In vitro antibacterial activity of acacia nilotica methanolic extract against wound infection pathogen

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Abstract

Background: Awound is a break in the skin, the first line of defense against infection. Minor wounds include cuts, scrapes, and puncher wounds. Other examples include incisions, lacerations, diabetic ulcer and burns. While most minor wounds heal easily, some can worsen into open sores that can become seriously infected. Acacia Nilotica is plant used by traditional healer in Sudan for treatment of many diseases.

Objective: The aim of this study was to screen antibacterial activity of methanolic extract of acacia nilotica against wound infection isolated pathogen and to determined MIC.

Materials & methods: Experimental and analytical study conducted had been in Khartoum teaching hospital during the period from May to June. Cup-plate agar diffusion method was done to screened antibacterial activity and determined MIC.

Result: A total of 90 bacterial pathogens isolated, 30 *Escherichia coli*, 30 *Pseudomonas aerugnosa*, and 30 *staphylococcus aureus*. The result of this study indicated that the methanolic extract of acacia nilotica with high antibacterial activity.

Conclusions: Methanolic extract of acacia nilotica showed high sensitivity against wound infection bacterial isolate

Keywords: wound infection, acacia nilotica, methanolic extract

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Introduction

A wound is a break in the skin, the first line of defense against infection. Minor wounds include cuts, scrapes, and puncture wounds. Other examples include incisions (clean cuts), lacerations (jagged, irregular cuts), diabetic ulcers, and burns.
While most minor wounds heal easily, some can worsen into open sores that can become seriously infected. You may be able to treat minor wounds at home, by washing the area with clean water and applying a bandage. But you should seek emergency care for any animal or human bite or a cut greater than ½ inch long where you can see fat, muscle, or bone.(1)
A wound infection is defined by the US Centre for Disease Control and Prevention (CDC) as surgical site infection (SSI). This is further defined as:
Superficial incision SSI – infection involves only skin and subcutaneous tissue of incision.
Deep incision SSI – infection involves deep tissues, such as facial and muscle layers.
Organ/space SSI – infection involves any part of the anatomy in organs and spaces other than the incision, which was opened or manipulated during the operation. (2)

Acacia nilotica is a medium sized tree found in the dry parts of Africa, India, Australia, Arabia and other areas. It is used in traditional African herbal medicines, which are traditional healing systems in India (3). According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. For along period of time, plant had been a valuable source of natural product for maintaining human health especially in the last decade, with more intensive studies for natural therapies(4)
Acacia nilotica it have been advocated in folk medicine for the Treatment of tuberculosis, leprosy, smallpox, dysentery, coughs Opthalmia, toothache, skin cancer as astringent, antispasmodic, and aphrodisiac since immemorial time. The present study investigates the antibacterial, antifungal, antiviral, and immunomodulatory potential of hot aqueous extract (HAE) of acacia nilotica (5)\

Materials and Methods

Experimental and analytical study conducted had been in Khartoum teaching hospital during the period from May to June. A total of 90 bacterial pathogens isolated, 30 *Escherichia coli*, 30 *Pseudomonas aerugnosa*, and 30 *staphylococcus aureus*

Preparation of extract of acacia nilotica

Each of the coarsely powdered plant material (50g) was exhaustively extracted for 20 hours with chloroform in Soxhlet apparatus. The chloroform extract was filtered and evaporated under reduced pressure using Rota-vap. The extracted plant material was then air-dried, repacked in the Soxhlet and exhaustively extracted with methanol. The methanolic extract was filtered and evaporated under reduced pressure again using Rota-vap.Each residue was weighed and the yield percentage was determined. The methanol residue (2g) was suspended in a mixture containing methanol: petroleum ether (2:1) to a final volume 20 ml (con.100mg/ml). The methanol residue (2g) was dissolved in methanol 20 ml (con.100mg/ml) and kept in refrigerator until used.

