

Antibacterial Activities of Garad (*Acacia nilotica* L.) Plant

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Abstract

Garad is reported to has some antimicrobial activity. The aim of the present study is to investigate the antibacterial activities of the plant. The cup plate Inhibition zone method was used for some solvents tested. The results showed that the Garad pods extract was more effective against *E. coli* and *S. paratyphi*. However, the pods, bark and leaves extracts were highly effective against *Staphylococcus* sp. The solvent tests showed that the methanolic leaves extracts were effective against *Staphylococcus* sp. The ethanolic bark, seeds and pods extracts of Garad were effective against *E. coli* and *S. paratyphi*. All the petroleum ether and hexane extracts of the plant parts were not effective against all the tested bacteria.

INTRODUCTION

A. nilotica is a plant 5 to 20 m high with a thick spherical crown, stems and branches usually sinister to black colored, grey-pinkish slash, fissured bark, exuding a reddish low quality gum. The plant has straight, light, thin, grey spines in axillary pairs, usually in 3 to 12 pairs, 5 to 7.5 cm long in young trees, mature trees commonly without thorns. The leaves are bipinnate, with 3 to 6 pairs of pinnulae and 10 to 30 pairs of leaflets each, rachis with a gland at the bottom of the last pair of pinnulae. Flowers in globulous heads 1.2 to 1.5 cm in diameter of a bright golden-yellow color set up either axillary or whorly on peduncles 2 to 3 cm long located at the end of the branches. Pods are strongly constricted, white-grey, hairy and thick (baravker *et al.*, 2008). *A. nilotica* is a pantropical and subtropical genus with species abundant throughout Asia, Australia, Africa and America. *A. nilotica* is an imperative multipurpose plant that has been used broadly for the treatment of various diseases(Singh~~etal.~~,2009b).

Natural medicinal plants promote self healing, good health and durability in ayurvedic medicine practices and have acknowledged that *A. nilotica* can provide the nutrients and therapeutic ingredients to prevent, mitigate or treat many diseases or conditions). It also serves as a source of polyphenols (Singh *et al.*, 2009a). The role of these polyphenols to the plant itself is not well implicit, but for the human kind they can be of prime strategies (Singh *et al.*, 2009a). The phytochemicals contribute chemically to a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes (Banso, 2009). This plant contain a profile of a variety of bioactive components such as gallic acid, ellagic acid, isoquercitin, leucocyanadin, kaempferol-7-diglucoside, glucopyranoside, rutin, derivatives of (+)-catechin-5-gallate, apigenin-6,8-bis-C-glucopyranoside, m-catechol and their derivatives (Singh *et al.*, 2009a). It has been reported that different parts of the plant are prosperous in tannins (ellagic acid, gallic acid and tannic acid), stearic acid, vitamin-C (ascorbic acid), carotene, crude protein, crude fiber, arabin, calcium, magnesium and selenium (Meena *et al.*, 2006). Traditionally the bark, leaves, pods and flowers are used against cancer, cold, congestion, cough, diarrhea, dysentery, fever, gall bladder, hemorrhoid, ophthalmia, sclerosis, tuberculosis and small pox, leprosy, bleeding piles, leucoderma and menstrual problems. They have spasmogenic, vasoconstrictor, anti/-hypertensive, -mutagenic, -carcinogenic, -spasmodic, -inflammatory, -oxidant and -platelet aggregatory properties (Singh *et al.*, 2009b). *A. nilotica* has anti-plasmodial, molluscicidal, anti-fungal, anti-microbial activity, inhibitory activity against HCV

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and HIV-I (Sultana *et al.*, 2007). The bark of the plant is used as astringent, acrid, cooling, styptic, emollient, anthelmintic, aphrodisiac, diuretic, expectorant, emetic and nutritive, in hemorrhage, wound ulcers, leprosy, leucoderma, skin diseases and seminal weakness. Gum is used as astringent, emollient, liver tonic, antipyretic and antiasthmatic (baravkar *et al.*, 2008). The bark is used extensively for colds, bronchitis, biliousness, diarrhoea, dysentery, bleeding piles and leucoderma (Del, 2009). phytochemical and pharmacological traits of this plant of high economic value.

