



## Phytochemical, antioxidant and antimicrobial activities of *Acacia etbaica* Schweinf aerial parts

Ahmed Abdalla<sup>\*1</sup>, Abdelazim A. Ahmed<sup>2</sup>

<sup>1</sup>Department of Botany, Faculty of Science, University of Khartoum, Khartoum, Sudan

<sup>2</sup>Department of Biology, Faculty of Science, University of Al Baha, Saudi Arabia

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### Abstract

This study was aimed at evaluating the phytochemical components, antioxidant and antimicrobial activities of leaves, twigs and stem barks of *Acacia etbaica* (*Vachellia etbaica*). Results should that the highest yields were obtained from the chloroform extract (23.5%) followed by methanol (20%), petroleum ether (2.25%), and ethyl acetate extract (1.7%) with most of them being sticky in texture. Leaves had a high amount of polyphenols (140.33mg GAE/L), tannin (8.13mg TAE/L), flavonoids (0.51%), and alkaloids (0.11%) as compared with the other parts of the plant. leaves had a high amount of saponins (0.74%). Stembark and leaf extracts showed high antioxidant activities compared with vitamin C. Chloroform and methanol extracts of all plant parts had potent antibacterial and antifungal activities when compared with extract of petroleum ether and ethyl acetate extracts. Petroleum ether extract of all parts strongly inhibited the growth of *B. subtilis* with an inhibition zone ranging from 23.3-26.2mm.

**\*Corresponding Author:** Ahmed Abdalla ✉ [ahmdawad90@gmail.com](mailto:ahmdawad90@gmail.com)

## Introduction

Plants were an important source of drugs in ancient times, although there are many new approaches to drug discovery (Veeresham, 2012). Novel, effective and affordable drugs are needed to combat infectious diseases especially in developing countries of the world; where up to one-half of deaths are due to infectious diseases (Awouafack *et al.*, 2013; Srivastava *et al.*, 2013). About 10,000 phytochemicals compounds such as tannins, flavones, triterpenoids, steroids, saponins and alkaloids have been identified, but a large percentage remains unknown (Zhang *et al.*, 2015). These secondary metabolites played critical roles in modern drug development, especially for antibacterial, antitumor, antimalarial, anticancer and anti tyrosinaemia agents (Veeresham, 2012). However, several phytochemicals are closely connected with antioxidant activity against many human diseases (Barbosa *et al.*, 2013; Zhang *et al.*, 2015). On the other hand, many researchers work for access to safe and effective antimicrobial compounds driven from plant. *Acacia* species possess bioactive compounds that exhibit physiological activity against bacteria and other microorganisms. *Acacias* belong to the family Leguminosae, firstly described by Linnaeus in 1773. It is estimated that there are roughly 1380 species of *Acacia* worldwide (Maslin *et al.*, 2003). Besides forage, timber, gum and food sources, *acacia* species were used in traditional and folklore medicine in many countries for wound healing, antiseptic, skin disease, gonorrhoea, stomach pain, Cough, Pneumonia and Cold and flu; Pharyngitis (Mattana *et al.*, 2010; Adiamo *et al.*, 2020; Yagi S and Yagi A, 2021). Furthermore, bark, flowers, leaves, pods, seeds and roots contain bioactive compounds such as phenolics, tannins, alkaloids and flavonoids, these compounds have positive effects against various chronic diseases (Adiamo *et al.*, 2020). Generally, phenolic compounds in the *Acacia* species possessed many biological activities including antiseptic, antifungal and bactericidal properties (Gill, 1999). *Acacia etbaica* Schweinf is a shrub tree and one of the most widespread plants. Mainly found in Sudan and Somalia, in Sudan common in Eastern Sudan and native people is used as a folk medicine for the

treatment of stomach pain, gonorrhoea and skin diseases (Yayneshet *et al.*, 2008; Galib *et al.*, 2017). Also, the leaves crush and mix with the latex of *Euphorbia abyssinica* and rub the paste on the affected part to treat swelling, a mixture made of stems clove to treat ring worm and also used to treat haemorrhoids by burn and place it on the affected part (Teklay *et al.*, 2013). This action may be attributed to the bioactive compounds found in this promising tree. Generally, the literature data on their antioxidant and antimicrobial activities of *Acacia etbaica* are scarce. Hence, the aim of this study was to evaluate the phytochemical components, antioxidant, and antimicrobial activity of this plant.

