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Antifungal Activity and Fourier Transform Infrared Spectrometric Characterization of Aqueous Extracts of *Acacia senegal* and *Acacia tortilis* on Phytopathogenic Fungi

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Objective: To evaluate the *in vitro* antifungal activity of aqueous extracts of *Acacia senegal* (*A. senegal*) and *Acacia tortilis* (*A. tortilis*) against three phytopathogenic fungi (viz., *Alternaria alternata* [*A. alternata*], *Helminthosporium rostratum* [*H. rostratum*] and *Fusarium solani* [*F. solani*]).
Methods: Crude aqueous extracts of *A. senegal* and *A. tortilis* at 1%, 2.5% and 5% concentrations were used for screening. Antifungal activities of the extracts were evaluated against three phytopathogenic fungal strains (*A. alternata, H. rostratum* and *F. solani*) by poisoned food technique. Scanning electron microscopy (SEM) of the treated and untreated mycelia was employed to analyze the ultrastructural changes and Fourier-transform infrared (FTIR) spectrometry analysis was performed to identify important functional groups.
Results: Aqueous extract of *A. tortilis* at high concentrations exhibited moderate inhibitory activity against the selected fungal strains. The aqueous extract of *A. senegal* showed no effect on *A. alternata*, while exhibited very mild activity against *H. rostratum* and *F. solani* at high concentrations (2.5% and 5%). Scanning electron microphotographs of the untreated fungal cells showed no

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structural changes (well–defined mycelium and conidia without any distortion), whereas the treated cells showed structural distortions, twisted and wrecked mycelia and showed the presence of vesicles on the surface. FTIR analysis showed the presence of important functional groups such as alcohols, carboxylic acids and aromatic compounds.

Conclusion: Results from this study indicate that the aqueous extracts of both *A. senegal* and *A. tortilis* have the potential to be used as natural fungicidal agents in the management of diseases caused by plant pathogenic fungi.

Keywords: Acacia senegal; Acacia tortilis; antifungal activity; aqueous extracts; plant pathogenic fungi; Fourier-Transform Infrared (FTIR) spectrometric; Scanning Electron Microscopy (SEM).

1. INTRODUCTION

Acacia senegal (L.) Willd (*A. senegal*) and *Acacia tortilis* (*A. tortilis*) commonly known as gum acacia are leguminous dryland trees widely distributed in arid and semiarid ecosystems of sub-Saharan Africa, Australia, and the Middle East [1]. These legumes have remarkable adaptability to drought, alkalinity, and salinity and substantially contributes to the replenishment of soil fertility in the arid regions [2,3]. Both plants produce a myriad of primary and secondary metabolites, which possess inhibitory properties against an array of microorganisms [4-8]. Traditionally, the gum from both *Acacia* species have been used as a food additive [9,10]. In addition to their use in food industry, they possess immense medicinal properties and have been used to treat inflamed skin surface, burns, sore throat, diarrhea, dysentery, gonorrhea, urinary tract infections, leprosy and renal diseases [11-14]. Furthermore, they are also used as antioxidants, antitussive and astringent agents [8,14,15].

Several studies have reported the antifungal activity of *Acacia* species (for example *A. nilotica, A. saligna, A. catechu,* and *A. arvensis*) [13,16-20]. The extracts of the *Acacia* plants were found to exhibit potent activity against wide range of fungal species including *Pythium aphanidermatum* [17], *Alternaria brassicae* [19], *Fusarium oxysporum ciceris* [19], *Rhizoctonia solani* [16,19], *Candida albicans* [13,18], *Trichophyton rubrum* [20], *Microsporum gypseum* [20] and *Epidermophyton floccosum* [20], *Fusarium culmorum* [16], *Penicillium chrysogenum* [16].

Alternaria alternata (A. alternata) is a necrotrophic fungi pathogen that causes diseases in a variety of economically important crops, including apple, broccoli, cauliflower, carrot, citrus, pear, potato, rice, strawberry, tomato and tobacco, ornamental plants and a number of weed species [21]. It generally affects the aerial parts of the plants such as leaves, petioles, floral parts and seeds [22,23]. *Helminthosporium rostratum* (*H. rostratum*) is another phytopathogenic fungi that affects an array of the plants including rice, maize, corn, sorghum, and millet [24]. *Fusarium solani* (*F. solani*) is a highly fungal species and has been known to infect several crops including peas, beans, potatoes, and many types of cucurbits [25].

