

**Antifungal activity and Fourier Transform  
Infrared Spectrometric Characterization of  
Aqueous Extracts of *Acacia senegal* and *Acacia  
tortilis* on phytopathogenic fungi**

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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 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*; Anti-fungal activity; Aqueous extracts; Plant pathogenic fungi; Fourier Transform Infrared Spectrometric (FTIR); Scanning Electron Microscopy (SEM).

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## 1. INTRODUCTION

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*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, gonorrhoea, urinary tract infections, leprosy, and renal diseases [11-14]. Furthermore, they are also used as antioxidants, antitussive and astringent agents [8, 14, 15].

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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], *Microsporium gypseum* [20], and *Epidermophyton floccosum* [20], *Fusarium culmorum* [16], *Penicillium chrysogenum* [16].

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*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].

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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.

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## 2. MATERIAL AND METHOD

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### 2.1 Plant Materials

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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.

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## 68 **2.2 Preparation of Aqueous Plant Extracts**

69 First, the fresh dry plant materials were crushed then 30 gm of the crushed material were  
70 soaked in 300 mL distilled water (10% w/v) at 37<sup>0</sup>C in closed containers for 24 hours. The  
71 soaked material was macerated with 50 mL distilled water (10% w/v) in separate labeled  
72 glass bottles and then subjected to shaking (250 rpm at 45<sup>0</sup>C for 24 hours) in an orbital  
73 shaker (Sartorius Certomat IS, Germany). The supernatant was filtered through Whatman's  
74 (No. 1) filter paper. The extracts were then concentrated and dried under reduced pressure  
75 and 40<sup>0</sup>C using rotary evaporator (Rotavapor® R-215, BUCHI). All the filtered extracts were  
76 preserved aseptically in glass bottles at 4<sup>0</sup>C until further use.

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

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## 83 **2.3 Fungal Material**

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

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## 89 **2.4 In vitro Antifungal Activity**

90 The antifungal activity of crude aqueous extracts of *A. senegal* and *A. tortilis* was evaluated  
91 against three fungal strains (*H. rostratum*, *F. solani*, and *A. alternata*) by poisoned food  
92 technique [30]. To 9 cm petri plate, 1 mL of the extract was added followed by 19 mL of  
93 molten potato dextrose agar and mixed gently by swirling. The modified agar was allowed to  
94 solidify, after which a mycelial plug (6 mm) was placed in the center of the plate. The  
95 mycelial plug was removed from the periphery of 9 days old actively growing colony. The  
96 above-mentioned experiments was carried out aseptically in a laminar air flow. After  
97 inoculation, petri plates were incubated at 25± 2<sup>0</sup>C for 7days. All fungal strains were  
98 subjected to different concentrations of aqueous extracts in triplicates. Results were noted  
99 by measuring the diameter of mycelial growth when the control plate showed full plate  
100 growth and percentage growth inhibition was calculated as follows:

101 % inhibition= (AC- AT)/AC × 100

102 Where AC = colony diameter in control plate,

103 AT = diameter of the colony in treatment plate

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## 105 **2.5 Scanning Electron Microscopy**

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

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## 116 **2.6 Fourier Transform Infrared Spectrometry Analysis**

117 Aqueous extracts with strongest antifungal activity were qualitatively analyzed by Fourier  
118 Transform Infrared Spectrometry Analysis (FTIR) spectrometer for the detection and  
119 confirmation of functional constituents in plant extracts. The aqueous extracts of *A. senegal*

120 and *A. tortilis* were passed into the FTIR and functional groups of the components were  
 121 separated based on its peak ratio. A sophisticated computer-controlled spectrophotometer  
 122 (Nicolet-6700, Thermo Scientific, USA) equipped with a beam splitter, a detector (DTGS)  
 123 and OMNIC software was used to generate the FTIR spectra in the mid-region of 500 - 4000  
 124  $\text{cm}^{-1}$ . The IR spectrums obtained from the analysis were used to interpret the functional  
 125 groups present in each of the aqueous extracts.  
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## 127 2.7 Statistical Analysis

128 All the experiments were performed in triplicate. Data are reported as means and standard  
 129 deviations (SD). One-way analysis of variance (ANOVA) was used for data analysis and the  
 130 significant differences ( $p < 0.05$ ) between the means were performed.  
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## 132 3. RESULTS