## Material and Methods

### *Plant materials*

The leaves, stembarks, and small twigs of *Acacia etbaica* were collected from Erkowit, eastern Sudan. Voucher specimens have been deposited at the Herbarium of the Department of Botany, Faculty of Science, University of Khartoum as reference materials. The plant materials were shade dried then coarsely powdered separately in a hammer mill (Abdalla and Ahmed, 2023).

### *Preparation of plant extracts*

Twenty grams of the dried powdered plant materials (the leaves, stembarks, and twigs) were defatted with petroleum ether and extracted successively by maceration in cold petroleum ether using a shaker apparatus at room temperature then the residual of the powder plant materials was extracted again with chloroform, ethyl acetate and methanol in order. Each extract was filtered and concentrated under reduced pressure using rotary evaporator. Finally, the extracts were air dried under room temperature and their yield, colour, and texture were determined.

### *Phytochemical analysis*

A preliminary phytochemical screening in different plant parts were evaluated by standard qualitative methods described by Sofowara (1993) and Harborne (1992). Total polyphenolic, flavonoids, tannins, alkaloid and saponin content was determined according to the

methods described by Dewanto and coworkers (2002) and Obadoni and Ochuko (2001), respectively.

#### *Antioxidant activity*

##### *DPPH radical-scavenging test*

Antioxidant activity of the extracts was estimated using 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) *in vitro* method described by Villano and coworkers (2007). Ascorbic acid was used as a reference antioxidant compound.

#### *Antimicrobial assay*

The bacterial cultures used were *Bacillus subtilis* ATCC 25924, *Escherichia coli* ATCC 25923, *Klebsiella pneumonia* ATCC 35657, *Salmonella typhi* ATCC 19430 and *Proteus vulgaris* ATCC 19430. *Aspergillus niger* ATCC 9763 and *Candida albicans* ATCC 7596 fungi were used. The media used for antibacterial tests were nutrient broth and Mueller Hinton agar and *Sabouraud dextrose* agar were used for antifungal tests.

#### *Antibacterial assay*

The antibacterial and antifungal assay for plant extract was also conducted using disc diffusion method as described by Perez *et al.*, 1990, with some minor modifications. Sterile filter paper discs (Whatman No.1, 0.5mm in diameter) were impregnated with 100µL of the solution of crude extract (5mg/ ml) dissolved in dimethyl sulfoxide (DMSO). Tetracycline (20mg/ml) was used as a positive control. A bacterial suspension was prepared and added to the sterilized medium before solidification. The media with bacteria were poured into sterilized Petri dishes under aseptic condition. Extract discs were then placed on the agar plates. Three replicates were carried out for each extract against each of the test organisms. After incubation period for 24 h at 37°C, the inhibition zones around the discs were measured and recorded.

#### *Antifungal assay*

The same method as for bacteria was adopted but instead of nutrient broth, *Sabouraud dextrose* was used. The molten medium was inoculated with the

specific organism and the medium was incubated at 25°C for two days for *Candida albicans* and *Aspergillus niger*.

#### *Statistical analysis*

The obtained results for each assay were expressed as the mean  $\pm$  standard deviation (SD). Significant differences among the groups were determined by one-way analysis of variance (ANOVA) and Duncan test as post-hoc,  $P < 0.05$  using SPSS 16 software.

### **Result and Discussion**

Table 1 (A-D) showed the physical appearances and percentage yields of various solvents extracts. The obtained total yields from petroleum ether, chloroform, ethyl acetate and methanol extracts were 2.25, 23.50, 1.70 and 20%, respectively. Leave extracts contained a high amount of phytochemicals compared with the other parts. The textures of the yielded compounds of all plant parts were sticky except the methanol extract of the stem-bark, was powder. The colour of the extracted compounds was either dark green or greenish-white or brown or yellowish. This variation in texture and colour could be due to the chemical and physical nature of the extracted phytochemical compounds. Table 2. illustrates the qualitative analysis of the phytochemical components in leaves, twigs and stem-bark of the plant. The studied plant parts were rich in the major secondary metabolites. Phenol, tannins, cyanogenic glycosides, cardiac glycosides, saponins, sterols, triterpenes and flavonoids were detected in all plant parts. Alkaloids, anthraquinones and coumarins were detected only in leaves not found in twigs. Several chemical and biological studies have been carried out in *Acacia* species in the world and revealed that this genus is an important producer of secondary metabolites with significant biological activity such anticancer, anti-inflammatory, antimalarial and anti-spasmodic activity (Seigler, 2003, Saeedi *et al.*, 2020). The total polyphenolic, flavonoids, tannins, alkaloids and saponin contents of the leaves and stembarks were evaluated and results are presented in Table 3. leaves and stembarks contained nearly equal amount of total polyphenols

