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FULL LENGTH ARTICLE

Preliminary phytochemical screening, plant growth inhibition and antimicrobial activity studies of *Faidherbia albida* legume extracts

Abeer M. Ismail [a], Eman A. Mohamed [a], Marwa R. Marghany [a], Fatma F. Abdel-Motaal [b], Ibrahim B. Abdel-Farid [b], Magdi A. El-Sayed [a,b,*]

[a] *Unit of Environmental Studies and Development, Aswan University, Aswan 81528, Egypt*
[b] *Botany Department, Faculty of Science, Aswan University, Aswan 81528, Egypt*

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Abstract Phytochemical analysis of the methanolic and aqueous extracts of *Faidherbia albida* legumes indicated the presence of terpenes, cardiac glycosides, monosaccharides and carbohydrates type of compounds in both extracts. While alkaloids and saponins were found in aqueous extract only, flavonoids were found to be absent in both extracts. The aqueous and methanolic extracts exhibited a potent growth stimulation effect. Inhibition of both the rootlet and shoot showed a dose dependent response. Aqueous extract has a greater inhibitory effect on rootlet growth than shoot growth. The methanolic extract has a greater inhibitory effect than the aqueous extract. Both extracts and some fractions were tested against three pathogenic bacterial species; *Staphylococcus aureus*, *Escherichia coli* and *Shigella dysenteriae*, also tested against three pathogenic fungal species; *Fusarium oxysporum*, *Alternaria alternate*, and *Aspergillus niger*. Most of the plant extracts stimulate the studied fungal growth specially the aqueous extract. Meanwhile it shows interesting results by inhibiting the growth of the studied pathogenic bacterial species with most extracts and fractions.

1. Introduction

Faidherbia albida previously known as *Acacia albida* (Del) is of the family Mimosoideae (Fabaceae). It is native to Southwest Africa, through West, North Africa to Egypt and East Africa. Common names include winter thorn and apple-ring acacia. The Hausa people of northern Nigeria call it 'Gawo' while in Fulfuldes it is called 'Chayski'. Phytochemical studies reveal that plants in this family contain tannins (Barry and McNabb, 1999) which account for their use in the making of dyes. In addition to this, (Tijani et al., 2008) reported the

* Corresponding author at: Unit of Environmental Studies and Development, Aswan University, Aswan 81528, Egypt. Tel./fax: +20 973480448.
E-mail address: magradi2000@yahoo.com (M.A. El-Sayed).
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presence of alkaloids and saponins in the stem bark extract of *F. albida*. In folkloric medicine, it is used to treat fevers by the Masai people of Kenya as well as in the treatement of diarrhoea in Tanganyika (Irvine, 1961). These activities have been reported by (Tijani et al., 2009). It is also used in treatement of colds and haemorrhage. A liniment, made by steeping the bark, is used for bathing and massage in pneumonia. The bark infusion is used in cases of difficult delivery, and is used as a febrifuge for cough (Irvine, 1961). (Kubmarawa et al., 2007) reported the antimicrobial activity of *F. albida* against *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Salmonella typhi*. In northern Nigeria, especially among the cattle rearing nomads, a decoction of the stem bark is taken orally for the management of the sleeping sickness (Tijani et al., 2008).

No previous study was made on the *F. albida* legumes, this study was made to evaluate the methanolic and aqueous extracts on the growth inhibition of the wheat root and shoot and their antimicrobial effects, besides screening the most important compound classes for further studies.

2. Materials and methods

2.1. Plant material

F. albida legumes were collected from the Botanical Desert Garden, at the Aswan University in March 2011. A voucher specimen was deposed at the Department of Botany, Faculty of Science, Aswan University.