Invitro testing of extract for antimicrobial activity

After preparation of bacterial suspensions, the antimicrobial activity of plant extracts will be tested using cup-plate agar diffusion method. The minimum inhibitory concentrations will be determined by agar plate dilution method

Testing of Extracts for Antibacterial Activity

The cup-plate agar diffusion method was adopted to assess the antibacterial activity of the prepared extracts. 0.6 ml of isolated bacterial suspensions (10^8-10^9) colony- forming units per ml was thoroughly mixed with 60 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar was distributed into sterile Petri dishes. The agars plate was left to set and in each of these plates 4 cups, 10 mm in diameter, was cut using a sterile cork borer No. 4 and the agar discs was removed .Alternate cups was filled with 0.1ml of each of the extracts using micro titer-pipette and was allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. Four replicates were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation the diameters of the results and growth inhibition zones was measured, averaged and the mean values was tabulated.

Determination of minimum inhibitory concentration (MIC)

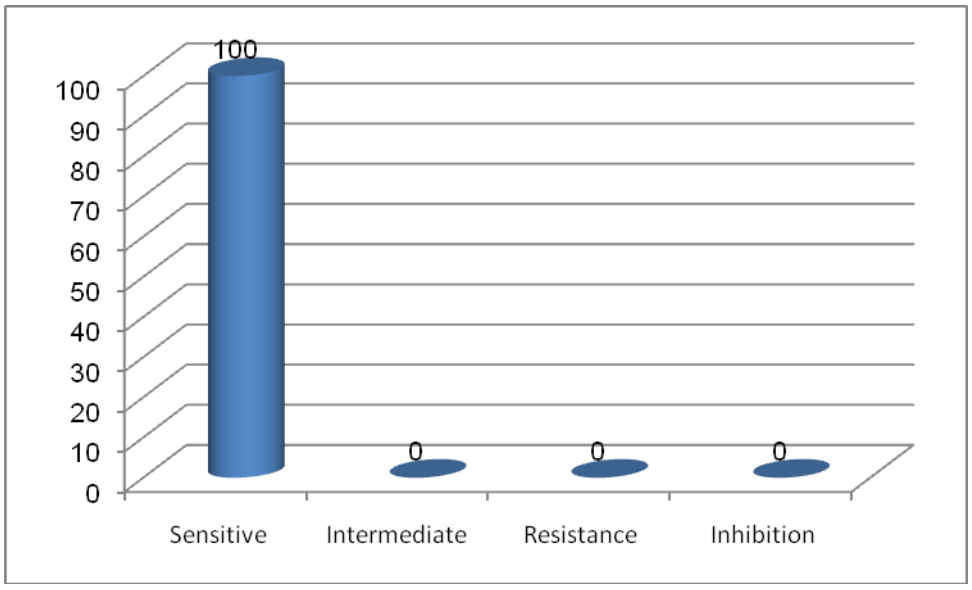
The principle of the agar plate dilution is the inhibition of growth on the surface of the agar by the plant extracts incorporated into the medium.
Plates were prepared in the series of increasing concentrations of the plant extract. The bottom of each plate was marked off into 6 segments. The organisms tested was grown in broth over night to contain 10^8 C F U/ml. Loop-full of diluted culture was spotted with a standard loop that delivers 0.001 ml on the surface of segment. The end point (MIC) is the least concentration of antimicrobial agent that completely inhibits the growth. Results were report as the MIC in mg/ml.

Result of Sensitivity

Table 1: *Escherichia coli* /* M.D.I.Z {mm}

	Frequency	Percent
Sensitive	30	100.0
Intermediate	0	0
Resistance	0	0
Inhibition	0	0
Total	30	100.0

* M.D.I.Z {mm}: Mean diameter of inhibition zones {mm}



Figures 1: Percentage of extract of acacia nilotica sensitivity and resistance of *Escherichia coli.*

Table 2: *Pseudomonas aerugnosa* / M.D.I.Z {mm}

	Frequency	Percent
Sensitive	30	100.0
Intermediate	0	0
Resistance	0	0
Inhibition	0	0
Total	30	100.0

