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Antibacterial Activity of *Acacia nilotica* Flower, Bark and Root Extracts

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Abstract. *Acacia nilotica* (*A. nilotica*) is an important medicinal plant and used for various diseases including diarrhea, dysentery, leprosy, cancers, ulcer, diabetes, diuretic, intestinal pains, cold, congestion, coughs, fever, hemorrhages and leucorrhea. Keeping in view the medicinal importance of this plant, flower, bark and root chloroform ($CHCl_3$), n-hexane (n-Hex), ethyl acetate (ETAC) and methanol (MeOH), the twelve extracts were prepared and evaluated for antibacterial potential by well diffusion method. Our results confirmed the susceptibility of bacteria i.e. *Staphylococcus aureus (S. aureus), Bacillus cereus (B. cereus), Escherichia coli (E. coli)* and *Salmonella enterica (S. enterica)* against prepared extracts in concentration dependent manners with zone of inhibition (ZoI) ranging from 0.31-5.22 mm at 40 mg/mL. Interestingly, all prepared extracts showed antibacterial activity at both tested concentrations (20 and 40 mg/mL), except flower $CHCl_3$ against *S. aureus* at 20 mg/mL. Comparatively, flower ETAC extract showed highest ZoI against all bacteria, expressing its antibacterial potential for future applications.

Keywords: *Acacia nilotica*, bacteria, medicinal plant

Introduction

The plants had a great involvement to provide the health benefits to human even when there was no perception of synthetic medicines and surgical management (Sher and Al-Yemeni, 2011). The World Health Organization (WHO) has reported about 21,000 plants that have great medicinal uses globally (Sufi *et al*., 2020). From 119 plant derived medicines, about 74% are used in current medicine. Around 80% population of the world (4 billion people) use herbal medicine for health maintenance (Bhushan *et al*., 2010). Herbal medicine is considered safe and effective, while trouble some side effects are associated with conventional medicine (Shruthi *et al*., 2012).

Acacia nilotica (A. nilotica) belongs to the Leguminosae family of plants and the sub-family Mimosaceae. In folk medicine, it is exposed to long term clinical trials (EI-Tahir *et al*., 1999). It is a composite species having nine sub species and six of them are innate to the African tropics, while others are innate to the Indian sub-continent (Raj *et al*., 2015). *A. nilotica* is also famous as Indian Tomentose Babool, Gum Arabic, Black Aiquant, Black Babul, Egyptian Thorn, Egyptian Mimosa, Nile *Acacia*, Prickly *Acacia*, Scented-Pod *Acacia* and Scented Thorn *Acacia* in English language while different names in Arabic are as Ummughilan and Usarequrz (Jame, 2018). It is widely found in Asia, Australia and Africa. It is also cultivated in the Indian sub-continent and also present on lateritic soil in the Himalayan foothills of India (Kaur *et al*., 2016).

A. nilotica is a multipurpose tree that provides fuel, timber, shade, food, fodder, gum, dye, honey and fences. It also influences the environment *via* soil enrichment, soil re-clamation, protection against wind, fire and also as a shelter for biodiversity and ornament. It is extensively used in ethno-medicine (Orwa *et al*., 2009; Cook *et al*., 2005). It is also a multipurpose nitrogen fixing, legume tree. It is found from the sea level to about 2000 m. It bears extreme temperature (>50 ºC) and air dryness, while is subtle to frost when it is young (Vijayasanthi *et al*., 2012). This plant is characterized by small and aromatic flowers that are rich in volatile terpenoids. The aroma of *Accacia* flower is used in cosmetics, perfumes and also used for the preparation of essential oils and aromatic products (Isla *et al*., 2021). *A. nilotica* has bright golden-yellow and globulus flowers, with 1.2 to 1.5 cm diameter (Baravkar *et al*., 2008). The branches and bark are dark with fissures and have spikes (about 2 cm long). The inflorescence comprises bright yellow flowers in auxiliary head on stalks that are half way up (Vijayasanthi *et al*., 2012).