Although several studies have evaluated the antimicrobial activities of gum exudates against a broad range of pathogens [7,26-29], antifungal activity against the plant pathogenic fungi *viz*., *A. alternata*, *H. rostratum,* and *F. solani* remains highly elusive. Therefore, the present study explored the *in vitro* antifungal activity of aqueous extracts of *A. senegal* and *A. tortilis* against three plant pathogenic fungi (*A. alternata*, *H. rostratum* and *F. solani*). Moreover, Fourier-transform infrared (FTIR) spectroscopy analysis for the identification of phytochemicals and scanning electron microscopic (SEM) studies of fungi treated with extracts were undertaken to characterize the ultrastructural damage caused by the aqueous extracts from both the plants.

2. MATERIALS AND METHODS

2.1 Plant Materials

The gum of *A. senegal* and *A. tortilis* were procured from authorized suppliers (forever drug store and Bin mingash respectively, Riyadh, Saudi Arabia). Gum resins were kept at -80°C freezer overnight following which they were crushed into a fine powder using mortar and pestle in a 40 µM mess, regular blender, and electric sieve system. After pulverization, the gum resins were stored at -20°C in separate, well-labeled containers until further processing.

2.2 Preparation of Aqueous Plant Extracts

First, the fresh dry plant materials were crushed then 30 gm of the crushed material were soaked in 300 mL distilled water (10% w/v) at 37°C in closed containers for 24 hours. The soaked material was macerated with 50 mL distilled water (10% w/v) in separate labeled glass bottles and then subjected to shaking (250 rpm at 45°C for 24 hours) in an orbital shaker (Sartorius Certomat IS, Germany). The supernatant was filtered through Whatman's (No. 1) filter paper. The extracts were then concentrated and dried under reduced pressure and 40°C using rotary evaporator (Rotavapor® R-215, BUCHI). All the filtered extracts were preserved aseptically in glass bottles at 4°C until further use.

The sterile aqueous extracts were diluted with distilled water (10% w/v) to obtain different final concentrations (1,2.5,5 mg/mL) on the base of the dry weight of dried aqueous extracts. Reconstituted aqueous extracts were passed through 0.45 µM bacterial filter papers (Millipore Inc., Riyadh, Saudi Arabia) before using them for *in vitro* assay.

2.3 Fungal Materials

The plant-fungal strains (*H. rostratum, F. solani* and *A. alternata*) used in this study were obtained from the Department of Plant Protection, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia. All the fungal strains were maintained on Potato dextrose agar.

2.4 *In vitro* Antifungal Activity

The antifungal activity of crude aqueous extracts of *A. senegal* and *A. tortilis* was evaluated against three fungal strains (*H. rostratum, F. solani* and *A. alternata*) by poisoned food technique [30]. To 9 cm petri plate, 1 mL of the extract was added followed by 19 mL of molten potato dextrose agar and mixed gently by swirling. The modified agar was allowed to solidify, after which a mycelial plug (6 mm) was placed in the center of the plate. The mycelial plug was removed from the periphery of 9 days old actively growing colony. The above–mentioned experiments was carried out aseptically in a laminar air flow. After inoculation, petri plates were incubated at 25±2°C for 7 days. All fungal strains were subjected to different concentrations of aqueous extracts in triplicates. Results were noted by measuring the diameter of mycelial growth when the control plate showed full plate growth and percentage growth inhibition was calculated as follows:

$$\% \text{ inhibition} = (AC - AT)/AC \times 100$$

Where,

AC = colony diameter in control plate,
AT = diameter of the colony in treatment plate

2.5 Scanning Electron Microscopy

Scanning electron microscopy (SEM) was employed to analyze the mycelia treated with crude aqueous extract and was compared with the untreated control. In brief, small agar pieces (6 mm) were aseptically cut out from the inhibition zone and were fixed in 2.5% (v/v) glutaraldehyde buffered with 0.1 M sodium phosphate buffer (pH 7.4). The suspension was centrifuged after 48 h, rinsed thrice with phosphate-buffered saline and was dehydrated in graded ethanol series (60% - 100%). The dehydrated specimen were freeze-dried and were mounted onto stubs using double-sided carbon type, and then were coated with a thin layer of gold. The processed specimens were investigated under a scanning electron microscope (JSM–6060LV–JEOL, Japan–LTD).