2.2. Extract preparation

The collected legumes were taken to the Unit of Environmental Studies and Development (UESD), Aswan University for the analysis. The legumes were collected dry, deseeded and crushed to powder. The crude powder (107.97 g, 105.18 g) for methanol and aqueous extract respectively, was extracted by the cold extraction method, with methanol and water, for three days at room temperature. The methanolic and aqueous extracts were placed in a rotatory evaporator to eliminate the solvent, to obtain a dark reddish residue of 66.25 g with the methanol extract and 89.24 g with the aqueous extract. The methanolic residue was prefractionated by Column Chromatography (CC), (6×120 cm) on silica gel eluting with *n*-hexane followed by a gradient of *n*-hexane–CH_2Cl_2 up to 100% CH_2Cl_2 (25%, 50%, 75% and 100% CH_2Cl_2) and CH_2Cl_2–MeOH up to 100% MeOH (25%, 50%, 75% and 100% MeOH). Meanwhile, the aqueous extract was prefractionated by CC (6×120 cm) on silica gel eluting with CH_2Cl_2 followed by a gradient of CH_2Cl_2–MeOH up to 100% MeOH and MeOH–H_2O Up to 100% H_2O.

2.3. Phytochemical screening

2.3.1. Test for terpenes

To 5 ml of the extract add 2 ml of chloroform and 3 ml of H_2SO_4 conc., formation of a reddish brown ring confirms the presence of terpenes (Obianime and Uche, 2008).

2.3.2. Test for flavonoids

A few drops of concentrated hydrochloric acid were added to a small amount of the extracts of the plant material. Immediate development of a red colour was taken as an indication of the presence of flavonoids (Shahid-Ud-Duaula and Anwarul, 2009).

2.3.3. Test for saponins

Frothing test: Exactly 0.5 g of the extract was dissolved in distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for saponins (Adegoke et al., 2010).

2.3.4. Test for steroids

Liebermann–Burchard reaction: Added 2 ml of acetic anhydride and 2 ml conc. H_2SO_4 to 5 ml of the extract. Change of colour from violet to blue confirms the presence of steroids (Boxi et al., 2010).

2.3.5. Test for cardiac glycosides

Added 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution and 1 ml of conc H_2SO_4. Appearance of a brown ring indicates the presence of cardiac glycosides (Obianime and Uche, 2008).

2.3.6. Test for proteins

Biuret test: Added 4% of NaOH and few drops of 1% $CuSO_4$ solution to 3 ml of the extract. Formation of violet or pink colour indicates the presence of proteins (Boxi et al., 2010).

2.3.7. Test for carbohydrates

2.3.7.1. Monosaccharide. Barfoed's test: Mix equal volumes of Barfoed's reagent and the extract solution. Heated for 1–2 min in a boiling water bath and cooled. Red colour wasobserved (Boxi et al., 2010).

2.3.7.2. Test for reducing sugars. Fehling test: Mixed 1 ml of Fehling's A and Fehling's B solutions, boiled for one minute. Added equal volume of test solution. Heated in a boiling water bath for 5–10 min. First a yellow then a brick red ppt. was observed (Boxi et al., 2010).

2.3.7.3. Test for carbohydrates. Molisch test: To 2–3 ml of the aqueous extract, add two drops of alpha napthol solution in alcohol, shake and add conc. H_2SO_4 from the sides of test tube. Violet ring is formed (Boxi et al., 2010).

2.3.8. Test for tannins and phenolic compounds

Red coloured solution with acetic acid indicates the presence of tannins and phenolic compounds (Boxi et al., 2010).

2.3.9. Test for alkaloids

0.5 g of the extract was stirred with 5 ml of the 1% aqueous HCl acid on a steam bath. A few drops of dragendorff's reagent were used to treat 1 ml of the filtrate. Turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids (Adegoke et al., 2010).