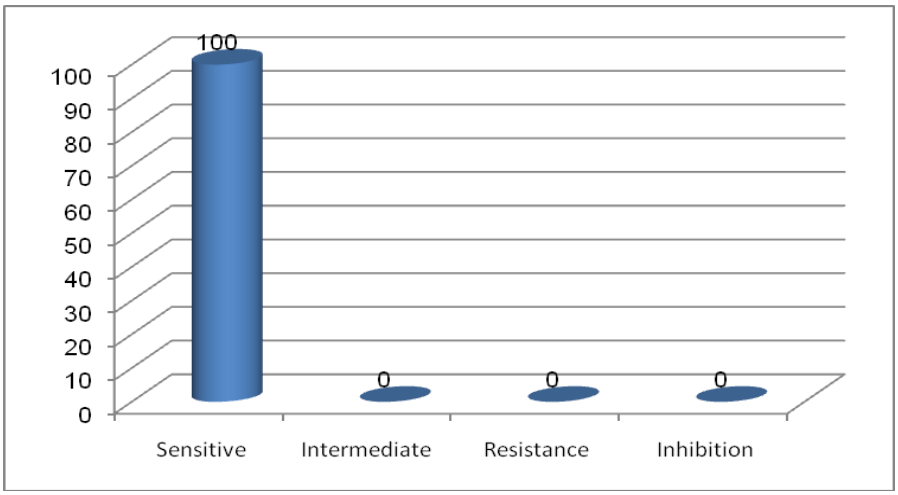


Figure 2: Percentage of extract of acacia nilotica sensitivity and resistance of *Pseudomonas aerugnosa.*

Table 3: *staphylococcus aureus* / M.D.I.Z {mm}

	Frequency	Percent
Sensitive	30	100.0
Intermediate	0	0
Resistance	0	0
Inhibition	0	0
Total	30	100.0

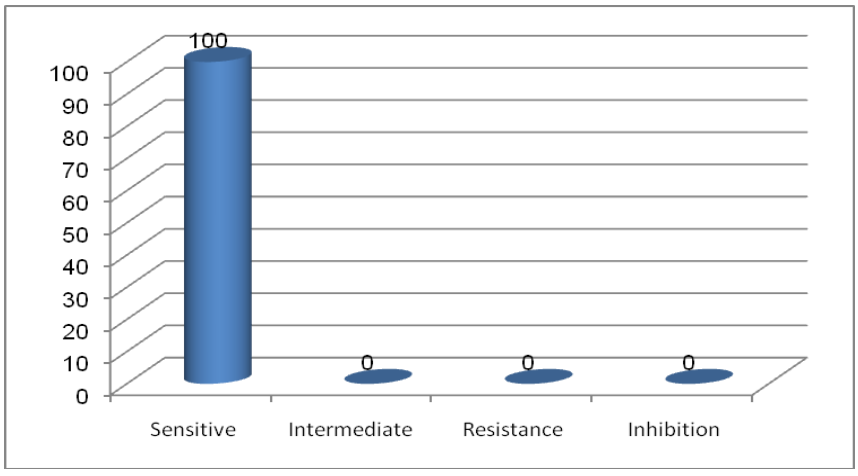


Figure 3: Percentage of extract of acacia nilotica sensitivity and resistance of *staphylococcus aureus.*

Table 4: Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
E.coli	30	32.50	53.50	41.0833	6.11257
P.aeruginosa	30	40.00	55.00	47.7167	4.73010
S.aureus	30	21.50	51.00	36.3667	7.39866
Total	30				