2.6 Infrared Spectrometry Analysis

Aqueous extracts with strongest antifungal activity were qualitatively analyzed by Fourier-Transform Infrared (FTIR) spectrometry analysis for the detection and confirmation of functional constituents in plant extracts. The aqueous extracts of *A. senegal* and *A. tortilis* were passed into the FTIR spectrophotometer and functional groups of the components were separated based on its peak ratio. A sophisticated computer-controlled spectrophotometer (Nicolet–6700, Thermo Scientific, USA) equipped with a beam splitter, a detector (DTGS) and OMNIC software was used to generate the FTIR spectra in the mid-region of 500-4000 cm^{-1}. The IR spectrums obtained from the analysis were used to interpret the functional groups present in each of the aqueous extracts.

2.7 Statistical Analysis

All the experiments were performed in triplicate. Data are reported as means and standard

deviations (SD). One-way analysis of variance (ANOVA) was used for data analysis and the significant differences ($p<0.05$) between the means were performed.

3. RESULTS

3.1 Antifungal Activity

The antifungal activity exhibited by aqueous extracts of *A. senegal* and *A. tortilis* is summarized in Table 1. The aqueous extract of *A. senegal* at 1%, 2.5% and 5% concentrations did not inhibit the growth of *A. alternata* (Table 1).

The fungal growth in the treated plates was comparable to the growth observed in the control plate (Fig. 1a). While the aqueous extract of *A. senegal* at 1% concentration showed no effect on the growth of *H. rostratum*, it exhibited very mild activity on the mycelial growth at 2.5% and 5% concentrations (Table 1). There was no difference observed in the confluence of fungal growth in the control plate and those treated at 1% concentration. The plates treated with 2.5% and 5% concentrations showed very weak inhibitory activity (Fig. 1b). The aqueous extract of *A. senegal* showed very mild inhibitory activity against *F. solani* which increased as the concentration increased from 1% to 5% concentration (Table 1). The diameter of growth observed across the treated plates (1%, 2.5% and 5%) and control plates was similar (Fig. 1c).

The aqueous extract of *A. tortilis* did not inhibit the growth of *A. alternata* at 1% and 2.5% concentrations. However, it induced moderate inhibition of fungal colonies at 5% and *H. rostratum* (Table 1). The confluence of fungal growth was comparable in the control, 1% and 2.5% treated plates whereas, it showed a clear zone of inhibition at 5% concentration (Fig. 2a). Similarly, the extract of *A. tortilis* showed no activity and minimal activity at 1% and 2.5% concentrations but it showed a moderate activity at 5% concentration (Table 1 and Fig. 2b). The aqueous extract of *A. tortilis* showed mild potency against *F. solani* which increased as the concentration increased. The growth in the control and 1% treated plates was comparable, while those treated with 2.5% and 5% clearly showed clear zones of inhibitions (Fig. 2c).

3.2 Scanning Electron Microscopy

The scanning electron micrographs of untreated mycelia (controls) of *A. alternate* along with the plates treated with aqueous extracts of *A. senegal* and *A. tortilis* with the maximum inhibitory effect of *A. tortilis* were selected for assessing the morphological changes.

The untreated-biomass (control) of *A. alternata* had normal tubular hyphae and intact mycelial and conidial growth and absence of structural changes (Fig. 3a). The SEM images of *A. alternata* treated with aqueous extract of *A. senegal* showed smooth external surface with no morphological changes (Fig. 3b). However, plates treated with aqueous extract of *A. tortilis* showed deformation of cellular structure as indicated by shrunken curly hyphae of variable sizes, deformed and wrinkled external surfaces, and compressed conidia (Fig. 3c).

3.3 Fourier-transform Infrared Spectroscopic Analysis of the Aqueous Extracts

Aqueous extracts of both *A. senegal* and *A. tortilis* were subjected to FTIR analysis to identify the functional groups of the active components present in extract based on the peak values in the region of IR radiation. In *A. senegal*, IR spectrum showed strong absorption peaks at 3437, 2930, 2143, 1634, 1433, 1153 and 1172, 574 and 439 cm-1 which corresponds to alcohols, carboxylic acids, alkynes, amides, alkanes, alkyl amines, halogen and cycloalkanes groups, respectively (Fig. 4a). For *A. tortilis*, broad peaks were recorded at 2930, 2367 and 2340, 2121, 1621, 1429, 1081 and 1032, and 574 cm^{-1} that corresponds to CH asymmetry, phosphine, alkyne, conjugated alkene, carboxylic acid, alkyl halides and nitriles functional groups, respectively (Fig. 4b).