2.4. Allelopathic potential of F. albida aqueous and methanolic extracts on wheat

2.4.1. In vitro seed germination and seedlings growth in Petri dishes bioassay

Seeds of wheat were washed thoroughly under running tap water for half an hour and surface sterilized for 30 s with

70% ethyl alcohol for one minute followed by shaking in 5% solution of commercial sodium hypochlorite (5%) for 5 min, the seeds were then rinsed several times with sterilized distilled water. 10 seeds were placed on sterilized filter paper in 11-cm Petri dishes. In every Petri dish 6 ml of the extract was added at the time of germination. Sterilized distilled water was used as control. Three replicates were prepared for each concentration (0, 10, 25, 50, 75 and 100 mg). Petri dishes were placed in an incubator at 25 °C ± 2 with 14/10 h of light/darkness. Seeds were considered as germinated, when the radicle protrusion was visible to the naked eye (El-Khatib and Abd-Elaah, 1998). Petri plates were monitored daily for seed germination and the measurements of shoot and root lengths were recorded at the sixth day from germination for both extracts. Data presented for seedling growth (root and shoot length) were based on all the seedlings from replicates of each treatment.

2.4.2. Antimicrobial activity test

The antimicrobial activity test was performed at the Unit of Environmental Studies and Development. The crude extracts (aqueous and methanolic) and their fractions (50% CH_2Cl_2, 100% CH_2Cl_2 and 25% MeOH of methanolic extract. And 30% MeOH, 50% MeOH and 50% H_2O of aqueous extract) were applied to the biological activity.

2.4.3. The biological activity of F. albida legumes with three pathogenic bacteria

This activity was assayed with three bacterial species, *Staphylococcus aureus*, *Shigella dysenteriae* and *Escherichia coli* using filter paper discs (6 mm diameter), placed on nutritive agar surface. Suspension of bacterial cells (1 ml) was mixed with the nutrient agar media until it solidifies and then they were the paper discs were applied. Each disc contained 1 mg of each fraction. The average of the inhibition zone around the disc has been calculated.

2.4.4. The biological activity of F. albida legumes with three pathogenic fungi

The biological properties of *F. albida* legume extracts were estimated with three mould species of *Fusarium oxysporum*, *Alternaria alternata* and *Aspergillus niger*. This activity was carried out using Czapek's agar medium in Petri dishes and mixed with each fraction by a final concentration (1 mg/plate) and then inoculated in the central zone with a fresh culture fungal disc of 3 mm diameter. All plates were incubated at 28 °C. The diameters of cultures were measured in control dishes and in the treated plates containing culture medium supplemented with variable fractions and the average growth rates were measured.

2.5. Statistical analysis

One way analysis of variance (ANOVA) (*F*-test) from Minitab version 12.21 was used to test the significant difference of all the data recorded in the study. Data are presented in the form of mean with standard deviation and considering p values < 0.05 as significant.

3. Results and discussion

3.1. Phytochemical tests

From phytochemical screening, we observed that the methanolic and aqueous extracts gave a positive result with the Molish test and the Barfoed test, which indicated the presence of monosaccharides and carbohydrates in both extracts. The Dragendorff's reagent failed to show the presence of alkaloids in the methanolic extract, but gave a positive result with the aqueous extract. The Frothing test would confirm the presence of saponins in the aqueous extract. Based on the general test for terpenes, indicates the presence of terpenes in both the extracts (Table 1). The ferric chloride test for flavonoids gave negative results in both extracts. Test for tannins and phenolic compounds gave positive results with the aqueous extract only. Bicurot test gave positive results, which indicates the presence of proteins in the methanolic extract. The test of cardiac glycosides, gave positive results in both extracts.

3.2. Allelopathic potential of F. albida aqueous and methanolic extracts on wheat

3.2.1. Effect of extracts on percentage of wheat seed germination

The aqueous and methanolic extracts of *F. albida* legumes significantly stimulate and increase the seed germination of wheat, and the increase was proportional to the concentration used along the experimental period from second to sixth day after germination, highest increase with the aqueous extract was noticed with the lowest concentrations (10% and 25%) and the highest concentration (100%). Two day treatment with the aqueous extract at 50% and 75% concentration suppressed germination to 90 and 93.33, respectively (Table 2). Meanwhile, increase with the methanolic extract was noticed at 25% and 75% concentrations. Suppression is observed with 10%, 50% and 100% at 83, 93 and 83, respectively after the second day (Table 2).