(140.33mg GAE/L). The twigs were significantly different from that of the leaves and the stem barks (88.67mg GAE/L). Generally, phenolic compounds are commonly found in plants, and they have been reported to have multiple biological effects, including antioxidant activity (Ali *et al.*, 2022). Previous studies on *Acacia species* have reported different amount of phenolic content (Almahy and Nasir, 2011; Anand and Mohan, 2014; Foyzun *et al.*, 2022).

**Table 1.** (A-D) Yields and physical characterization of the extracted compounds.

(A) Petroleum ether extract

Plant Part	Weight (g/20 g)	Yield%	Colour	Texture
Leaves	0.29	1.45	Dark green	Sticky
Twigs	0.11	0.55	Brown	Sticky
Stem-bark	0.05	0.25	Yellowish	Sticky
Total yields <b>2.25</b>				

(B) Chloroform extract

Plant Part	Weight (g/20 g)	Yield%	Colour	Texture
Leaves	2.32	11.6	Dark green	Sticky
Twigs	1.28	6.4	Brown	Sticky
Stem-bark	1.10	5.5	Brown	Sticky
Total yields <b>23.5</b>				

(C) Ethyl acetate extract

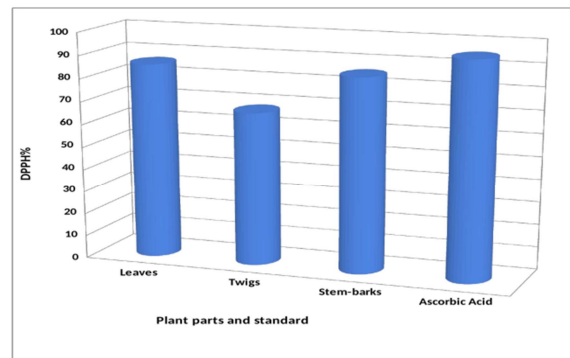
Plant Part	Weight (g/20 g)	Yield%	Colour	Texture
Leaves	0.22	1.1	Dark green	Sticky
Twigs	0.08	0.4	Dark green	Sticky
Stem-bark	0.04	0.2	Greenish- White powder	
Total yields <b>1.7</b>				

(D) Methanol extract

plant Part	Weight (g/20 g)	Yield%	Colour	Texture
Leaves	2.10	10.5	Dark green	Sticky
Twigs	1.1	5.5	Light green	Sticky
Stem-bark	0.80	4.0	Yellowish-Brown	Sticky
Total yields <b>20</b>				

All the studied plant parts were not rich in flavonoid contents. The detected amounts of flavonoids were 0.51, 0.32 and 0.44% in the leaves, twigs and stembarks, respectively. Flavonoids are low molecular weight polyphenolic substances based on the flavan nucleus and are plentiful in the heartwood of several *Acacia species*, belonging to the family Leguminosae (Clark-Lewis and Porter, 1972; Pietarinen *et al.*, 2005). The percentage tannin contents in each plant

part were 8.13, 2.73 and 3.80%, respectively. The amount of tannin content in the leaves was three times that of the twigs. It was significantly different from that of twigs and stem barks. Haroun and others, who studied twelve *Acacia species* and found that they contained more than the 10% tannin needed for commercial exploitation (Haroun *et al.*, 2013).



**Fig. 1.** Antioxidant activity of leaves, twigs, and stembarks.

The other species, such as *Acacia caesia* showed high activity against ten human pathogenic bacteria and fungi. Also, different parts of *Acacia nilotica* are known to possess astringent, antibacterial, insect repellent, antioxidant, antidiabetic, and antiviral properties (Bachaya *et al.*, 2009; Thambiraj and Paulsamy, 2015; Khaleel *et al.*, 2021).