4. DISCUSSION

Plant diseases caused due to fungiform impose significant damage to many economic crops worldwide. Synthetic fungicides are the first-line defense for protecting the plants against fungal infections. However, commercially available fungicidal agents are toxic and produce undesirable effects on other organisms, soil, plants and water [31]. Moreover, the development of resistance towards the synthetic fungicides of pathogenic fungi has been a matter of great concern. Plant-derived antifungal agents represent a vast untapped source with tremendous potential.

Table 1. Antifungal activity of different concentrations of aqueous extracts of *Acacia senegal* and *Acacia tortilis* and the percentage mycelial inhibition

	Alternaria alternata		*Helminthosporium rostratum*		*Fusarium solani*	
Acacia senegal	**Growth (mm)**	**Inhibition %**	**Growth (mm)**	**Inhibition %**	**Growth (mm)**	**Inhibition %**
Control	8.0 (0.00)	0.0	8.0 (0.00)	0.0	8.0 (0.00)	0.0
1.0%	8.0 (0.00)	0.0	8.0 (0.00)	0.0	7.4 (0.13)	1.5
2.5%	8.0 (0.00)	0.0	7.5 (0.06)	5.9	7.7 (0.23)	4.1
5.0%	8.0 (0.00)	0.0	7.4 (0.13)	7.3	7.9 (0.20)	7.9
Acacia tortilis						
Control	8.0 (0.00)	0.0	8.0 (0.00)	0.0	8.0 (0.00)	0.0
1.0%	8.0 (0.00)	0.0	8.0 (0.00)	0.0	7.3 (0.26)	8.8
2.5%	8.0 (0.00)	0.0	7.6 (0.08)	5.3	6.5 (0.43)	18.5
5.0%	4.2 (0.21)	47.9	4.5 (0.00)	43.8	6.5 (0.23)	19.4