3.2.2. Effect of extracts on shoot and root growth of wheat

The effect of different concentrations of aqueous and methanolic extracts of *F. albida* legumes on shoot and root length was evaluated after six days of the treatment (Table 2). Data revealed that shoot and root length of wheat was significantly decreased at all concentrations of both extracts. The reduction was more prominent at the highest extract concentration.

In conclusion, this is the first study on the effect of different concentrations of aqueous and methanolic extracts of *F. albida* legumes indicating that the increase in seed germination in wheat was correlated with the dose of the extracts. The wheat seeds were more sensitive to the methanolic extract than the aqueous extract. The reduction of shoot and root lengths of wheat on exposure to different concentrations of both the extracts was reduced more dramatically with the methanolic extract. Root length of wheat is more sensitive to higher concentrations of the extracts than the shoot length, the methanolic extract was more suppressive than the aqueous extract. This may be attributed to different allelochemicals in both the extracts. Further studies are required.

Table 1 Results of phytochemical screening of methanol and aqueous extracts.

Sample	Methanol extract	Aqueous extract
Test for terpenes	+ + +	+ +
Test for flavonoids	–	–
Test for saponins	–	+ + +
Test for steroids	+ + +	–
Test for cardiac glycosides	+	+
Test for proteins	+	–
Test for carbohydrates		
Monosaccharides	+ + +	+ +
Reducing sugars	–	–
Carbohydrates	+	+
Test for tannins and phenolic compounds	–	+
Test for alkaloids	–	+

+ + +, High concentration; + +, moderate concentration; +, low concentration; –, absence.

3.2.3. Antimicrobial tests

3.2.3.1. The biological activity of F. albida legumes against three pathogenic bacteria. This study shows the antibacterial activity of *F. albida* legume extracts against 3 bacterial species, *E. coli*, *S. dysenteriae* and *S. aureus* using filter paper discs (6 mm diameter), placed on nutritive agar surface. *E. coli* is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination (Vogt and Dippold, 2005).

In this study, *E. coli* was highly inhibited using the fraction 50% H_2O from aqueous extract and the fraction 25% MeOH from methanol extract followed by 100% CH_2Cl_2, the lowest inhibition effect was recorded with aqueous plant extract and its fraction 30% MeOH (Fig. 1).

The second studied bacterium is *S. dysenteriae* which is a genus of Gram-negative, nonspore forming, non-motile and rod-shaped bacteria. *Shigella* causes dysentery that results in the destruction of the epithelial cells of the intestinal mucosa. Some strains produce enterotoxin and shiga toxin, similar to the verotoxin of *E. coli* O157:H7 (Hale and Keusch, 1996).

F. albida fraction of 50% MeOH from the methanol extract recorded a high inhibition zone compared with other fractions followed by the methanol crude extract, on the other hand, *S. dysenteriae* was very resistant to 50% H_2O of the aqueous extracts (Fig. 1).

S. aureus MRSA is a member of the *Firmicutes*, and is frequently found in the human respiratory tract and on the skin. It is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning. The emergence of antibiotic-resistant forms of pathogenic *S. aureus* (e.g. MRSA) is a worldwide problem in clinical medicine. Therefore, it was interesting to inhibit this type of bacteria by *F. albida* extracts specially the fraction of 50% MeOH from the aqueous extract and 100% CH_2Cl_2 from the methanol crude extract (Fig. 1).

Table 2 Effect of *aqueous* and methanolic extracts on the germination and seedling growth of wheat.