### 133 3.1 Antifungal activity

136 The antifungal activity exhibited by aqueous extracts of *A. senegal* and *A. tortilis* is  
 137 summarized in Table 1. The aqueous extract of *A. senegal* at 1%, 2.5%, and 5%  
 138 concentrations did not inhibit the growth of *A. alternata* (Table 1).  
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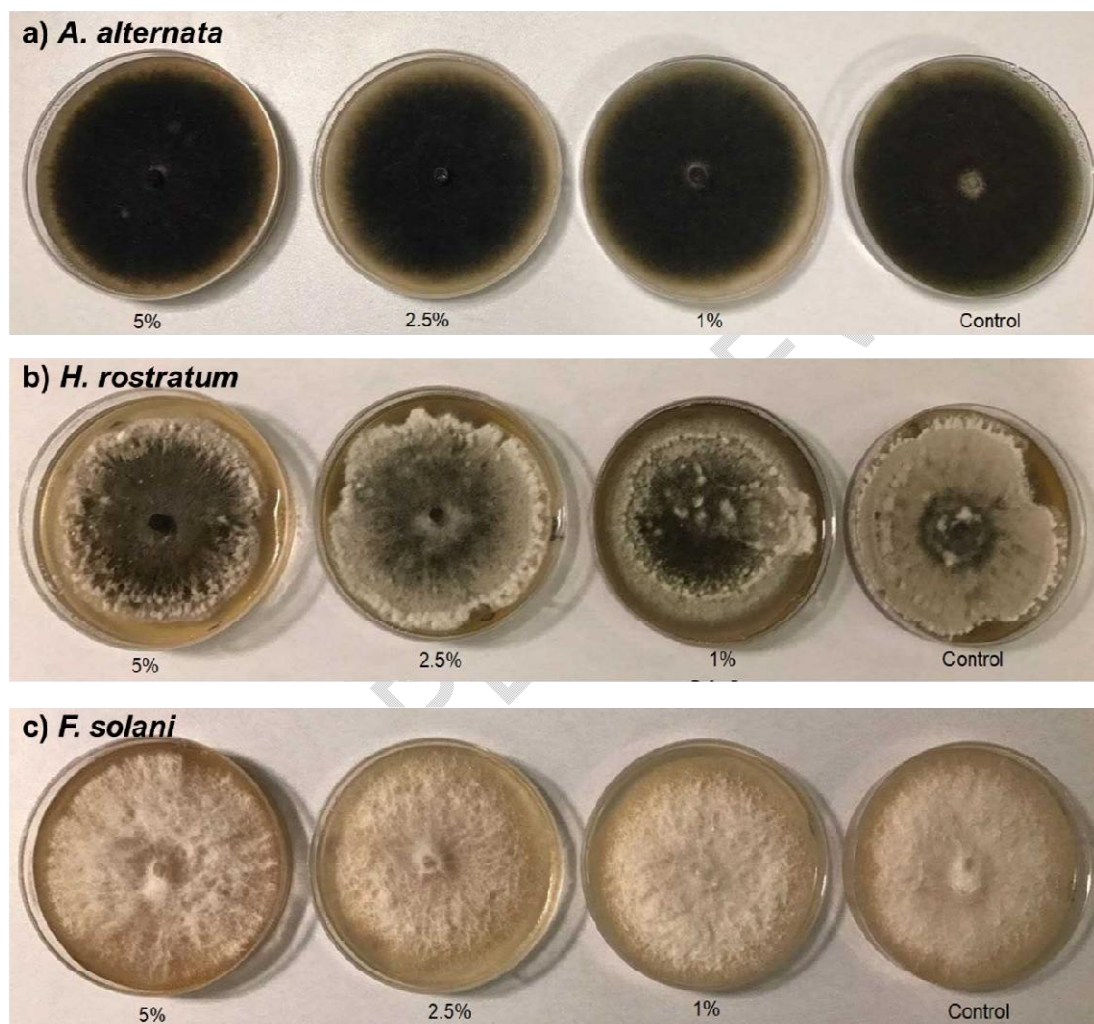
140 **Table 1. Antifungal activity of different concentrations of aqueous extracts of *Acacia***  
 141 ***senegal* and *Acacia tortilis* and the percentage mycelial inhibition**  
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	<i>Alternaria alternata</i>		<i>Helminthosporium rostratum</i>		<i>Fusarium solani</i>	
<i>Acacia senegal</i>	Growth (mm)	Inhibition %	Growth (mm)	Inhibition %	Growth (mm)	Inhibition %
<b>Control</b>	8.0 (0.00)	0.0	8.0 (0.00)	0.0	8.0 (0.00)	0.0
<b>1.0%</b>	8.0 (0.00)	0.0	8.0 (0.00)	0.0	7.4 (0.13)	1.5
<b>2.5%</b>	8.0 (0.00)	0.0	7.5 (0.06)	5.9	7.7 (0.23)	4.1
<b>5.0%</b>	8.0 (0.00)	0.0	7.4 (0.13)	7.3	7.9 (0.20)	7.9
<b><i>Acacia tortilis</i></b>						
<b>Control</b>	8.0 (0.00)	0.0	8.0 (0.00)	0.0	8.0 (0.00)	0.0
<b>1.0%</b>	8.0 (0.00)	0.0	8.0 (0.00)	0.0	7.3 (0.26)	8.8
<b>2.5%</b>	8.0 (0.00)	0.0	7.6 (0.08)	5.3	6.5 (0.43)	18.5
<b>5.0%</b>	4.2 (0.21)	47.9	4.5 (0.00)	43.8	6.5 (0.23)	19.4