**Values are presented as means (SD) of triplicates*

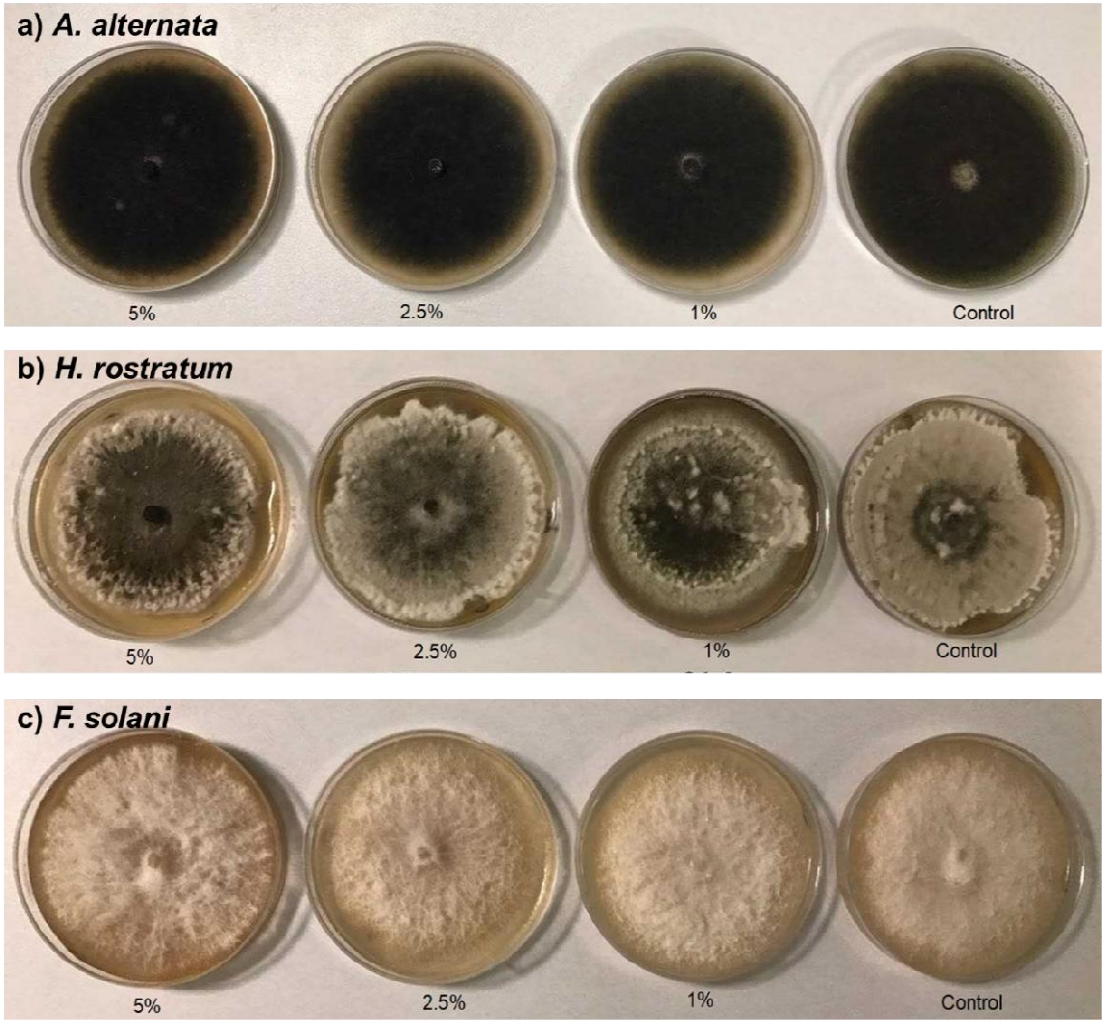

Fig. 1. *In vitro* activity of aqueous extract of *Acacia senegal* on three phytopathogenic fungi
a) A. alternata, b) H. rostratum and c) F. solani

