



RESEARCH ARTICLE

IN VITRO ASSESSMENT OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF EXTRACTS OF YEMENI *FAGONIA SCHWEINFURTHII* HADIDI

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Abstract



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Background: Antioxidant and antimicrobial properties of plant extracts are attributed to bioactive components derived from medicinal plants. This study inspected into the antimicrobial and antioxidant effects of extracts from *Fagonia schweinfurthii* Hadidi aerial portions.

Method: Several solvents, including n-hexane, ethyl acetate, and methanol, were utilized sequentially to extract secondary metabolites from *F. schweinfurthii* aerial parts. Antimicrobial activity was evaluated using the well diffusion method and broth serial dilution, whilst antioxidant activity was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl radical scavenging method.

Results: The findings exposed that the studied fungal strains (*Candida albicans* and *Trichophyton rubrum*) were resistant to all plant extracts. *F. schweinfurthii* methanol and ethyl acetate extracts demonstrated inhibitory effects on Gram-positive and Gram-negative tested bacteria with minimum inhibitory concentration between 2.5 and 20 mg/ml. In addition, the most sensitive bacterium was *Proteus vulgaris*, with an inhibitory concentration (2.5 and 5 mm). While the most resistant bacterium was *Staphylococcus epidermidis*. Due to the physical and chemical properties of the solvents, different extracts of *F. schweinfurthii* aerial parts exhibited diverse antioxidant capabilities in the antioxidant activity experiment. Methanol and ethyl acetate extracts exhibited IC₅₀ values of 236 ± 0.2 µg/ml and 359.4 ± 0.6 µg/ml, respectively.

Conclusion: According to the findings of this study, the aerial portions of *F. schweinfurthii* could be considered a possible source of natural antioxidants as well as a valuable source of antibacterial agents against bacteria that cause ear infections.

Keywords: Antimicrobial, Antioxidant, DPPH, *F. schweinfurthii*.

INTRODUCTION

During their physiological processes, plants produce a variety of secondary metabolites, including alkaloids, polyphenols, flavonoids, terpenoids, and carotenes, among others¹. These secondary metabolites are a potent source of anticancer², antioxidant³, antiviral⁴, anti-inflammatory⁵, and antimicrobial agents⁶. The prevalence of life-threatening infections produced by microbial pathogens has increased globally, and among developed regions, it is now a significant cause of death in immunosuppressed patients⁷. In order to minimize the spread of infectious diseases worldwide, antimicrobial drugs are crucial⁸. Although fewer, or even occasionally, ineffective antimicrobial treatments are available for the infection caused by pathogenic

bacteria, the development and spread of multidrug-resistant (MDR) strain have become a significant public health threat^{9,10}. Folk medicine offers a valuable and underdeveloped resource for research-ing and developing potential new treatments for microbial infections to reduce the evolution of drug resistance and adverse medication effects.

Additionally, because these may be more readily available, more economical, and more accessible, the use of medicinal plants opened the potential for the developing world¹¹. Free radicals can negatively impact lipids and proteins, leading to peroxidation, which can result in unfavorable alterations to cells and gene mutation¹², premature aging, tissue damage, and inflammation¹³. These free radicals ultimately cause diabetes mellitus, Alzheimer's disease, cancer, and

other neurological disorders^{14,15,16}. Because of their capacity to stop or reduce oxidative damage, many synthetic dietary antioxidants have gained recognition. The most widely used, such as tert-butyl hydroquinone (TBHQ), butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), and tocopherol, have been banned in the food industry because they are thought to cause liver damage and cancer¹⁷. It was difficult to build the sustainability idea to screen natural, abundant, low-value raw products. Vegetables, fruits, and biomass from forests all provide significant sources of bioactive compounds, notably natural antioxidants^{13,18}. *Fagonia schweinfurthii* Hadidi (family Zyophyllaceae) and its closely related species are found throughout the deserts and dry regions of South West U.S.A., Chile, and tropical Africa¹⁹. People in the desert have traditionally used the plant to cure skin eruptions, sores, skin diseases, antipyretic, analgesic, ear infections, venereal ailments, and other conditions²⁰. The antibacterial, antiviral, analgesic, anti-inflammatory, cooling, antioxidant, anticancer, and thrombolytic effects of *Fagonia* species have been demonstrated²¹. Phytochemical investigation suggests that *F. schweinfurthii* extracts contain flavonoids, tannins, saponins, alkaloids, steroids, amino acids, carbohydrates, and cardiac glycosides²². Pareek *et al.*,²³ investigated the DPPH radical scavenging activity of *F. schweinfurthii* Hadidi aerial parts ethanol extracts, where results showed antioxidant activity with an IC₅₀ of 200.27 µg/ml. Mothana *et al.*,²⁴ reported that methanol extract of Yemeni *Fagonia indica* leaves displayed a significant concentration-dependent DPPH scavenging activity.