143 \*Values are presented as means (SD) of triplicates.  
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145 The fungal growth in the treated plates was comparable to the growth observed in the  
 146 control plate (Figure 1a). While the aqueous extract of *A. senegal* at 1% concentration  
 147 showed no effect on the growth of *H. rostratum*, it exhibited very mild activity on the mycelial

148 growth at 2.5% and 5% concentrations (Table 1). There was no difference observed in the  
149 **confluence** of fungal growth in the control plate and those treated at 1% concentration, the  
150 plates treated with 2.5% and 5% concentrations showed very weak inhibitory activity (Figure  
151 1b). The aqueous extract of *A. senegal* showed very mild inhibitory activity against *F. solani*  
152 which increased as the concentration increased from 1% to 5% concentration (Table 1). The  
153 diameter of growth observed across the treated plates (1%, 2.5%, and 5%) and control  
154 plates was similar (Figure 1c).  
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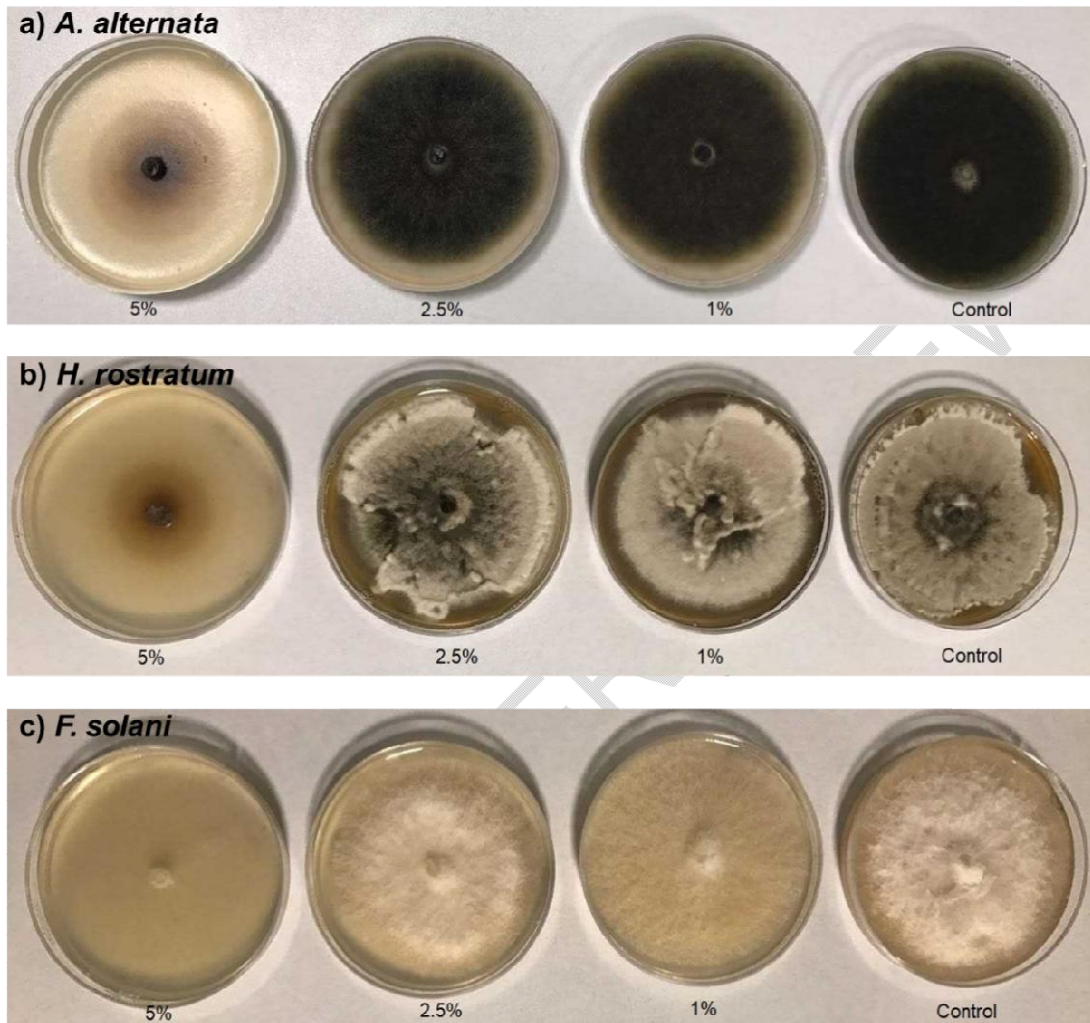
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157 **Figure 1: In vitro activity of aqueous extract of *Acacia senegal* on three**  
158 **phytopathogenic fungi; a) *A. alternata*, b) *H. rostratum* and c) *F. solani***  
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160 The aqueous extract of *A. tortilis* did not inhibit the growth of *A. alternata* at 1% and 2.5%  
161 concentrations. However, it induced moderate inhibition of fungal colonies at 5% and *H.*  
162 *rostratum* (Table 1). The **confluence** of fungal growth was comparable in the control, 1%,  
163 and 2.5% treated plates, whereas it showed a clear zone of inhibition at 5% concentration  
164 (Figure 2a). Similarly, the extract of *A. tortilis* showed no activity and minimal activity at 1%  
165 and 2.5% concentrations but it showed a moderate activity at 5% concentration (Table 1 and  
166 Figure 2b). The aqueous extract of *A. tortilis* showed mild potency against *F. solani* which  
167 increased as the concentration increased. The growth in the control and 1% treated plates