Objectives of the study:

1. The overall aim of this study was to find environmentally friendly alternatives antimicrobial phytochemical compounds from Garad plants.
2. The extracts from the different parts of the plant will be examined for their activities against different bacteria isolates, using different concentrations.

MATERIALS AND METHODS

Source of materials

Plants sources

Fruits (pods and seeds) of Garad were purchased from Elhasahiesa local market. Other Garad tree parts (Leaves & bark) were collected from nearby Garad tree-in Elhasahiesa Faculty of Education.

Microorganisms sources

The cultures of Bacteria (*Escherichia coli*, *Salmonella paratyphi*, and *Staphylococcus* sp.) were obtained from the Food Science and Technology Laboratory, Faculty of Science and Technology, University of Gezira.

Media used

The media used in this study were prepared locally, using Oxoid Corporation substances. The media include the followings:

The Nutrient Agar Medium

This medium was used for isolation and maintenance of bacteria, and for other experiments whenever needed. The medium consists of the following materials: (g/L)

Animal tissues	5
Beaf extract	1.5
Yeast extract	1.5
Na Cl	5
Agar	15

Preparation of the Medium

Twenty eight grams of the prepared media were added to 1 liter distilled water. The medium was then dispensed in 100 ml samples in conical flasks covered with cotton plugs and aluminium foil before being sterilized in the autoclaved at 121⁰ C (151/in²) for 15 minutes.

Methods

The Inhibition Zone Method (Cup Plate)

This method was used for measuring the inhibition zone against the growth of the tested bacteria (*E. coli*, *S. paratyphi*, and *Staphylococcus* sp.) using the Nutrient Agar (NA) medium. In this method a standardized cell suspensions of each bacterium were prepare and then added to the solidified medium into sterile Petri dishes and spreaded using sterile L-shape glass rod. Sterile Whatman glass fiber disks (No.5) were saturated with each extract, then allowed to dry and transferred centrally on the surface of the solidified medium in each plate. The plates were then incubated at room temperature for 72 hours and the inhibition zones were measured as described by Barry *et al.*, (1970) and Cruickshank *et al.*, (1975). The test of the antibiotic compounds was made following the same method. Three replicates were made for each treatment.

RESULTS

The present study investigated the biological activity of the extracts of Garad plant parts against three bacteria (*E.coli* , *Staphylococcus* sp. and *S. paratyphi*).

The effects of the aqueous extracts of the Garad plant parts on inhibition zone of the different three bacteria are shown in Table (1), from the data only the pods extracts were highly effective in suppressing the inhibition zone of *E.coli*, while the extracts of all other parts were less effective table (1a). Table (1b) show the effect of Garad aqueous plant parts extracts on inhibition zone of *S. paratyphi*. The extracts of pods, seeds and the bark extracts were less effect in inhibiting growth of *S. paratyphi*, although the pod extract were more effective than the other. On contrast the leaves extract were not effective. Table (1c) show the effect of Garad aqueous plant parts extracts on inhibition zone of *Staphylococcus* sp. the results shows that, the pods, bark and leave extracts were all highly effective in suppressing growth of *Staphylococcus* sp. on the other hand, the extract of seed were less effective.

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Table (2) shows the effect of the Garad plant part methanolic extracts on the inhibition zone of three bacteria (*E. coli*, *S. paratyphi*, and *Staphylococcus* sp.). From the results it is clear that only the leaf extracts were the highly effective against *Staphylococcus* sp.

Table (3) shows the effect of the Garad plant part ethanolic extracts on the inhibition zone of three bacteria (*E. coli*, *S. paratyphi*, and *Staphylococcus* sp.). From the results it is clear that the bark, the seeds and the pods showed effective results against both *E.coli* and *S. paratyphi*. However, the *Staphylococcus* sp., showed some resistance to the extracts.

Table (4) show the effect of the Garad plant part petroleum ether extracts on the inhibition zone of the three bacteria. From the data it is clear that all the petroleum ether extracts were not effective against all the tested organisms.