Al Ghanem²⁵ tested *F. mollis* petroleum ether, methylene chloride, ethyl acetate, acetone and methanol extracts for antibacterial activity against pathogenic bacterial strains (*Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*) and fungal strains (*Candida albicans*, *Mucor spp.*, *Aspergillus fumigatus*, and *A. Aspergillus niger*). All microorganisms were suppressed by acetone, except *Escherichia coli* and *S. aureus*. The plant's methanol extract displayed broad-spectrum antibacterial action on studied microbes. On the other hand, *Aspergillus fumigatus* and *Aspergillus niger* were unaffected by Petroleum ether extract, which inhibited the growth of *Candida albicans* and *Mucor spp.* While the methylene chloride, ethyl acetate, and methanol showed no antifungal action. On the other hand, *A. fumigatus* experienced growth inhibition from acetone extract, whereas the other fungi were unaffected.

This study aims to investigate *F. schweinfurthii*'s potential as an antioxidant and antimicrobial agent for the treatment of ear infections.

MATERIALS AND METHODS

F. schweinfurthii aerial parts were obtained in September 2019 from resident areas in Sana'a, Yemen. Dr. Hassan Ibrahim recognized the plant in the Biology Department, Sciences College, Sana'a University.

Test Organisms: Two Gram positive bacteria (*S. aureus* ATCC 259 23 and *S. epidermidis* RCMB

009(2), Gram negative bacteria (*E. coli* ATCC 25922, *P. vulgaris* ATCC 13315), as well as two fungi, including one filamentous fungus belonging to dermatophytes (*Trichophyton rubrum* RCMB 025002) and one yeast species (*Candida albicans* ATCC 10231), obtained from the culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt, were used in this study.

Preparation of plant extract:

One kilogram of fresh aerial parts was cleaned thoroughly with tap water, dried at room temperature, and ground into a fine powder with an electric blender. Before subsequent applications, the powder was kept in a cool place and away from light. The powdered aerial parts were soaked in 5 L of n-hexane, ethyl acetate, and methanol respectively. The samples were submerged at room temperature for three to seven days with constant stirring. This procedure was repeated three times to increase the extraction efficiency; following filtration, the extracts were concentrated at a temperature of 40°C in a rotary evaporator and dried in an oven at 37°C. For further examination, the dried extracts were weighed and kept at 4°C²⁶.

In vitro antimicrobial activity assay:

Agar Well Diffusion Assay:

Antimicrobial activity on Sabouraud Dextrose Agar (SDA) for fungi and Mueller Hinton Agar (MHA) for bacteria was investigated using the agar well diffusion method. Both media were made following the manufacturer's instructions, boiled to dissolve, and autoclaved at 12°C for 15 minutes at 15 psi. After cooling to 45°C, the sterilized media was aseptically placed into an appropriate number of labeled sterile Petri dishes and allowed to harden.