168 was comparable, while those treated with 2.5% and 5% clearly showed clear zones of  
169 inhibitions (Figure 2c).  
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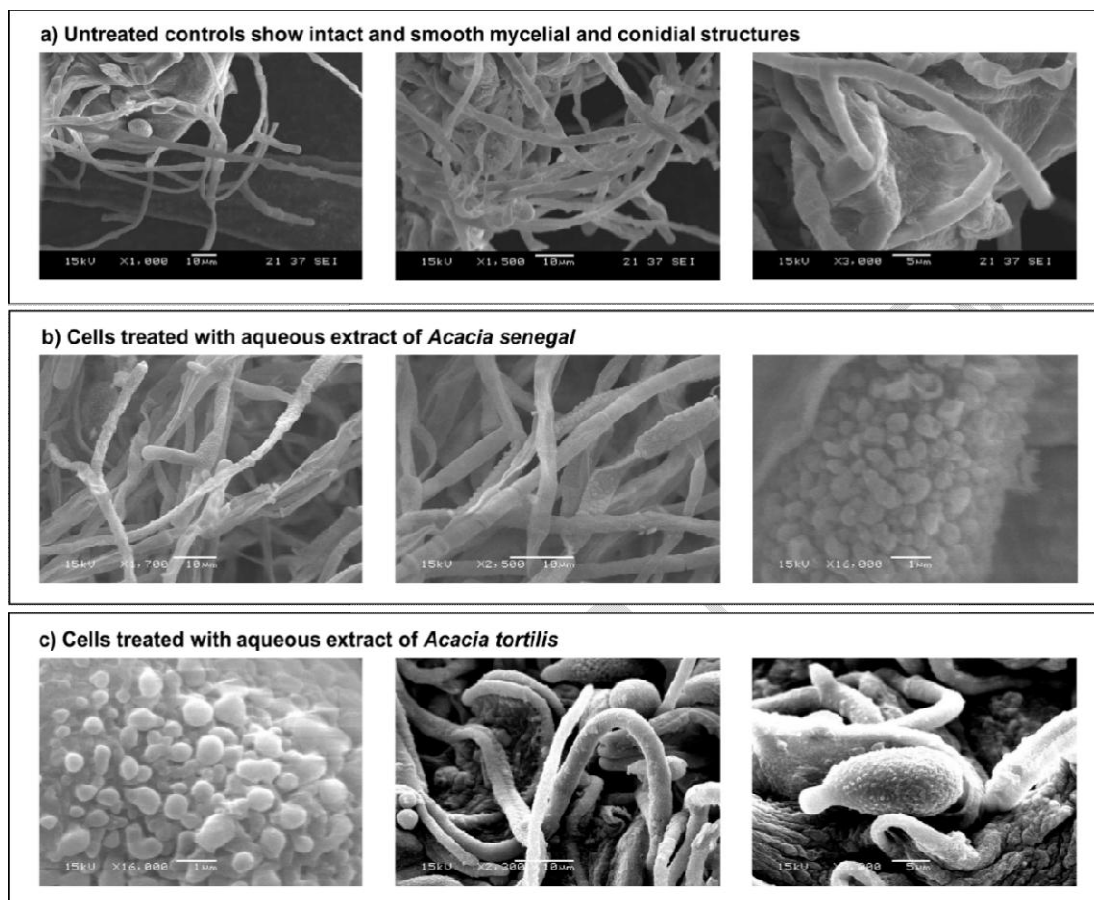
172 **Figure 2: *In vitro* activity of aqueous extract of *Acacia tortilis* on three**  
173 **phytopathogenic fungi; a) *A. alternata*, b) *H. rostratum* and c) *F. solani***

### 174 3.2 Scanning electron microscopy

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

178 The untreated-biomass (control) of *A. alternata* had normal tubular hyphae and intact  
179 mycelial and conidial growth and absence of structural changes (Figure 3a). The SEM  
180 images of *A. alternata* treated with aqueous extract of *A. senegal* showed smooth external  
181 surface with no morphological changes (Figure 3b). However, plates treated with aqueous  
182 extract of *A. tortilis* showed deformation of cellular structure as indicated by shrunken curly