**Table 2.** The major chemical classes in leaves, twigs, and stem barks of *Acacia etbaica*.

Chemical class	Leaves	Twigs	Stembarks
Alkaloids	+	-	-
Flavonoids	++	+	+
Anthraquinones	+	-	-
Triterpenes	++	+	+
Sterols	++	+	+
Saponins	+	++	+
Cardiac Glycosides	++	+	+
Coumarins	+	-	-
Tannins	+++	+	+
Cyanogenic Glycosides	++	+	+
Phenol	++	+	+

**key:** Indicates absence of the constituents, +: Low concentration, ++: Medium concentration, +++: High concentration.

Bark decoctions of this tree are used in African traditional medicine for the treatment of diarrhea, dysentery, respiratory ailments, sore throats, dry

coughs, children’s fevers and toothache. Also, the bark decoction can be used for eye complaints, as a nerve stimulant and aid for digestion. Ayurvedic medicine practices have declared that green pods and seeds of *acacia* species can provide the nutrients and therapeutic ingredients to prevent, mitigate and treat various diseases (Singh, Yadav, 2009). Antifungal activity of *Acacia nilotica* bark extract against *Candida albicans* is revealed by (Pai *et al.*, 2010).

**Table 3.** Total polyphenolic, flavonoids, tannins, alkaloid and saponin contents of different plant parts.

Plant part	Total polyphenolics (mg GAE/L)	Total Tannins (mg TAE/L)	Total Flavonoid (%)	Total Alkaloid (%)	Total Saponin (%)
Leaves	140.33± 0.57 <sup>a</sup>	8.13± 0.05 <sup>a</sup>	0.51± 0.01 <sup>a</sup>	0.11± 0.01 <sup>a</sup>	0.74 ± 0.01 <sup>a</sup>
Twigs	88.67± 1.15 <sup>b</sup>	2.73 ± 0.05 <sup>c</sup>	0.32 ± 0.01 <sup>c</sup>	0.03± 0.01 <sup>c</sup>	0.14 ± 0.01 <sup>c</sup>
Stembarks	140.33± 0.57 <sup>a</sup>	3.80± 0.01 <sup>b</sup>	0.44± 0.02 <sup>b</sup>	0.04± 0.01 <sup>b</sup>	0.21± 0.02 <sup>b</sup>

On the other hand, low levels of tannins in foliage can increase the utilization of crude protein by protecting it from digestion in the rumen thereby increasing the flow of essential amino acids to the small intestine for absorption (Waghorn, 1990).

It was observed that all parts contained low amount of alkaloids. Relatively, the leaves had a high amount of alkaloids (0.11%) compared with that of twigs (0.03%) and stem barks (0.04%). Alkaloids are known as organisms natural defense, also involved in protective function in animals and are used as medicine especially the steroidal alkaloids (Stevens *et al.*, 1992; Heinrich *et al.*, 2021).

Previous studies showed that alkaloids can be isolated from different parts of *acacia* spices (Sadgrove, 2022; Wang *et al.*, 2022). The saponin contents in leaves, twigs and stembarks were 0.51, 0.32 and 0.44%, respectively. Saponin is useful in medicine and pharmaceutical industry due to its foaming ability that produces frothy effects in the food industry (George *et al.*, 2002).

Also used in the manufacture of cosmetics, food additives and steroid saponin used as raw materials for the production of steroid drugs (Guclu-Ustundag and Mazza, 2007). Generally, *Acacia* species have potentials with synergism against phytopathogenic fungi and insect pests, they have anti-fertility effects on rats (Mwangengwa *et al.*, 2021; Rajagopal *et al.*, 2022). Fig. (1) shows the antioxidant activities of the methanol extracts of leaves, twigs, and stembarks. It was clear that all the extracts produced high scavenging activity compared with vitamin C as a standard.

The highest DPPH scavenging activity was observed in leaves extract (86%) followed by stembarks (85.3%), twigs (67.5%) while the scavenging activity of vitamin C was (64.5%). No significant differences between leaves extract and stembarks extract in their DPPH radical scavenging activity. In the light of urgency of newer natural antioxidant compounds to be discovered day by day with their improved biological activity, *Acacia* provides a valuable insight for such discoveries. However, in most of previous studies showed antioxidant properties in different types of plant materials (Agrawal *et al.*, 2010; El-toumy *et al.*, 2011; Ali, 2022).