The test organisms were subcultured into Sabouraud Dextrose Broth (SDB) for fungi and Nutrient Broth (NB) for bacteria before being incubated at 25°C for 24-48 hours and 37°C for 18-24 hours, respectively, before being analyzed. Using the McFarland standard, each organism was standardized to a turbidity of 0.5 x 10⁸ cells/ml in saline solution (0.85 % NaCl) (through visual comparison). Using a sterilized cotton swab, 0.1 ml of the standardized suspensions were used to inoculate the surfaces of the 90mm-diameter MHA and SDA plates respectively. Each agar plate was punctured with sterile cork borer tools, each with 6mm diameter well. Each hole was filled with a 50 µl, 20 mg/ml methanol, ethyl acetate, and n-hexane extract of *F. schweinfurthii*.

Commercial antibiotics (gentamicin and ketoconazole) were employed as positive controls for bacteria and fungi, respectively, to test the sensitivity of the isolates, while DMSO was used as negative controls. After allowing the extract to diffuse into the agar for 5 hours at room temperature, the plates were incubated at 37°C for 18-24h for bacteria and 25°C for 24-48 h for fungi, except yeast (*Candida* species), which was incubated at 37°C. The inhibition zones were measured in millimeters after incubation using a meter rule.

The entire experiment was done three times, and the zone of inhibition's mean values was calculated²⁷.

Minimum Inhibitory Concentration Determination:

The MIC was obtained specifically for the extracts and isolates that demonstrated inhibitory action. The MIC of the effective extracts was measured using the broth dilution method²⁷. The extracts were tested against the bacteria at various concentrations ranging from 2.5 to 20 mg/ml. The extract-free broth served as a negative control, while the conventional antibacterial drug (gentamycin) served as a positive control. The minimum inhibitory concentration was determined after 24 h incubation at 37°C as the lowest concentration that demonstrated no observable growth using turbidity as a measure.

Determination of antioxidant activity: The free radical scavenging activity of *F. schweinfurthii* methanol, ethyl acetate, and n-hexane extracts was determined *in vitro* using the 2,20-diphenyl-1-picrylhydrazyl (DPPH) test. Sample stock solutions (1.0 mg/mL) in methanol were diluted to final concentrations of (500, 250, 125, 62.5 µg/mL). One milliliter of a 0.3 mM DPPH methanol solution was added to 2.5 ml of various concentration sample solutions and allowed to react at room temperature.

The absorbance values at 518 nm were taken after 30 minutes and transformed to percentage antioxidant activity (AA) using the following formula²⁸:

$$\% \text{ inhibition} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

DPPH solution (1.0 mL; 0.3 mM) plus methanol (2.5 mL) was used as a control and the reference compound ascorbic acid was also measured. All measurements were made in triplicate and averaged.

RESULTS**Antimicrobial Activity Assay:**

Antimicrobial activity of *F. schweinfurthii* aerial parts was tested against gram +ve bacteria (*S. aureus* and *S. epidermidis*), gram -ve bacteria (*E. coli* and *Proteus vulgaris*), and fungal strains (*Trichophyton rubrum* and *Candida albicans*) causing an ear infection. Antimicrobial activity of *F. schweinfurthii* methanol, ethyl acetate, and n-hexane extracts at 20 mg/ml was also compared to conventional antibiotics (Gentamicin) and antifungals (Ketoconazole) and results are provided in Table 1 and Table 2.

Table 1: Antibacterial activity of *F. schweinfurthii* aerial parts extracts at 20 mg/ml.

Plant extracts	<i>E. coli</i>	<i>P. vulgaris</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
Methanol	7.8±0.6	13.4±0.8	9.13±0.75	15.3±1.21
Ethyl acetate	7.5±0.5	10.3±1.1	-	11.2±0.8
n-hexane	-	11.8±1.27	-	-
Gentamicin	34.6±2.8	29.7±2.9	33.2±2.4	27.5±1.9

Table 2: Antifungal activity of *F. schweinfurthii* aerial parts extracts at 20 mg/ml.