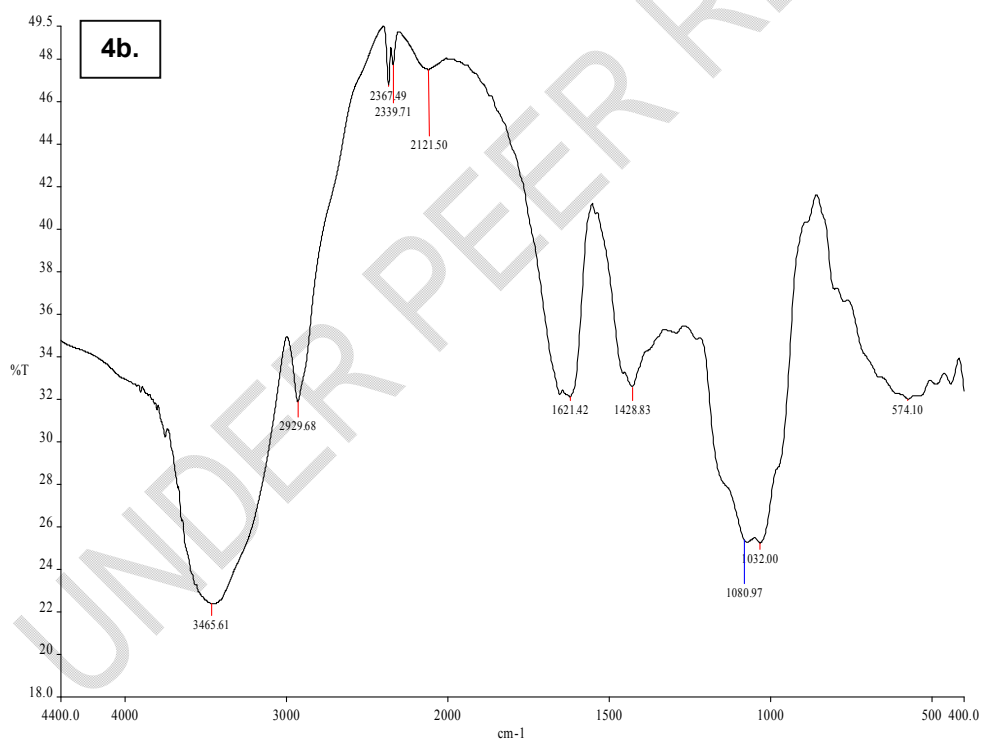
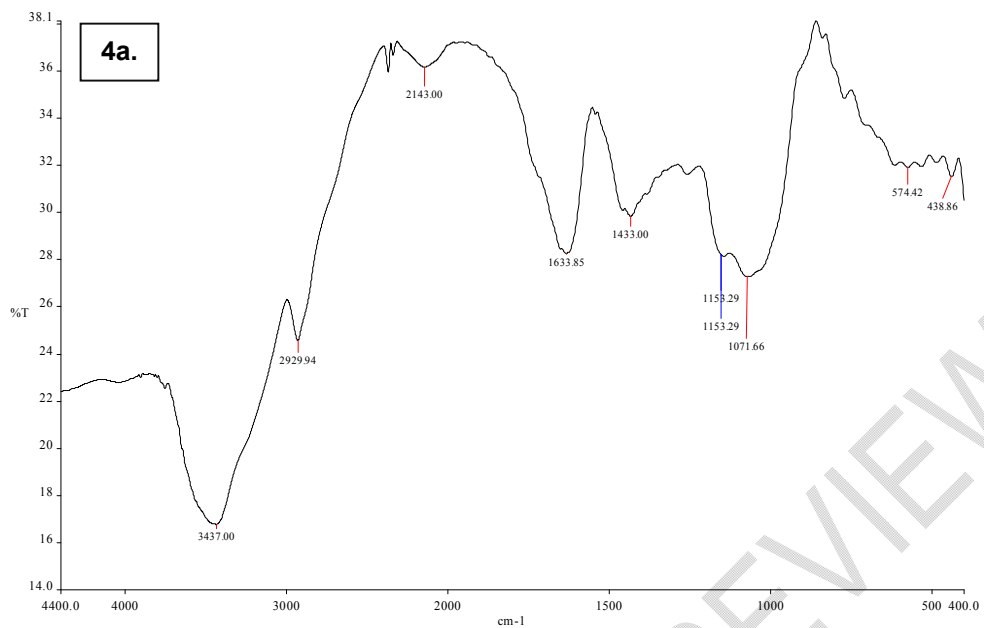
183 hyphae of variable sizes, deformed and wrinkled external surfaces, and compressed conidia  
184 (Figure 3c).  
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187 **Figure 3: Scanning electron microphotographs of *Alternaria alternata*; a)** Untreated  
188 controls show intact and smooth mycelial and conidial structures, **b)** Cells treated with  
189 aqueous extract of *Acacia senegal*, and **c)** Cells treated with aqueous extract of *Acacia*  
190 *tortilis*

### 191 3.3 Fourier-transform infrared spectroscopic analysis of the aqueous extracts

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



**Figure 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*.**

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207 **4. DISCUSSION**

208 Plant diseases caused due to fungiform impose significant damage to many economic crops  
209 worldwide. Synthetic fungicides are the first-line defense for protecting the plants against  
210 fungal infections. However, commercially available fungicidal agents are toxic and produce  
211 undesirable effects on other organisms, soil, plants, and water [41]. Moreover, the  
212 development of resistance of pathogenic fungi towards the synthetic fungicides has been a  
213 matter of great concern. Plant-derived antifungal agents represent a vast untapped source  
214 with tremendous potential.