Table (5) show the effect of the Garad plant parts hexane extracts on the inhibition zone of the three bacteria (*E. coli*, *S. paratyphi*, and *Staphylococcus* sp.). From the data it is clear that all the hexane extracts were not effective against all the tested bacteria. Table (6) show the effect of the anti- Gram negative and Gram positive antibiotic (as control) against *E.coli*, *S. para typhi* and *Staphylococcus* sp. From the results it is clear that Cefotaxime, Piperacillin/ Tazobactam, Chloramphenicol and Tetracycline were effective against *E.coli*, while, Ampicillin/ Sulbactam and Ciprofloxacin, were highly effect against the same bacterium. Co- Trimoxazole, Piperacillin/ Tazobactam, Chloramphenicol, Ciprofloxacin, Ceftizoxime and Tetracycline, were effective, Ampicillin/ Sulbactam, Ofloxacin, Gentamicin, Amikacin and Levofloxacin were highly effect against *S. paratyphi*. Then Tetracycline, Gentamicin, Linezolid were effective against *Staphylococcus* sp.

Table 1 : Effect of Garad parts aqueous extracts on inhibition zone (cm) of the three bacteria:

a- *E.coli*

Conc. (mg/ml)	Bark	Seeds	Pods	Leaves
0	0.5	0.5	0.5	0.5
25	0.5	0.5	0.6	0.5
50	0.5	0.5	1.00	0.6
75	0.9	0.9	1.8	0.9
100	1.5	1.4	2.3	1.6

b- *Salmonella paratyphi*

0	0.5	0.5	0.5	0.5
25	0.5	0.5	0.7	0.5
50	0.7	0.6	1.00	0.5
75	1.1	1.00	1.3	0.5
100	1.5	1.3	1.8	0.5

c- *Staphylococcus sp.*

0	0.5	0.5	0.5	0.5
25	0.5	0.5	0.6	0.6
50	0.9	0.6	0.9	0.9
75	1.5	0.9	1.6	1.4
100	1.9	1.4	2.00	1.9

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Table 2: Effect of Garad parts methanolic extracts on inhibition zone (cm) of the three bacteria

Organisms	Bark	Seeds	Pods	Leaves
<i>E.coli</i>	1.1	1.6	1.6	0.5
<i>Salmonella para typhi</i>	0.5	0.6	0.5	0.5
<i>Staphylococcus sp.</i>	0.9	0.5	1.6	2.00

Table 3: Effect of Garad parts ethanolic extracts on inhibition zone (cm) of the bacteria

Organisms	Bark	Seeds	Pods	Leaves
<i>E.coli</i>	1.2	1.2	1.3	0.5
<i>Salmonella para typhi</i>	1.1	1.1	1.1	0.5
<i>Staphylococcus sp.</i>	0.5	0.7	0.8	0.5

Table 4: Effect of Garad petroleum ether extracts on inhibition zone (cm) of the bacteria.

Organisms	Bark	Seeds	Pods	Leaves
<i>E.coli</i>	0.5	0.7	0.7	0.6
<i>Salmonella para typhi</i>	0.5	0.6	0.7	0.6
<i>Staphylococcus sp.</i>	0.5	0.7	0.5	0.5

Table 5: Effect of Garad hexane extracts on inhibition zone (cm) of the tested bacteria:

Organisms	Bark	Seeds	Pods	Leaves
<i>E.coli</i>	0.5	0.5	0.5	0.6
<i>Salmonella para typhi</i>	0.5	0.5	0.5	0.5
<i>Staphylococcus sp.</i>	0.5	0.5	0.5	0.5

Table 6 : Effect of anti- Gram negative and Gram positive antibiotic (on inhibition zone-cm) against *E.coli*, *S. paratyphi* & *Staphylococcus* sp.