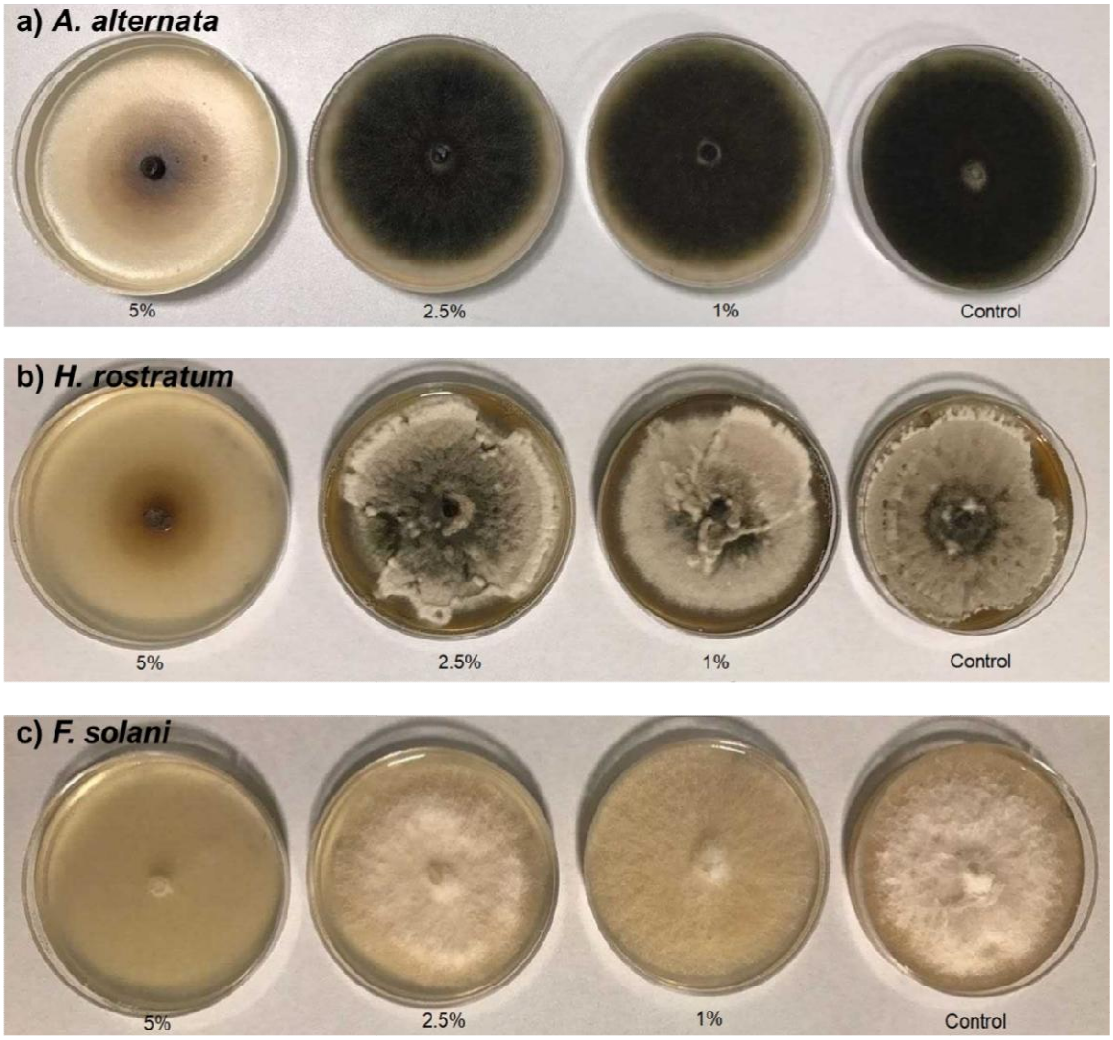

Fig. 2. *In vitro* activity of aqueous extract of *Acacia tortilis* on three phytopathogenic fungi
a) A. alternata, b) H. rostratum and c) F. solani

The present study assessed the antifungal activity of crude aqueous extracts of *A. senegal* and *A. tortilis* against three phytopathogenic fungal strains viz., *A. alternata, H. rostratum* and *F. solani*. Results from this study showed that aqueous extracts of *A. senegal* and *A. tortilis* varied in their effectiveness in inhibiting fungal growth. While SEM microphotographs confirmed the presence of ultrastructural changes in the treated cells, FTIR spectroscopy showed the presence of important functional groups responsible for antifungal activity.

The arid and semi-arid regions of the Middle East is a rich source of many medicinal plants [1]. Gum acacia is one of the most important medicinal plants available in the entire region. It contains various primary and secondary metabolic constituents (alkaloids, catechins, chalcones, flavones, flavonoids, polyphenols, and tannins) which have been traditionally used for the treatment of various plant and human diseases [15]. Two different species of gum acacia (*A. senegal* and *A. tortilis*) were selected based on their traditional use [32]. Three different concentrations (1%, 2.5% and 5%) of the aqueous extracts of both plants were screened for their in vitro activity against three important pathogenic plant fungi (*A. alternata, H. rostratum* and *F. solani*). The extract of A. senegal showed no effect on the growth of *A. alternata* at all the three concentrations tested (1%, 2.5% and 5%) whereas, it showed very mild activity on *H.*

rostratum and *F. solani* at 2.5% and 5% concentrations. Aqueous extract of A. tortilis exhibited moderate activity against *A. alternata* at 5% concentration and mild to moderate activity against *H. rostratum* and *F. solani*. Although both Acacia species showed antifungal activity, the aqueous extract of *A. tortilis* showed relatively higher potency in inhibiting the mycelial growth of all the three tested fungal strains. Limited effectiveness of aqueous extract of *A. senegal* against *A. alternata* and the least activity against *H. rostratum* and *F. solani* could be partially due to incomplete extraction of the active principles [33]. Other factors include solubility, pH, volatility, diffusion characteristics in the growth medium, and fungal strains [34,35].

Furthermore, SEM and FTIR studies were conducted to characterize the ultrastructural changes in the mycelia and identify the functional groups in the plant extracts respectively. SEM studies of the cells treated with aqueous extract of *A. tortilis* showed a deleterious effect on mycelial and conidial structures. Results from the FTIR analysis revealed the presence of several functional groups (alcohols, carboxylic acids, alkynes, amides, alkanes, alkyl amines, halogen and cycloalkanes groups) that can act alone or in synergy, as demonstrated by other studies [5,36-38]. As this is the first study to investigate the antifungal activity of crude aqueous extracts of A. senegal and A. tortilis against A. alternata, *H. rostratum* and *F. solani*, formal comparison of the data obtained in this study is not possible. However, the results of antifungal activity of the Acacia species assessed in this study are similar to those reported in previous studies [16,39]. In the study by Baig et al. aqueous extracts of *A. nilotica* was found to exhibit moderate activity against *Aspergillus flavus* and *Aspergillus niger*. The antifungal activity increased as the concentration increased from 10% to 25% [39].