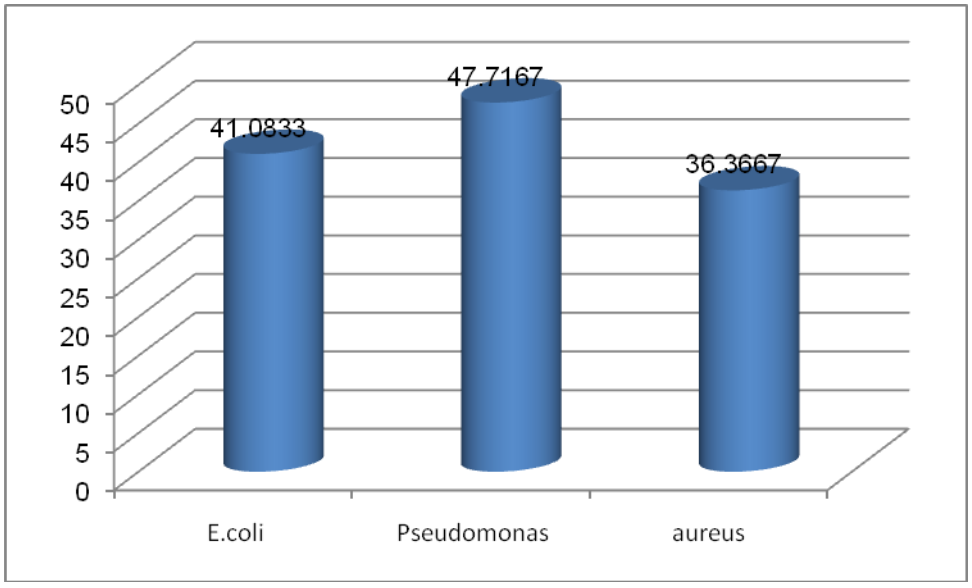


Figure 4: Descriptive Statistics.

Result of MIC

Table 5: MIC of *E.coli* crosses tabulation

			E.coli				Total
			Inhibition	Resistance	Intermediate	Sensitive	
Concentration	3.12	Count	4	1	9	16	30
		% of Total	3.3%	.8%	7.5%	13.3%	25.0%
	1.56	Count	10	0	19	1	30
		% of Total	8.3%	.0%	15.8%	.8%	25.0%

	0.8	Count	29	0	1	0	30
		% of Total	24.2%	.0%	.8%	.0%	25.0%
	0.4	Count	30	0	0	0	30
		% of Total	25.0%	.0%	.0%	.0%	25.0%
Total		Count	73	1	29	17	120
		% of Total	60.8%	.8%	24.2%	14.2%	100.0%

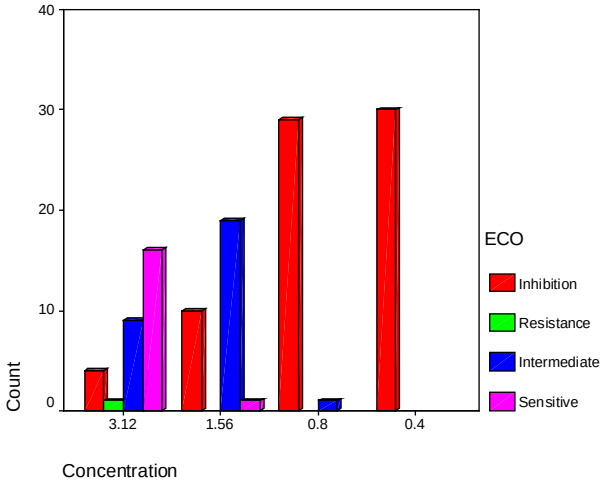


Figure 5: MIC of *E.coli.*

Table 6: MIC of *P.aeruginosa* Cross tabulation

			P.aeruginosa				Total
			Inhibition	Resistance	Intermediate	Sensitive	
Concentration	3.12	Count	6	0	14	10	30
		% of Total	5.0%	.0%	11.7%	8.3%	25.0%
	1.56	Count	8	5	10	7	30
		% of Total	6.7%	4.2%	8.3%	5.8%	25.0%
	0.8	Count	27	0	2	1	30
		% of Total	22.5%	.0%	1.7%	.8%	25.0%
	0.4	Count	30	0	0	0	30
		% of Total	25.0%	.0%	.0%	.0%	25.0%
Total		Count	71	5	26	18	120
		% of Total	59.2%	4.2%	21.7%	15.0%	100.0%