Plant extract	<i>T. rubrum</i>	<i>C. albicans</i>
Methanol	-	-
Ethyl acetate	-	-
n-hexane	-	-
Ketoconazole	11.8±0.6	21.3±1.7

All three *F. schweinfurthii* aerial extracts were ineffective against *Trichophyton rubrum* and *Candida albicans*. On the other hand, Ketoconazole showed an inhibitory zone (11.8±0.6 and 21.3±1.7) against *Trichophyton rubrum* and *Candida albicans* respectively. The methanol extract was the most effective against *S. aureus*, *S. epidermidis*, *P. vulgaris*, and *E. coli*, with inhibition zone values of

15.3±1.21, 9.13±0.75, 13.4±0.8, and 7.8 ±0.6 as compared with other tested extracts. Ethyl acetate extract was less efficient against *S. aureus* (11.2 ±0.8), *P. vulgaris* (10.3±1.1), and *E. coli* (7.5±0.5), with no activity against *S. epidermidis*. In contrast, n-hexane was inactive against all tested bacteria except *P. vulgaris* (11.8±1.27).

Table 3: MIC (mg/ml) of effective *F. schweinfurthii* extracts against susceptible tested bacterial strains.

Plant extracts	<i>E. coli</i>	<i>P. vulgaris</i>	<i>S. epidermidis</i>	<i>S. Aureus</i>
Methanol	20.0	2.5	10	2.5
Ethyl acetate	20.0	5.0	-	5.0
n-hexane	-	5.0	-	-
Gentamicin	0.0024	0.0048	0.0097	0.0048

Minimum Inhibitory Concentration (MIC):

The bacterial strains sensitive to *F. schweinfurthii* extracts were tested for the minimum inhibitory concentration (MIC). The MIC for *F. schweinfurthii* extracts against sensitive bacterial strains is indicated in Table 3. The minimum inhibitory concentration of methanol extract was 20 mg/ml against *E. coli* and 2.5 mg/ml against *S. aureus* and *Proteus vulgaris*. In contrast, MIC for *S.*

epidermidis was 10 mg/ml. Ethyl acetate extract's MIC against *E. coli* was 20 mg/ml, and it's MIC against *S. aureus* and *P. vulgaris* was 5 mg/ml. On the other hand, n-hexane extract had MIC value of 5 mg/ml against *P. vulgaris*.

Antioxidant activity assay:

The antioxidant activity of the plant extracts was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The antioxidant activity of extracts from *F.*

schweinfurthii aerial parts was concentration dependent, with methanol extract showed the highest activity level ($IC_{50}=236\pm0.2$ $\mu\text{g/ml}$), followed by ethyl acetate extract ($IC_{50}=359.4\pm0.6$ $\mu\text{g/ml}$), while, n-hexane extract had no antioxidant activity (Table 3). In contrast to other concentrations examined, the highest DPPH scavenging potential (86.83%, 66.56%) was

observed at 500 $\mu\text{g/ml}$ of methanol and ethyl acetate extracts, respectively. While, the lowest DPPH scavenging potential ($18.62\pm0.54\%$, $9\pm0.2\%$) was achieved with the methanol and ethyl acetate extracts at 62.5 $\mu\text{g/ml}$ correspondingly. All the studied extracts had appreciable antioxidant scavenging abilities at lower levels than ascorbic acid.

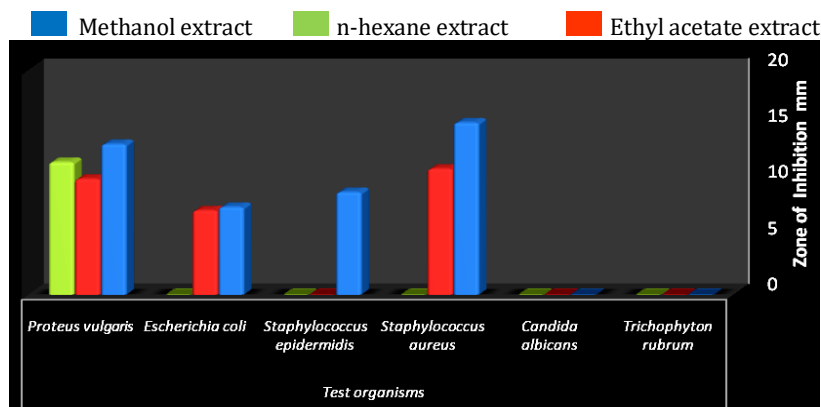


Figure 1: Antimicrobial activity of *F. schweinfurthii* aerial parts extracts.