Phytoconstituents such as bioactive alkaloids, flavonoids, phenolics, polysaccharides, saponins, terpenoids and tannins are richly found in *Acacia* species (Seigler, 2003).

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A. nilotica fruit is used for the treatment of sore throat, bronchitis, cold, pneumonia, dysentery, diarrhea, leprosy, ophthalmia and venereal diseases. The decoction of plant bark is extensively used as an astringent douche in cystitis, gonorrhea, vaginitis and leucorrhoea (Saeedi *et al*., 2020). The infusion of flowers is used for the preparation of sedative, digestive, analgesic and anti-rheumatic tea (Isla *et al*., 2021). Thus, the objective of this study was to evaluate the antimicrobial effects of *A. nilotica* extracts against four pathogenic bacteria, *Staphylococcus aureus (S. aureus), Bacillus cereus (B. cereus), Escherichia coli (E. coli)* and *Salmonella enterica (S. enterica)*, as there is need for formulation of new antimicrobial agents to combat bacterial infections. Though a few studies on the antibacterial potential of *A. nilotica* are found in the literature however, antibacterial activity of *A. nilotica* species from Mirpur, Azad Jammu and Kashmir (AJK) has not been reported yet.

Materials and Methods

Collection and preparation of plant extracts. Flower, bark and roots of *A. nilotica* were collected from Mirpur AJK during September, 2021, to prepare test extracts by following (Ilyas *et al*., 2021). Collected parts were shade dried after proper cleaning. Then these parts of the plant were ground separately by using electric grinder (Fig. 1). Powdered parts of plant were dipped into different solvents, $CHCl_3$, n-Hex, ETAC and MeOH for ten days, with daily shaking. By using filter paper, dipped plant parts were filtered separately and filtrate was vapourized by rotary evapourator to obtain semi solid plant extracts. Rotary evapourated plant extracts were air dried and stored at 4 °C for use according to experimental design.

Study area. Mirpur is a partly plane and partly hilly area having sub-tropical climate. Average rainfall in Mirpur is 1000 mm. Mirpur is consisted of brush forests and range lands and about 40% area of Mirpur is cultivated (Hayat and Khan, 2013). It is situated at 459 m above the sea level.

Chemicals. During this study nutrient broth (Merck, Germany) and nutrient agar (OXOID CM0003, UK), $CHCl_3$ (commercial grade), n-Hex (commercial grade), ETAC (commercial grade), MeOH (commercial grade) and dimethyl sulfoxide (DMSO) (Sigma Aldrich) were used.

Bacterial culture. In this study, two gram negative (*E. coli* ATCC 8739 and *S. enterica* ATCC 43971) and two gram positive bacterial isolates (*S. aureus* ATCC 2592 and *B. cereus* ATCC 10876) were used, these bacteria were a kind gift of Dr. Iftikhar Ahmed (National Culture Collection of Pakistan). Bacterial culture were streaked on fresh nutrient agar medium, kept at 37 °C for 24 h and stored at 4 °C. Later on, bacterial colonies were suspended into nutrient broth from freshly prepared bacterial culture and then incubated at 37 °C for using in microbial assay.

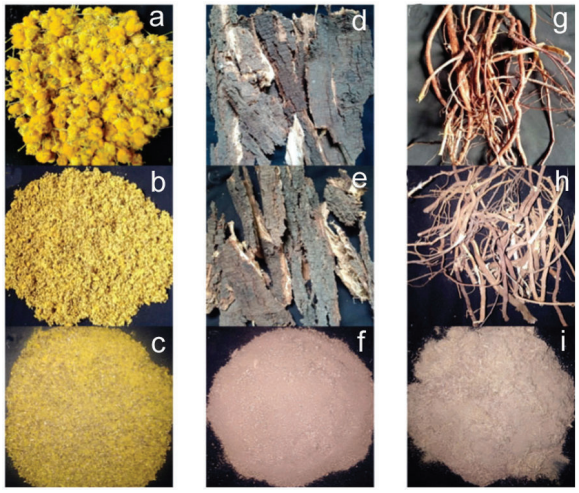

Fig. 1. *A. nilotica* parts collected for extracts preparation, **(a-c)** fresh, dry and grinded flower and **(d-f)** fresh, dry and grinded bark and **(g-i)** fresh, dry and grinded roots, respectively.