F. albida extract	Aqueous extract								Methanol extract							
	Percentage seed germination per day					Shoot length (cm)	Root length (cm)		Percentage seed germination per day					Shoot length (cm)	Root length (cm)	
Concentration	2nd	3rd	4th	5th	6th	6th			2nd	3rd	4th	5th	6th	6th		
Control	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	6.677 ± 1.571	7.223 ± 1.273		100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	6.66 ± 1.05	8.31 ± 2.014	
10%	100 ± 0 ***	100 ± 0 ***	100 ± 0 ***	100 ± 0 ***	100 ± 0 ***	6.13 ± 1.013	6.637 ± 0.165		83.33 ± 11.55	93.33 ± 5.77	93.33 ± 5.77	93.33 ± 5.77	93.33 ± 5.77	6.47 ± 0.48	6.19 ± 2.372	
25%	100 ± 0 ***	100 ± 0 ***	100 ± 0 ***	100 ± 0 ***	100 ± 0 ***	6.147 ± 0.935	5.743 ± 0.852		86.87 ± 15.28	96.67 ± 5.77	100 ± 0 ***	100 ± 0 ***	100 ± 0 ***	9.59 ± 0.45 *	6.56 ± 1.117	
50%	90 ± 10	100 ± 0 ***	100 ± 0 ***	100 ± 0 ***	100 ± 0 ***	5.337 ± 0.074	5.077 ± 0.938		93.33 ± 5.77	96.67 ± 5.77	96.67 ± 5.77	96.67 ± 5.77	96.67 ± 5.77	3.87 ± 0.33 *	4.38 ± 1.270 *	
75%	93.33 ± 5.71	96.69 ± 5.77	96.69 ± 5.77	96.69 ± 5.77	96.69 ± 5.77	5.540 ± 1.293	4.7033 ± 0.6121*		90 ± 0 ***	96.67 ± 5.77	100 ± 0 ***	100 ± 0 ***	100 ± 0 ***	2.54 ± 0.72 **	2.90 ± 0.665 *	
100%	100 ± 0 ***	100 ± 0 ***	100 ± 0 ***	100 ± 0 ***	100 ± 0 ***	3.190 ± 0.620 *	3.2367 ± 0.5501 **		83.33 ± 5.77 **	96.67 ± 5.77 **	96.67 ± 5.77 **	96.67 ± 5.77 **	96.67 ± 5.77 **	1.78 ± 1.51 *	2.87 ± 1.089 *	

* Significant at $p < 0.05$.
** Highly significant at $p < 0.0$.
*** Very highly significant at $p < 0.001$ and value after ± is the standard deviation.

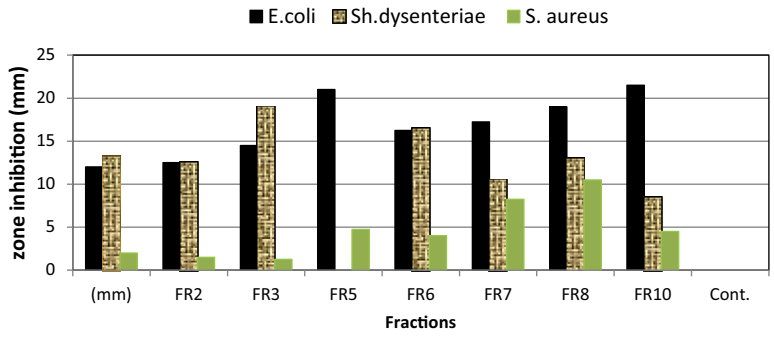

Figure 1 The antibacterial activity of *Faidherbia albida* legume extracts {1-Aqueous plant extract and its fractions 2–30% MeOH, 3–50% MeOH, 4–50% H_2O, 5-methanolic plant extract and its fractions 6–50% CH_2Cl_2, 7–100% CH_2Cl_2, 8–25% MeOH and control (contains solvent only)} against 3 bacterial species, *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus*.

