7	6	7

Control plate is 75mm

**Table 2** Phytochemical component of aqueous extract of *Ficus sycomorus*

Phytochemical component	Reaction	Colour of compound
Tannin	+++	Dark green
Flavonoid	++	Yellowish
Saponin	++	Frunth
Alkaloid	++	Precipitation
Glycoside	++	Reddish brown
Anthraquinone		

+++ = Highly significant

++ = Significant amount

- = Absent

## Discussion

The results obtained in this study showed that the aqueous extract of *Ficus sycomorus* leaf is active on fungi *Malassezia glubosa*. In Table 1 it was shown that the aqueous extract of *Ficus sycomorus* leaf have effect on the test isolate *Malassezia glubosa*, because even at the least concentration (from the research work of 10mg/ml), zone of inhibition of 40mm was recorded and noted down. It was also observed in this table that the higher the concentration, the more effect it has on the vegetative growth of *Malassezia glubosa*, thereby revealing retardation of the vegetative growth of the yeast which may be as a result of the active water solution and antifungal properties associated with each of the plant leaves concentration.<sup>26</sup> Also, in the table it was shown that concentration at 40, 60 and 100mg/ml tend to have more effect (with zone of inhibition 22mm, 17mm and 7mm) than the concentration at 10 and 20mg/ml (with zone of inhibition 40mm and 33mm). However, the table have recorded the antifungal activity of aqueous extract of *Ficus sycomorus* and is in correspondence with the work of Sheila et al.,<sup>27</sup> that tested the aqueous extract of *Ficus sycomorus* (sandpaper tree) against some selected isolate of fungi (*Aspergillus niger* and *Candida albicans*) with 10mm and 8mm as the maximum zone of inhibition at high concentration of 100mg/ml. Sheila et al.,<sup>27</sup> also reported that moderate activity against *Candida albicans* observed in our study can justify the medicinal application of *Ficus sycomorus* Linn. Indicated for treatment of fungal infection of the gut and oral candidiasis. *Ficus sycomorus* have also been reported to possess antifungal activity at high concentration<sup>28</sup> and anti diarrhea activity.<sup>29</sup> The sedative and anticonvulsant properties of *Ficus sycomorus* have been reported.<sup>30</sup> The table also corresponds with the work of Nwankwo IU & Ukeagbu<sup>31</sup> that tested the aqueous extract of *Ficus sycomorus* (Sandpaper tree) against some selected isolates of bacteria (*Escherichia coli*, *Streptococcus pneumoniae*, *P. aeruginosa* *Staphylococcus aureus* and *Proteus mirabilis*) and showed strong inhibition of *Streptococcus pneumoniae* (30.17±0.01). Basel et al.,<sup>32</sup> also reported the antimicrobial activity of *Ficus sycomorus* against *staphylococcus aureus* and *A. baumani*. Nkafamiya et al.,<sup>12</sup> also published that the leaves of *Ficus sycomorus* has a higher protein, crude fibre and mineral contents than some Nigerian vegetables. The inhibition noticed is the reflection of the water soluble antifungal element in the respective leaves and these water soluble antifungal principles in the plant are responsible for the antifungal activities. This research work scientifically agreed that the aqueous crude extract of *Ficus sycomorus* are significantly used as the antifungal agent as well as antimicrobial agents. The extract could also be well active against several species of Fungi when use at high concentration ranging from

10-100 mg/ml as compared with previous research.

The phytochemical analysis of the aqueous leaves extract of *Ficus sycomorus* showed that the extract contains a highly significant amount of tannins, a significant amount of flavonoids, saponins, alkaloids, glycoside and anthraquinones. The presence of these constituents has been reported in *Ficus sycomorus* leaves and stem bark in Sokoto and Maidugri, Nigeria.<sup>28</sup> Similarly the absence of anthraquinone in *Ficus sycomorus* leaf and the presence of alkaloids, flavonoids and glycosides have also been documented in Zaria, Jos and Abuja, Nigeria.<sup>33</sup> The presence of tannins in the *Ficus sycomorus* plant extract may account for its effectiveness in wound healing.<sup>30</sup> The presence of these compounds has effect on the vegetative growth of fungi. These phyto compounds present might have little or no side effect for human use, thus, the main reason why sand paper tree is highly regarded as a medicinal source in most part of Nigeria.

## Conclusion

Conclusively, this study showed that the crude extract of *Ficus sycomorus* is active to the fungus *Malassezia glubosa* at different concentrations. It showed susceptibility at concentrations ranging from 10mg/ml(40mm), 20mg/ml(33mm), 40mg/ml(22mm), 60mg/ml(17mm), and 100mg/ml(7mm). However, the crude extract of *Ficus sycomorus* at different concentrations were all susceptible to *Malassezia glubosa* but the highest antifungal activity was recorded at 100mg/ml with zone of inhibition (7mm), therefore base on the research, *Ficus sycomorus* act as an fungicidal agent on *Malassezia glubosa*. The present work has shown that the studied plants are potentially a good source of antifungal agent; demonstrate the importance of this plant in medicine and in assisting primary health care in this part of the world. *Ficus sycomorus* have enormous phytochemical constituents including tannins, flavonoids, saponins, glycosides and alkaloids. *Ficus sycomorus* is highly medicinal as well as antifungal properties at increasing concentration with little or no side effect.

## Recommendations

- Further research should be carried out on the antifungal activity of the methanolic extract of *Ficus sycomorus* plant in comparison to the aqueous extracts.
- Further research should be carried out on other pathogens of health importance.
- It is also recommended that, further research should be carried out on the antifungal activity of the Ethanolic extract of *Ficus sycomorus* plant in comparison to the aqueous extracts.

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## Conflicts of interest

Author declares that there is no conflicts of interest.

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