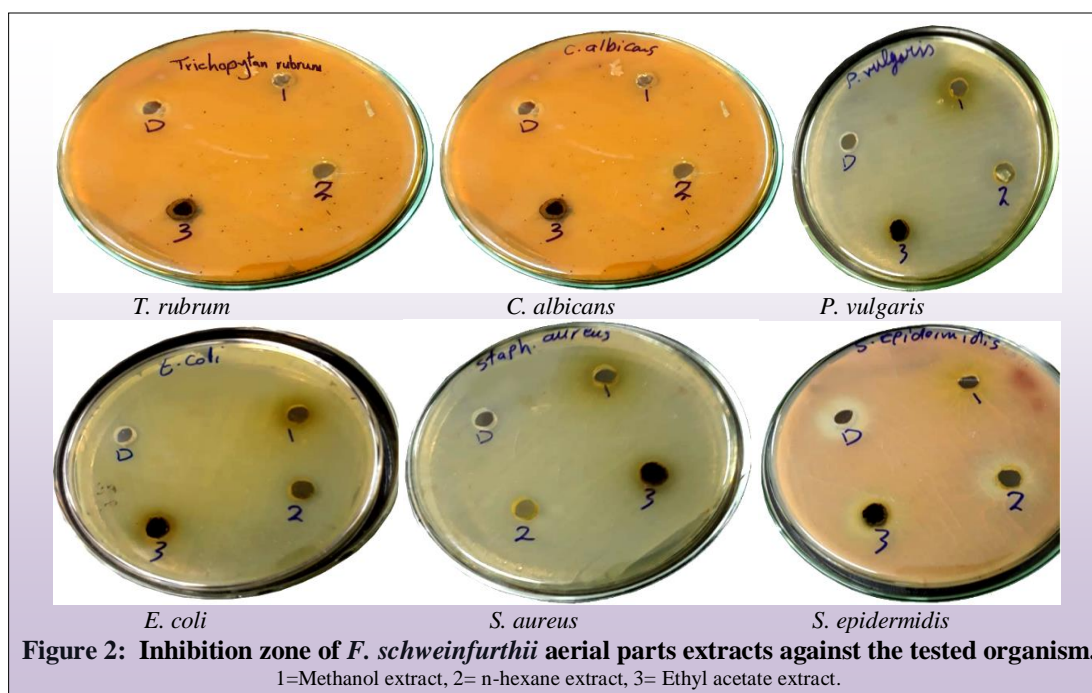


Figure 2: Inhibition zone of *F. schweinfurthii* aerial parts extracts against the tested organism.

1=Methanol extract, 2= n-hexane extract, 3= Ethyl acetate extract.

Table 4: % of DPPH inhibition by *F. schweinfurthii* aerial parts extracts.

Sample Conc. (mg/ml)	Ascorbic acid	Methanol	Ethyl acetate
500	95.58 \pm 0.03	86.84 \pm 1.22 ^a	66.56 \pm 0.21 ^a
250	95.31 \pm 0.18	59.8 \pm 1.02 ^a	40.57 \pm 0.81 ^a
125	95.14 \pm 0.15	34.94 \pm 3.26 ^a	14.37 \pm 0.30 ^a
62.5	95.02 \pm 0.17	18.57 \pm 0.25 ^a	9 \pm 0.20 ^a

Note: ^ap value <0.001 compared to reference drug (Ascorbic acid)