Stock preparation. Dry plant extracts were measured to prepare stock solution of 40 mg/mL concentration in DMSO. By using this stock solution, different concentrations were prepared according to experimental study.

Antibacterial assay. Bacterial suspension was spread on agar media plate by sterile cotton swab. Five circular wells per plate were bored with sterile cork borer (6 mm diameter). Well-1 was used as control (DMSO), while well-2 and well-4 were loaded with high concentrations (40 mg/mL) of extracts and well-3 and well-5 loaded with low concentrations (20 mg/mL) of extracts and incubated at 37 °C (Sharif *et al*., 2021). Each well was loaded with 50 µL of DMSO (well 1) and different concentrations of extract with same volume (well 2-5). Later on, all plates were photographed and zone of inhibition (ZoI) was measured after 24 h. The

data was shown after subtraction of control (if any) from extract ZoI.

Results and Discussion

In current study, different parts of *A. nilotica* were used to prepare 12 extracts to explore their antibacterial potential for control of bacterial infections.

Antibacterial activity. In this study antibacterial activity of gram positive and gram negative bacteria were studied by using *A. nilotica* extracts through well diffusion method.

Antibacterial effects of flower extracts. The flower $CHCl_3$ extract was determined for its antibacterial potential at concentration 40 mg/mL and 20 mg/mL and depicted ZoI were 0.31 and 0 mm against *S. aureus*, 0.56 and 0.41 mm against *B. cereus*, 0.63 and 0.25 mm against *E. coli*, 0.88 and 0.5 mm against *S. enterica,* respectively (Table 1).

Moreover, ZoI for flower n-Hex extract were 0.5 and 0.42 mm against *S. aureus*, 0.5 and 0.25 mm against *B. cereus*, 0.58 and 0.5 mm against *E. coli*, 0.58 and 0.5 mm against *S. enterica*, respectively. For flower ETAC extract ZoI were 3.58 and 2.75 mm against *S. aureus*, 4.03 and 3.36 mm against *B. cereus*, 5.22 and 4.39 mm against *E. coli*, 3.84 and 2.86 mm against *S. enterica*, respectively. However, ZoI for flower MeOH extract 2.47 and 1.03 mm against *S. aureus*, 2.94 and 1.94 mm against *B. cereus*, 4.92 and 3.33 mm against *E. coli*, 2.75 and 1.44 mm against *S. enterica* was noted, respectively (Table 1).

Antibacterial effects of bark extracts. The bark $CHCl_3$ extract was determined for its antibacterial potential at concentration 40 mg/mL and 20 mg/mL and depicted ZoI were 3.72 and 3.20 mm against *S. aureus*, 3.45 and 2.69 mm against *B. cereus*, 4.61 and 4.09 mm against *E. coli* and 4.34 and 3.72 mm against *S. enterica*, respectively (Table 2). Moreover, ZoI for bark n-Hex extract was 2.02 and 1.56 mm against *S. aureus*, 1.69 and 1.22 mm against *B. cereus*, 3.19 and 2.44 mm against *E. coli*, 2.34 and 1.86 mm against *S. enterica,* respectively. For bark ETAC extract ZoI were 3.80 and 2.97 mm against *S. aureus*, 3.17 and 2.45 mm against *B. cereus*, 4.23 and 3.48 mm against *E. coli*, 3.61 and 3.06 mm against *S. enterica*, respectively.