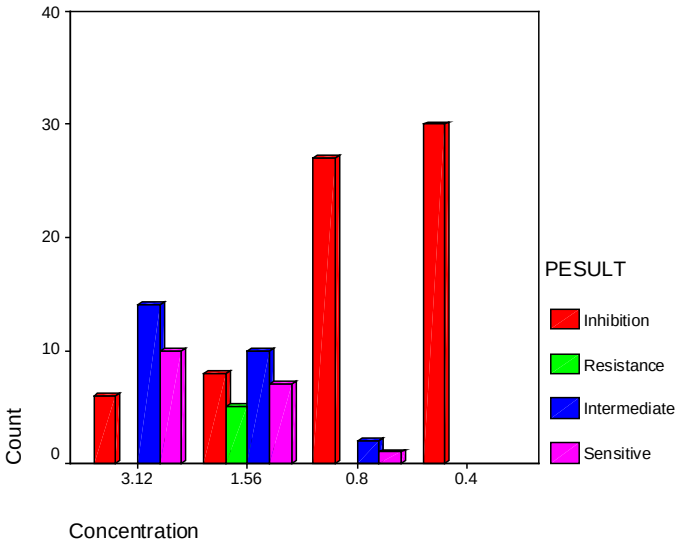


Figure 6: MIC of ***P.aeruginosa.***

Table 7: MIC OF *S.aureus* Cross tabulation

			S.aureus				Total
			Inhibition	Resistance	Intermediate	Sensitive	
Concentration	3.12	Count	1	1	16	12	30
		% of Total	.8%	.8%	13.3%	10.0%	25.0%
	1.56	Count	7	7	12	4	30
		% of Total	5.8%	5.8%	10.0%	3.3%	25.0%
	0.8	Count	27	0	3	0	30
		% of Total	22.5%	.0%	2.5%	.0%	25.0%
	0.4	Count	30	0	0	0	30
		% of Total	25.0%	.0%	.0%	.0%	25.0%
Total		Count	65	8	31	16	120
		% of Total	54.2%	6.7%	25.8%	13.3%	100.0%

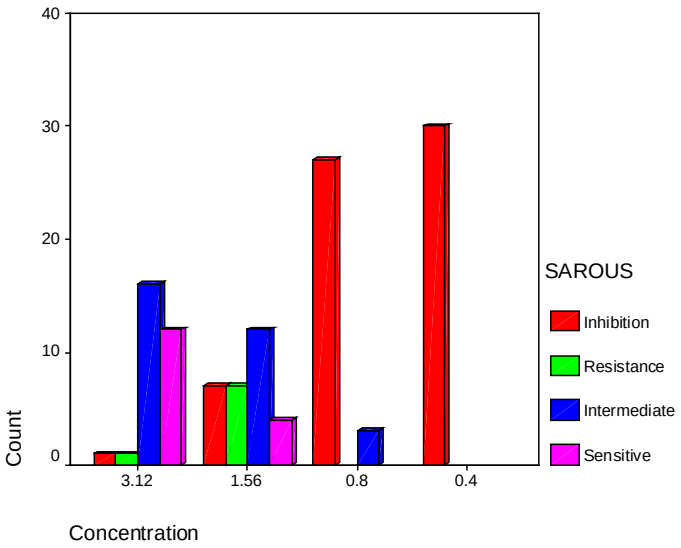


Figure 7: MIC OF *S.aureus.*

DISCUSSION

The current study was carried out to screen the antibacterial activity of acacia nilotica methanolic extract used against wound infection bacterial isolates *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.The result showed high activity 100% (Table 1, 2, 3 of sensetivity) of methanoilic extract of acacia nilotica and *pseudomonas aeruginosa* was more response (47%) than *Escherichia coli* (41%) & *Staphylococcus aureus* (36%).(Table 4)

Minimum inhibitory concentration for all bacteria was 0.4: *Escherichia coli* 60.8% Table 5, *pseudomonas aeruginosa* 59.2% Table 6, and *Staphylococcus aureus* 54.2% Table 7. This finding indicate high sensitivity, available and safety.

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