DISCUSSION

The systematic and proper screening of numerous extracts derived from various medicinal plants is necessary to search for novel antibiotics. The methanol extract of *F. schweinfurthii* showed potential antibacterial activity against all tested bacterial strains that cause ear infections (*S. aureus*, *S. epidermidis*, *P.*

vulgaris, and *E. coli*). In contrast, the ethyl acetate extract was only effective against *S. aureus*, *P. vulgaris*, and *E. coli*. However, n-hexane extract, was only effective against *P. vulgaris*. These results supported those of Shad et al.,²⁹ who found that n-hexane extract of *F. oliveri* had no effect against *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhus*, and *B. subtilis*. Similarly, Ur Rehman et al.,³⁰ reported that

methanol extract of *Fagonia cretica* exhibited the greatest antibacterial activity against tested bacterial strains. The methanolic extract demonstrated the highest antibacterial activity, indicating that the polar components of the crude extract predominated over the non-polar ones, confirming the traditional methods of use that rely on the aqueous extract as preferable for public use. However, the findings of this investigation contradicted those of Kouser and Quershi³¹ that the methanol extract of *Fagonia indica* has no activity against *S. aureus* or *S. epidermis*. Still, the n-hexane and ethyl acetate extracts show activity against *S. epidermis* with MICs of 2.5 and 1 mg/ml, respectively. Different plant species, growing areas, and bacterial strains could take all account for these antibacterial activity variances. The presence of tannin, alkaloids, saponins, and flavonoids in *F. schweinfurthii* extracts may explain its antibacterial activity³², which is similar to the findings of Doughari and Manzara³³ who found a correlation between antibacterial activity and phytoconstituents (alkaloid, saponin, phenol). In immunocompromised individuals, persistent opportunistic fungal infections have become a major cause of morbidity and mortality^{34,38-40}. Extracts of *F. schweinfurthii* aerial parts were tested for antifungal activity against *Candida albicans* and *Trichophyton rubrum*. Ketoconazole was employed as an antifungal standard. *F. schweinfurthii* aerial parts extracts did not inhibit fungal growth, meaning they are inactive against tested fungal strains. These findings matched those²⁵, which indicate that ethyl acetate and methanol extracts of *Fagonia mollis* exhibited no antifungal activity against *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, and *Mucor spp.* Similarly, Shad et al.,²⁹ found that methanol extract of *Fagonia Oliveri* had no activity against *Candida albicans*, and hexane fraction had no activity against many fungal strains tested (*T. longifusus*, *C. albicans*, *C. glaberata*, *F. solani*, and *A. flavus*). The current investigation results demonstrated that n-hexane extract lacked antioxidant activity while methanol and ethyl acetate extracts had concentration-dependent antioxidant activity. A similar outcome was obtained by El-Amier & Abo Aisha³⁵ who discovered that as plant extract concentration increased, *F. arabica*, *F. criticus*, and *F. mollis* methanolic extracts' capacity to scavenge free radicals increased constantly. The maximum antioxidant activity was found in the methanol extract (IC₅₀=236±0.2 µg /ml), followed by the ethyl acetate extract (IC₅₀=359.4±0.6 µg/m), whereas the n-hexane extract had no antioxidant activity. Current findings on antioxidant activity correspond with those of Pareek et al.,²³. However, the results of the present study contradicted those of a study³⁶. Diverse plant species, locations used for collecting, and extraction techniques could all contribute to this variation in activity. Flavonoids and phenolic compounds may be responsible for *F. schweinfurthii*'s antioxidant action³⁷. Additionally, methanol extract of Yemeni *Fagonia indica* leaves has been revealed to include flavonoids and saponins responsible for the antioxidant action²⁴.

Limitations of the study

Difficulty in getting standard bacteria and fungi.

CONCLUSIONS

This study shows that several extracts from *F. schweinfurthii* aerial parts have various antioxidant and antibacterial activities. According to the findings, methanol extract had the highest levels of antibacterial and antioxidant capabilities. Additionally, *F. schweinfurthii* extracts could inhibit bacteria linked to ear infections and may provide scientific support for the plant's traditional usage in folk medicine. Also, it is proposed that additional research be conducted on the isolation and identification of antioxidant components contained in plant materials for application in both the animal and human nutraceutical industries.

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AUTHOR'S CONTRIBUTIONS

Raweh SM: writing original draft, methodology. **Alhaj HA:** research design, data collection. **Al-Kaf AG:** statistical analysis, conceptualization. Final manuscript was read and approved by all authors.

DATA AVAILABILITY

Data will be made available on reasonable request.

CONFLICT OF INTEREST

There is no conflict of interest related to this work.

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