Table (4) shows the antimicrobial assayed against five bacterial strains and two fungal strains. The antibacterial activities of leaves, twigs and stembarks were compared with antibiotic Tetracycline at a concentration of 20mg /ml. Petroleum ether extracts exerted high antibacterial activity against *B. subtilis* (23.3 ± 1.2 - 26.2 ± 2.9mm) and negative activity towards *K. pneumonia* and *C. albicans*.

Ethyl acetate extracts of the stembarks exhibited positive activity towards all tested organisms. In general, chloroform and methanol extracts had antimicrobial activity against all strains while ethyl acetate and petroleum ether extracts had either low or no activity. In contrast, Tadege and coworkers reported that the leaf extract of *acacia etbaica* showed antibacterial activity against selected Enterobacteriaceae (Tadege *et al.*, 2020).

**Table 4.** Inhibition zones (mm) of different extracts from *Acacia etbica* against five bacterial and two fungal strains.

Extracts	Plant parts	Inhibition zone (mm)						
		Bs	Ec	Kp	St	Pv	Ca	An
Petroleum ether	Leaves	26.2 ± 2.9	0	0	0	0	0	0
	Twigs	24.7 ± 2.9	0	0	10 ± 0.1	11.7 ± 2.9	0	0
	Stembarks	23.3 ± 1.2	10 ± 0.1	0	0	0	0	12 ± 0.1
	Tetracycline	30	26	29	17	16	21	23
Chloroform	Leaves	16 ± 1.8	20 ± 0.1	10.7 ± 1.2	19 ± 0.1	18.7 ± 1.2	21.4 ± 1.2	21 ± 1.8
	Twigs	17.4 ± 2.3	9 ± 0.2	9 ± 0.5	18.7 ± 0.6	19.7 ± 0.6	22.3 ± 2.6	15 ± 0.1
	Stembarks	19.7 ± 0.6	20.3 ± 0.6	15.7 ± 0.6	10 ± 0.2	17.3 ± 2.3	17 ± 1.8	18.7 ± 2.3
	Tetracycline	30	26	29	17	16	21	23
Ethyl acetate	Leaves	0	13 ± 1.8	0	19.4 ± 1.2	18.4 ± 1.6	13.7 ± 2.3	15.7 ± 1.2
	Twigs	0	0	0	18.7 ± 0.2	0	0	0
	Stembarks	26 ± 1.8	18.7 ± 1.2	15.7 ± 0.6	16 ± 1.8	18.4 ± 0.6	13 ± 1.8	11 ± 0.1
	Tetracycline	30	26	29	17	16	21	23
Methanol	Leaves	18.4 ± 1.2	16.7 ± 1.2	12 ± 1.2	11 ± 1.5	20.4 ± 0.6	14 ± 1.8	20 ± 2
	Twigs	18.4 ± 0.6	11.7 ± 2.9	11 ± 1.7	19.7 ± 0.6	18.4 ± 0.6	20 ± 1	15.7 ± 0.6
	Stembarks	19.7 ± 1.6	18.4 ± 2.9	14.7 ± 4.2	13 ± 5.2	20.4 ± 0.6	20.7 ± 1.2	18.7 ± 1.2
	Tetracycline	30	26	29	17	16	21	23

Bs- *Bacillus subtilis*, Ec- *Escherichia coli*- Kp, *Klebsiella pneumonia*, St- *Salmonella typhi*, Pv- *Proteus vulgaris*, An- *Aspergillus niger*, Ca- *Candida albicans*

### Conclusion

Phytoconstituents such as glycosides, saponins, flavonoids, coumarins, tannins, triterpenes and sterols were presented; these compounds are known to have curative activity against several pathogens and therefore can be suggested for the treatment of different diseases. The plant parts under the study were rich in bioactive compounds and can be used as good source of antioxidant and antimicrobial agents. However, from this study it can be concluded that the *Acacia etbaica* may serve as a new potential source of chemical components and medicines due to the presence of most of the major secondary metabolites in all the plant parts. Last, this study provides scientific insight to further determine the phytochemical, antimicrobial and antioxidant principles in the one of the promising tree in Sudan.

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