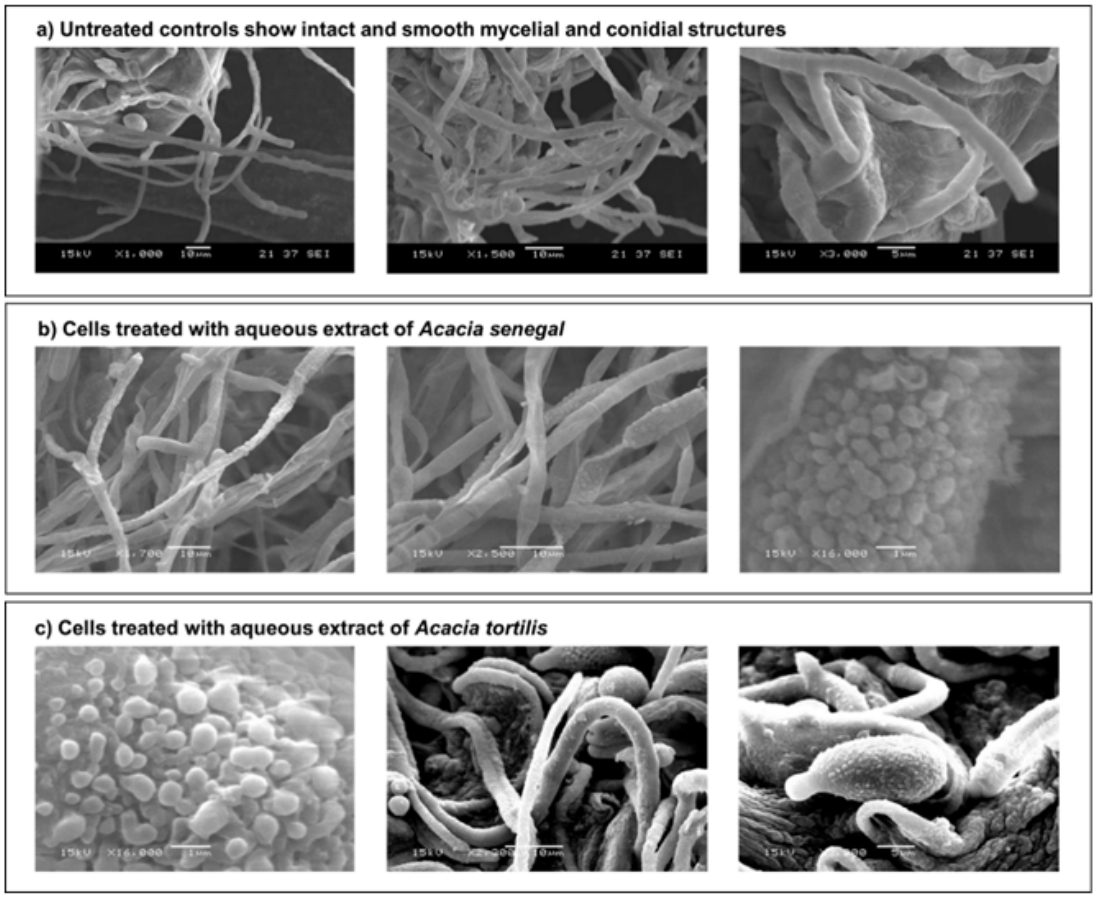

Fig. 3. Scanning electron microphotographs of *Alternaria alternata*

a) Untreated controls show intact and smooth mycelial and conidial structures, b) Cells treated with aqueous extract of Acacia senegal and c) Cells treated with aqueous extract of Acacia tortilis