However, ZoI for bark MeOH extract 3.45 and 2.84 mm against *S. aureus*, 3.44 and 2.5 mm against *B. cereus*, 3.30 and 2.5 mm against *E. coli*, 3.61 and 2.92 mm against *S. enterica* was noted, respectively (Table 2).

Antibacterial effects of roots extracts. The root $CHCl_3$ extract was determined for its antibacterial potential at concentration 40 mg/mL and 20 mg/mL and depicted ZoI were 4.38 and 3.97 mm against *S. aureus*, 2.73 and 2.52 mm against *B. cereus*, 3.88 and 3.72 mm against *E. coli*, 3.80 and 3.38 mm against *S. enterica*, respectively (Table 3). Moreover, ZoI for root n-Hex extract was 3.02 and 2.5 mm against *S. aureus*, 1.36 and 0.95 mm against *B. cereus*, 2.39 and 1.91 mm against *E. coli*, 2.31 and 1.80 mm against *S. enterica*, respectively. However, ZoI for root ETAC extract it was 4.47 and 3.95 mm against *S. aureus*, 3.16 and 2.69 mm against *B. cereus*, 3.92 and 3.02 mm against *E. coli*, 3.58 and 3.02 mm against *S. enterica*, respectively. For root MeOH extract, 3.30 and 2.73 mm against *S. aureus*, 2.91 and 1.91 mm against *B. cereus*, 3.20 and 2.5 mm against *E. coli*, 3.17 and 2.08 mm against *S. enterica* was noted, respectively (Table 3).

A study conducted by Gmaraldeen *et al*. (2016) reported the *in vitro* antibacterial activity of *A. nilotica* MeOH fruits extract against clinical isolates achieved by cup-plate agar diffusion method against gram-negative and gram-positive bacteria. (Gmaraldeen *et al*., 2016). In a more recent study, *A. nilotica* fruits were evaluated for their strong anti *H. pylori* activity (31 mm) as compared to the positive control (21.67 mm) (Al-Rajhi *et al*., 2023).

Table 1. Antibacterial potential of *A. nilotica* flower extracts showing zone of inhibition (mm)

Bacteria	Flower $CHCl_3$		Flower *n*-Hex		Flower ETAC		Flower MeOH	
	Zone of inhibition (mm)							
	40 mg/mL	20 mg/mL	40 mg/mL	20 mg/mL	40 mg/mL	20 mg/mL	40 mg/mL	20 mg/mL
S. aureus	0.31±0.11	0±0	0.5±0	0.42±0.12	3.58±0.24	2.75±0.20	2.47±0.29	1.03±0.09
B. cereus	0.56±0.11	0.41±0.13	0.5±0	0.25±0	4.03±0.35	3.36±0.19	2.94±0.30	1.94±0.14
E. coli	0.63±0.21	0.25±0	0.58±0.12	0.5±0	5.22±0.18	4.39±0.16	4.92±0.29	3.33±0.35
S. enterica	0.88±0.13	0.5±0	0.58±0.12	0.5±0	3.84±0.21	2.86±0.27	2.75±0.25	1.44±0.11

A. nilotica has various complex phyto-constituents including alkaloids, volatile essential oils, phenols, phenolic glycosides, amines, cyanogenic cyclitols, fatty acids, seed oils, fluoroacetate, gums, non-protein amino acids, terpenes (including essential oils, diterpenes, phytosterol, triterpenegenins and saponins), hydrolyzable tannins, flavonoids, oleosins, steroids and condensed tannins (Hameed *et al*., 2017; Sadiq *et al*., 2015; Seigler, 2003). The literature has revealed the qualitative phytochemicals present in different parts of *A. nilotica* plant extract; the bark contains terpenoids, alkaloids, tannins, saponins, glycosides and sterols; leaves constitute tannins, sterols, alkaloids, saponins, flavonoids and cardiac glycosides; roots comprise saponins, terpenes, flavonoids, tannins, sterols, alkaloids, phenols and anthraquinones, pods have alkaloids, tannins, flavonoids, saponins, sterol and carbohydrate, while flowers showed the presence of phenolic compounds (Jame, 2018). These phyto-constituents play a vital role for its medicinal applications.