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

221 The arid and semi-arid regions of the Middle East is a rich source of many medicinal plants  
222 [1]. Gum acacia is one of the most important medicinal plants available in the entire region. It  
223 contains various primary and secondary metabolic constituents (alkaloids, catechins,  
224 chalcones, flavones, flavonoids, polyphenols, and tannins) which have been traditionally  
225 used for the treatment of various plant and human diseases [15]. Two different species of  
226 gum acacia (*A. senegal* and *A. tortilis*) selected based on their traditional use [42]. Three  
227 different concentrations (1%, 2.5%, and 5%) of the aqueous extracts of both plants were  
228 screened for their in vitro activity against three important pathogenic plant fungi (*A.*  
229 *alternaria*, *H. rostratum*, and *F. solani*). The extract of *A. senegal* showed no effect on the  
230 growth of *A. alternaria* at all the three concentrations tested (1%, 2.5%, and 5%) whereas, it  
231 showed very mild activity on *H. rostratum* and *F. solani* at 2.5% and 5% concentrations.  
232 Aqueous extract of *A. tortilis* exhibited moderate activity against *A. alternata* at 5%  
233 concentration, and mild to moderate activity against *H. rostratum* and *F. solani*. Although  
234 both Acacia species showed antifungal activity, the aqueous extract of *A. tortilis* showed  
235 relatively higher potency in inhibiting the mycelial growth of all the three tested fungal strains.  
236 Limited effectiveness of aqueous extract of *A. senegal* against *A. alternaria* and the least  
237 activity against *H. rostratum* and *F. solani* could be partially due to incomplete extraction of  
238 the active principles [31]. Other factors include solubility, pH, volatility, diffusion  
239 characteristics in the growth medium, and fungal strains [32, 33].

240 Furthermore, SEM and FTIR studies were conducted to characterize the ultrastructural  
241 changes in the mycelia and identify the functional groups in the plant extracts respectively.  
242 SEM studies of the cells treated with aqueous extract of *A. tortilis* showed a deleterious  
243 effect on mycelial and conidial structures. Results from the FTIR analysis revealed the  
244 presence of several functional groups (alcohols, carboxylic acids, alkynes, amides, alkanes,  
245 alkyl amines, halogen, and cycloalkanes groups) that can act alone or in synergy, as  
246 demonstrated by other studies [5, 34-36]. As this is the first study to investigate the  
247 antifungal activity of crude aqueous extracts of *A. senegal* and *A. tortilis* against *A.*  
248 *alternaria*, *H. rostratum* and *F. solani*, formal comparison of the data obtained in this study is  
249 not possible. However, the results of antifungal activity of the *Acacia* species assessed in  
250 this study are similar to those reported in previous studies [16, 37]. In the study by Baig et al.  
251 aqueous extracts of *A. nilotica* was found to exhibit moderate activity against *Aspergillus*  
252 *flavus* and *Aspergillus niger*. The antifungal activity increased as the concentration increased  
253 from 10% to 25% [37]. Similarly, in a study by Al-Huqail et al. the aqueous extract of *A.*  
254 *saligna* (Labill) inhibited the growth of *Penicillium chrysogenum*. Furthermore, the  
255 percentage inhibition of the fungal mycelium increased as the extract concentration

256 increased [16]. The antifungal activity exhibited by both *A. senegal* and *A. tortilis* may be  
257 attributed to the presence of numerous phytoconstituents such as polyphenols, and  
258 flavonoid compounds which are reported to be abundantly present among *Acacia* species  
259 [16, 38-40].

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## 261 **5. CONCLUSION**

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

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## 273 **COMPETING INTERESTS**

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275 The author declare no conflict of interests.

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299 [role-of-Gum-Acacia-in-the-food-industry-Technical-paper-ENG-BD-1.pdf](https://www.allandetrobert.com/wp-content/uploads/2017/07/INFO_The-role-of-Gum-Acacia-in-the-food-industry-Technical-paper-ENG-BD-1.pdf) (accessed  
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