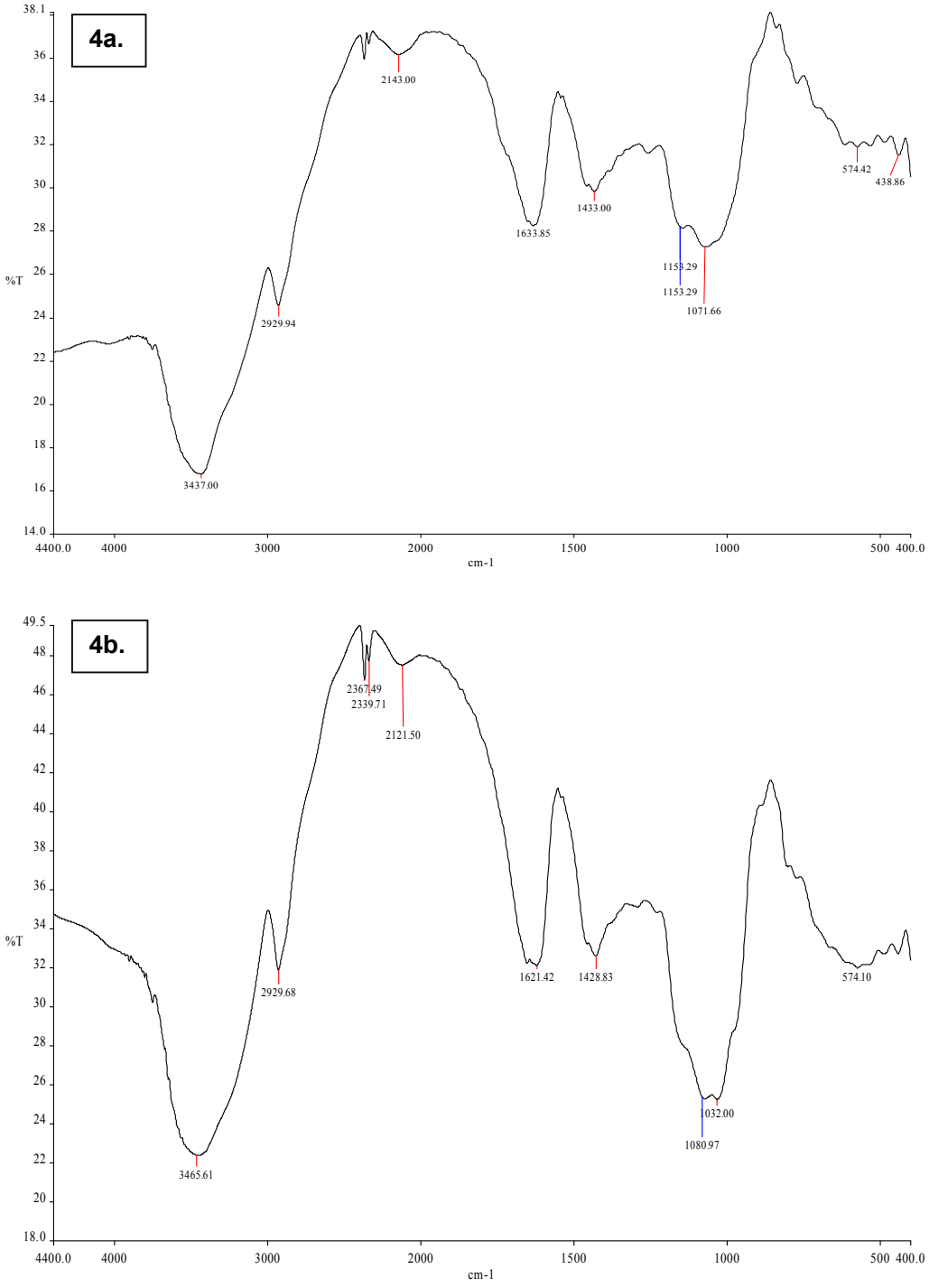

Fig. 4. Fourier-transform infrared spectrum of the aqueous extract of *Acacia* species

a) FTIR spectrum of the aqueous extract of Acacia senegal and b) FTIR spectrum of the aqueous extract of Acacia tortilis

Similarly, in a study by Al-Huqail et al. the aqueous extract of *A. saligna* (Labill) inhibited the growth of *Penicillium chrysogenum*. Furthermore, the percentage inhibition of the fungal mycelium increased as the extract concentration increased [16]. The antifungal activity exhibited by both *A.*

senegal and *A. tortilis* may be attributed to the presence of numerous phytoconstituents such as polyphenols and flavonoid compounds which are reported to be abundantly present among Acacia species [16,40-42].

5. CONCLUSION

The results from this study provide evidence that the aqueous extracts of *A. senegal* and *A. tortilis* varied in their efficacy in inhibiting the mycelial growth of tested fungal species. Although the selected concentration of aqueous extracts was unable to completely inhibit the selected phytopathogens, they can potentially be explored alone or in combination as a source of natural fungicidal material. The high proportion of active extracts in the *Acacia* species corroborates the validity of the use of these plant species as natural-plant derived fungicides. Further, large-scale *in vitro* and *in vivo* studies are warranted to replicate the findings of this study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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