The antimicrobial activity of plants phytochemicals like tannins, flavonoids, glycosides, saponins and alkaloids is reported (Javed *et al*., 2020; Farhadi *et al*., 2019; Nazemiyeh *et al*., 2008; Avato *et al*., 2006; Karou *et al*., 2005). The flavonoids present in the fruit, flower, and leaves are the key constituents responsible for antimicrobial potency (Saeedi *et al*., 2020). Guo *et al*. (2019) prepared a class of new norfloxacin-1,3,4-oxadiazole hybrids 5a–t derivatives and determined their antibacterial potential against *S. aureus* and Methicilin resistant *S. aureus* (MRSA). The antibacterial assays represented compound 5k as excellent antibacterial agent against *S. aureus* and MRSA. The compound 5k confirmed an advantage over commonly used vancomycin in killing *S. aureus* and MRSA in the time-kill kinetics study (Guo *et al*., 2019).

A. nilotica is used as an anti-inflammatory, antihypertensive, antidiarrhoeal, antispasmodic, antifungal, antibacterial, antiplasmodial, antipyretic, analgesic, antiviral, anticancer, antiscorbutic, astringent, antioxidant, natriuretic, antispasmodial, hypoglycemic and nerve stimulant (Jame, 2018; Malviya *et al*., 2011; Lam and Ng, 2010; Lopes *et al*., 2009), antiplatelet aggregation, vasoconstrictor, antihypertensive, anti-hepatitis C virus (Lee *et al*., 2011) wound healing (Tung *et al*., 2009), antinociceptive (Dongmo *et al*., 2005), chemopreventive, antimutagenic (Meena *et al*., 2006) and anthelmintic (Bachaya *et al*., 2009). Banso (2009) reported the antimicrobial activity of ethanol extracts of the stem bark against human pathogenic microbes. (Mohamed *et al*., 2010) reported antibacterial activity of 30 methanolic extracts of 23 different plants from 19 plant families against *S. aureus*, *E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*). Traditionally the pods,

Table 2. Antibacterial potential of *A. nilotica* bark extracts showing zone of inhibition (mm)

Bacteria	Bark $CHCl_3$		Bark n-Hex		Bark ETAC		Bark MeOH	
	Zone of inhibition (mm)							
	40 mg/mL	20 mg/mL	40 mg/mL	20 mg/mL	40 mg/mL	20 mg/mL	40 mg/mL	20 mg/mL
S. aureus	3.72± 0.09	3.20±0.44	2.02±0.21	1.56±0.17	3.80±0.19	2.97±0.25	3.45±0.30	2.84±0.21
B. cereus	3.45±0.28	2.69±0.30	1.69±0.11	1.22±0.09	3.17±0.12	2.45±0.25	3.44±0.23	2.5±0.24
E. coli	4.61±0.24	4.09±0.25	3.19±0.27	2.44±0.11	4.23±0.28	3.48±0.27	3.30±0.29	2.5±0
S. enterica	4.34±0.32	3.72±0.46	2.34±0.46	1.86±0.27	3.61±0.39	3.06±0.24	3.61±0.35	2.92±0.29

Table 3. Antibacterial potential of *A. nilotica* roots extracts showing zone of inhibition (mm).