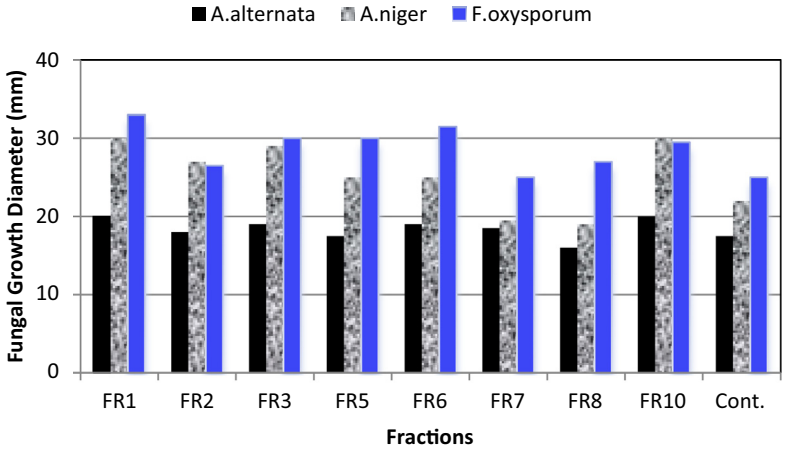

Figure 2 The growth rate of, *Alternaria alternata*, *Aspergillus niger*, *Fusarium oxysporum* cultivated on Czapek's agar medium supplemented with 1 mg *F. albida* legume extracts {1-Aqueous plant extract and its fractions 2–30% MeOH, 3–50% MeOH, 4–50% H_2O, 5-methanolic plant extract and its fractions 6–50% CH_2Cl_2, 7–100% CH_2Cl_2, 8–25% MeOH compared with the control (contain solvent only)}.

3.3. The biological activity of F. albida legumes against three pathogenic fungi

The biological activity was estimated using 3 fungal species cultivated on C'zapekes agar medium (control plates) and on the same medium supplemented with 1 mg of *F. albida* extracts (experimental part). The diameters of the colonies and the growth rates were measured (Fig. 2).

A. alternata has been recorded causing leaf spot and other diseases on over 380 host species. It is an opportunistic pathogen on numerous hosts causing leaf spots, rots and blights on many plant parts. It can also cause upper respiratory tract infections (Wiest et al., 1987) and asthma in people with sensitivity. Our results exhibit that almost all fractions stimulate the growth of *A. alternata* except the fraction 100% CH_2Cl_2 from the methanol extract which slightly inhibits the growth compared with the control. It was interesting to find that the fraction 25% MeOH and 100% CH_2Cl_2 stimulate the sporulation of *A. alternata* at the third day of growth, while 50% CH_2Cl_2 and the aqueous plant extract (crude) stimulated less sporulation than the above fractions but was better than the control (Fig. 2).

A. niger is a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called black mould on certain fruits and vegetables and is a common contaminant of food. *A. niger* is less likely to cause human disease than some other *Aspergillus* species, but, if large amounts of spores are inhaled, a serious lung disease, aspergillosis can occur. In this study, most of the extracts and fractions also stimulate the growth of *A. niger* except 50% CH_2Cl_2 and 100% CH_2Cl_2. The fraction 25% MeOH and aqueous plant extract (crude) were highly stimulators of the growth and sporulation.

F. oxysporum is infamous for causing a condition called *Fusarium* wilt (Snyder and Hansen, 1940). Similar to the above two fungal species, almost all fractions stimulate the growth of *F. oxysporum* specially the aqueous plant extract (crude) while 50% CH_2Cl_2 had the same growth rate as the control.

In conclusion, most of the plant extracts stimulate the studied fungal growth specially the aqueous plant extract (crude). These results may be revered to the high sugar concentration especially in the aqueous one. Therefore, we suggested using the aqueous extract of *F. albida* legume in the fungal media to stimulate the growth and sporulation of the fungal species. The most interesting of these results was that *F. albida* legume

extracts were able to inhibit the growth of dangerous bacterial species like *E. coli*, *S. dysenteriae*, *S. aureus*.

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