Antibiotic	<i>E.coli</i>	<i>S. paratyphi</i>	<i>Staph. sp.</i>
Ampicillin/Sulbactam (AS)	2.0	2.0	0.0
Co- Trimoxazole (BA)	0.0	1.0	0.0
Cefotaxime (CF)	1.6	0.0	0.0
Piperacillin/Tazobactam (TZP)	1.8	1.7	-
Chloramphenicol (CH)	1.5	1.8	-
Ciprofloxacin (CP)	2.4	1.0	0.0
Ceftizoxime (CL)	0.0	1.0	-
Tetracycline (TE)	1.7	1.2	1.0
Ofloxacin (OF)	0.0	2.5	-
Gentamicin (GM)	0.0	3.00	1.6
Amikacin (AK)	0.0	2.0	-
Levofloxacin (LE)	0.0	2.3	0.0
Co- Trimoxazole (BA)	-	-	0.0
Cephalexin (PR)	-	-	0.0
Linezolid (LZ)	-	-	1.2
Cloxacillin (CX)	-	-	0.0
Roxithromycin (RF)	-	-	0.0
Linomycin (LM)	-	-	0.0

- Not tested

DISCUSSION

Medicinal plants represent a rich source of antimicrobial agents (Mahesh & Satish, 2008; Adnan *et al.*, 2010). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann *et al.*, 2008; Gilani *et al.*, 2010; Hussain *et al.*, 2012).

The present study was investigated the biological activities of the extracts of Garad plant parts against three bacteria (*E. coli*, *Staphylococcus sp.* and *S. paratyphi*).

The results of the biological activities indicated that the extracts of Garad plant parts were showing different effects against the tested bacteria.

The effects of the aqueous extracts of the Garad plant parts on inhibition zone of the different three bacteria was made. From the results: the pods extracts were highly effective in suppressing the inhibition zone of *E.coli*, while the extracts of all other parts were less effective. The extracts of pods, seeds and the bark extracts were less effect in inhibiting growth of *S. paratyphi*, although the pod extract were more effective than the other. On contrast the leaves extract were not effective. However; the pods, bark and leave extracts were all highly effective in suppressing growth of *Staphylococcus sp.* on the other hand, the extract of seed were less effective.

The study of phytochemical properties and antibacterial activity of aqueous pods extract of *A.nilotica* revealed the presence of three principal phytochemicals (tannins, saponins and flavonoids) that have been reported to possess antibacterial activity (Giovana *et al*, 2013). This could be the reason for antibacterial activity of aqueous pods extract of *A.nilotica*. This study therefore reveal the potentials of *A.nilotica* pod extract as antibacterial agent especially in the management of ailments caused by organisms such as *S. pyogenes*, *B.subtilis*, *C. pyogenes*, *K.pneumoniae* and *C. albicans*.

The pods of *Acacia nilotica* can be used in future to develop antibiotics that can be of benefit to humans and animals.

The aqueous extract of *A. nilotica* antibacterial activity was compared against a standard drug, tetracycline. Tetracycline showed a better antibacterial property with highest zone of inhibition of 48 mm for *S.pyogenes* at 250 mg/ml and least zone of inhibition of 16mm for *S. aureus* and *S. typhi* as compared to the highest zone of inhibition of 25 mm at the 1000 mg/ml for *B. subtilis* and *K. pneumonia* and least zone of inhibition of 12 mm for *S. aureus* and *S. typhi*, Respectively for *A. nilotica* (Sofowora, 1993;Nwze *et al.*, 2004)

The assays of the stem bark extracts confirms the antimicrobial activity against *Streptococcus viridans*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Shigella sonnei* using the agar diffusion method. *A. nilotica* could be a potential source of antimicrobial agents (Banso, 2009).

A. nilotica demonstrates highest activity against three bacterial (*E. coli*, *S. aureus* and *Salmonella typhi*) and two fungal strain (*Candida albicans* and *Aspergillus niger*) (Kalaivani and Methew, 2010).