Bacteria	Roots $CHCl_3$		Roots n-Hex		Roots ETAC		Roots MeOH	
	Zone of inhibition (mm)							
	40 mg/mL	20 mg/mL	40 mg/mL	20 mg/mL	40 mg/mL	20 mg/mL	40 mg/mL	20 mg/mL
S. aureus	4.38±0.16	3.97±0.29	3.02±0.26	2.5±0.29	4.47±0.33	3.95±0.22	3.30±0.14	2.73±0.24
B. cereus	2.73±0.29	2.52±0.48	1.36±0.36	0.95±0.38	3.16±0.50	2.69±0.39	2.91±0.44	1.91±0.42
E. coli	3.88±0.18	3.72±0.34	2.39±0.39	1.91±0.27	3.92±0.19	3.02±0.06	3.20±0.22	2.5±0
S. enterica	3.80±0.44	3.38±0.19	2.31±0.40	1.80±0.31	3.58±0.34	3.02±0.60	3.17±0.43	2.08±0.51

leaves, bark and flowers have been reported to be used in the management of different disease conditions (Meena *et al*., 2006). Leaves of *Acacia* plants possess antibacterial activity against a wide range of bacteria, however the extent of antibacterial activity varies depending upon the extract type (Upadhyay *et al*., 2013; Solomon-Wisdom and Shittu, 2010), while in current study flower, bark and roots extracts showed significant antibacterial activity against selected bacterial strains.

In this study, we evaluated the antibacterial potential of *A. nilotica* extracts against selected bacteria showing ZoI ranging from 0.31-5.22 mm at 40 mg/mL. All extracts exhibited concentration dependent antibacterial activity (40 mg/mL > 20 mg/mL). For flower extracts ZoI range was 0.31-5.22 mm, for bark extracts 1.69-4.61 mm, while 1.36-4.47 mm for root extracts at 40 mg/mL. The tested plant extracts showed varying degree of action against gram positive bacteria and gram negative bacteria, showing the gram negative bacteria as more susceptible to flower and bark extracts at highest tested concentration (40 mg/mL). The bacterial susceptibility trend, for flower extracts was as *E. coli* > *S. enterica* > *B. cereus* > *S. aureus* at 40 mg/mL and *E. coli* > *B. cereus* > *S. enterica* > *S. aureus* at 20 mg/mL. However the bacterial susceptibility pattern at both tested concentrations (40 and 20 mg/mL) was noted as; *E. coli* > *S. enterica* > *S. aureus* > *B. cereus* for bark extracts and *S. aureus* > *E. coli* > *S. enterica* > *B. cereus* for root extracts. Among all tested extracts, more antibacterial potency against selected bacteria was presented by ETAC and $CHCl_3$ extracts. Flower ETAC extract was evaluated to be most potent antibacterial extract (5.22 mm ZoI against *E. coli*) among all tested extracts. However, overall results of tested plant parts showed the bark as more potential antibacterial agent as compare to flowers and roots. The present study favors the bark and root extracts of the plant to control bacterial infections in future.

The similar results in their study exhibiting weak or no antibacterial activity of leaves extract fractions against tested bacterial isolates, while reported by (Okoro *et al*., 2014) the bark and root extracts as strong antibacterial agents and also *A. nilotica* extracts which is not toxic for humans. Therefore, results of our study that *A. nilotica* has antibacterial potential is verified here. Thus, it is confirmed that all prepared extracts showed significant antibacterial activities against tested bacteria proposing the possibility to be useful in future for bacterial infections.

Conclusion

A. nilotica flower, bark and roots (twelve extracts) were evaluated for antibacterial potential against *S. aureus*, *B. cereus*, *E. coli* and *S. enterica.* Results confirmed the antibacterial activity of all prepared extracts at both tested concentrations (20 and 40 mg/mL) except *S. aureus* against flower $CHCl_3$ at 20 mg/mL. Comparatively, flower ETAC extract showed highest ZoI against all bacteria, while overall results expressed the bark as more potential antibacterial agent as compare to flowers and roots, expressing its antibacterial potential for future applications and in pharmaceutical industry to control bacterial infections. Various scientific studies stated the traditional system of medicine; however, additional details and clinical research is needed to confirm its several bioactivities in order to establish it as a standard drug.

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Conflict of Interest. The authors declare that they have no conflict of interest.

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