The use of different solvents for the different extracts was also made in the present study. The solvents used include (Methanol, ethanol, petroleum ether and hexane). Only the inhibition zone method was used for this test. From the results it was found that the Methanolic and the ethanolic extracts of Garad were more effective against the tested organism than the other solvents (petroleum ether and hexane). The methanolic Garad leaves extracts were only effective against *Staphylococcus* sp. All the other solvents were less effective. Methanol extracts were also reported as the most effective by different investigators (Abdel- Rahim *et al.*,2012; Zainal *et al.*1988; Ahmed, 2004). Solomon and Shittu (2010) has investigated in vitro antimicrobial activity of the crude ethanolic leaf extract of *Acacia nilotica* against *Campylobacter coli* isolated from goats. The highest zone of inhibition in their study was observed with the 70 mg/ml concentration. Banso (2009) has studied the antimicrobial activity of ethanolic extracts of the stem bark against *Streptococcus viridans*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Shigella sonnei* using the agar diffusion method and found that the minimum inhibitory concentration of the stem bark extract ranged between 35 and 50 mg/ml while the minimum bactericidal concentration ranged between 35 and 60 mg/ml (Banso, 2009). Khan *et al* (2009) has explored the antimicrobial activities of the crude ethanolic extracts of five plants against multidrug resistant (MDR) strains of *Escherichia coli*, *Klebsiella pneumoniae* , *Candida albicans* and ATCC strains of *Streptococcus mutans*, and different strains of microorganism. They found that Garad (*A. nilotica*) has minimum Inhibitory concentration range 9.75-313 µg/ml (Khan, 2009). Mashram (2009) has observed the antimicrobial activity of *Acacia nilotica*, against three bacteria (*S. aureus*, *B. subtilis* and *E. coli*). He found that the leaf and bark extracts showed a zone of inhibition between 7.5-16 and 8-15.5 mm, respectively and were most active against *E. coli*.

Mahesh and Satish (2008) have observed the antibacterial activity study of methanolic extracts of *Acacia nilotica*, and showed that the highest antibacterial activity was against *B. subtilis*. and *Staphylococcus aureus* with an inhibition zone of 15±0.66mm and the leaf extract showed the highest activity against *Bacillus subtilis* with an inhibition zone of 20±1.20mm. Methanolic extract of the *A. nilotica* is active against two animal viruses: Newcastle Disease and Fowl pox Viruses (Mohamed *et al.*, 2010). Saini (2008) examined the comparative antimicrobial studies of different *Acacia* species. He found that *A. nilotica* was exhibited the highest activity against the three tested bacterial (*Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*). The *Acacia nilotica* plant extracts were also reported by Shanab (2007) to have potent antibiotic activity against three bacterial species (gram positive; *Bacillus subtilis*, *Staphylococcus albus*, *Streptococcus faecalis*; a gram negative, *Escherichia coli*). Methanolic extracts of

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the plant were found to contain keampherol which is responsible for the antioxidant activity of the plant (Rajbir and Bikram (2008).

Methanol extracts of *Acacia nilotica* pods were found to cause a decrease in arterial blood pressure at dose (3–30 mg/kg). It also produces an inhibitory effect on force and rate of contraction in guinea-pig paired atria (Gilani,1999). However, *Acacia* species can be regarded as promising resources for antibacterial drugs due to their highly active nature. The antibacterial activity may be indicative of the presence of some metabolic toxins or broad-spectrum antibiotic compounds.

Prashanth *et al.* (2001) and Rajakaruna *et al.* (2002) reported that the bacterium *Bacillus subtilis*, was more sensitive to methanol or hexane extracts of 10 plants. On the other hand, no inhibition was observed in the *Erwinia* sp. Some organisms exhibited only slight susceptibility. *E. coli* was inhibited by methanol extract of flowers of *Cassia auriculata* and hexane extract of *Punica granatum*. *Proteus vulgaris* was inhibited by methanol extract of *P. pterocarpum* and *Syzigium lineare*. *Klebsiella pneumonia* were inhibited by hexane extracts of *Ola scandens*, methanol extracts of *P. pterocarpum* and *Syzigium cumini*. The methanol extracts were more effective than hexane extracts.

In Sudan many studies were carried out for testing the antimicrobial activity of some medicinal plants. Ahmed (2004) tested the extracts of 10 plants against Gram positive and Gram negative bacteria as well as *Candida albicans*. He found a marked effect against the Gram positive *Staph. aureus* followed by *E. coli* and *Candida albicans*, respectively.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. The *In vitro* tests indicated that there are different effects of Garad parts (bark, leaves, seeds and pods) extracts against the tested organisms.
2. The methanolic extracts of the plant parts was the only effective among the different solvents used.

Recommendations

- 1- The extracts of Garad plant can be used as antimicrobial agent.
- 2- It could be suggested that Garad extracts which traditionally used for curing many known disease would be used for treating disease.
- 3-The pods of *Acacia nilotica* can be used in future to develop antibiotics that can be of benefit to humans and animals.

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