

Anti bacterial activity of *Psidium guajava* and *carthamus tinctorius* against respiratory tract infection

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Abstract:

The aim of this study was to enrich the information about the antimicrobial activity of some medicinal plants of the Sudan. Also, to verify the claimed activity of certain Sudanese medicinal plants used in traditional medicine as antimicrobial agents, to subject these plants for further antimicrobial studies against clinical isolates, to determine their minimum inhibitory concentration (MICs) and to compare their activity with the commonly used antibacterial agents in the Sudan. The chloroform, methanol and aqueous extract of *Psidium guajava* leaves (family: myrtaceae) and *Carthamus tinctorius* seeds (family: Asteraceae) were screened for their antimicrobial activity against four standard bacteria: two gram positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and two gram negative (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) using the cup plate agar diffusion method. All six extracts were active against one or more of the four bacteria. Four of these extracts inhibited the growth of all microorganisms. *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the most sensitive organisms to all extracts, while *Streptococcus pyogenes* showed less susceptibility. The antibacterial activity of five reference drugs was determined against the tested four bacteria and their activity was compared to the activity of plants extracts.

Introduction:

Antibacterial activity is a substance that kills or inhibits the growth of bacteria. Some plants have been investigated scientifically for antimicrobial activity and as a large numbers of plant products have been shown to inhibit the growth of pathogenic microorganisms. Recently, works in medical field returned back to nature particularly in the use of medical plant to treat human ailments ⁽¹⁾.

Some plants are known as medicinal because they contain active substances that cause certain reactions that cure diseases of the human ⁽²⁾. Many countries use plants in treatment of diseases as in China ⁽³⁾, South America ⁽⁴⁾ and Iran ⁽⁵⁾. In African and other developing

countries, traditional medicines from plants continue to form the basis of rural medicinal care because traditional medicines are easily available and cheap ⁽¹⁾. The number of medicinal plants around the world range between 250,000 to 300,000 and more than two third of this plant growing in developing countries ⁽⁶⁾. Almagaboul *et al* ⁽⁷⁾ investigated 40 extracts belonging to 18 families from Sudan for antibacterial activity against one or more microorganisms. Ahmed *et al* ⁽⁸⁾ tested antibacterial activity of *Juliflora* (alkaloid isolated from *Prosopis juliflora*) invitro against six Gram negative and ten Gram positive bacteria at comparable concentration of penicillin, streptomycin, erythromycin, sulphamethoxazole, ampicillin and tetracycline. Abdelrahim, *et al* ⁽⁹⁾ and

Verma, *et al* ⁽¹⁰⁾ also reported a microbial effect of guava sprout extract upon many bacterial species.

Different extracts from traditional plants have been tested. Many report showed the effectiveness of traditional herbs against microorganisms ⁽¹¹⁾.

The chemotherapeutic index compares the maximum dose that can be tolerated by the host without causing death, with minimum dose that cures the particular infection ⁽¹²⁾.

Antimicrobial activity is measured in vitro ⁽¹³⁾ in order to determine:

- a. The potency of an antimicrobial agent in solution.
- b. The sensitivity of given microorganism to known concentration of the drug.

Determination of these quantities may be undertaken by one method (dilution method) ^(14, 15, 16).

The aim of agar dilution method is to determine the lowest concentration of assayed antimicrobial agent (minimum inhibitory concentration (MIC)) that, under defined test condition, inhibits the visible growth of the bacterium being investigated. MIC values are used to determine susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agent.

In this method, the medium is inoculated with test organism and the samples to be tested are mixed. The zones of inhibition results are dependent upon both the diffusibility of the agent in the medium and the degree of susceptibility of the organism. The speed of growth and the size of inoculums can influence to a marked degree the size of inhibitory zones.

The common bacteria causing respiratory infections are *staphylococcus aureus*, *streptococcus pyogenes*, *klebsiella pneumoniae* and *pseudomonas aeruginosa* ⁽¹⁷⁾.

Materials and methods:

Chemicals and reagents:

- Alcohol, hydrogen peroxide, crystal violet, distill water, immersion oil, lugol's iodine, methanol, methyl red, para- dimethyl-aminobenzaldehyde (Kovac's reagent), petroleum ether, suffranine.

Culture media: Blood base agar, DNase media, kliger iron agar, Koser's citrate, MacConkey agar, mulltor hintton agar, nutrient agar, nutrient broth, urea agar.

Medicinal plants extracts:

- *Carthamus tinctorius*
- *Psidium guajava*.

Bacterial Microorganism:

- *Klebsiella pneumonia* ATCC53657
(Gram –ve bacteria).
- *Pseudomonas aeruginosa* ATCC27853
(Gram –ve bacteria).
- *Staphylococcus aureus* ATCC25923
(Gram+ ve bacteria).
- *Streptococcus pyogenes* ATCC26722
(Gram+ ve bacteria).
- American type culture collection (ATCC)
Rockville, Maryland, USA.

Chemotherapeutic agents: amoclan, ampicillin, ciprofloxacin, erythromycin, gentamycin.

Methods:

Plants material:

The two plants used in this study were collected from Gazira state (*psidium guajava* from Al.

Kasamber, *Carthamus tinctorius* from Al-Sharafa Elbaher).

Hundred grams of each plant sample were powdered by grinder and extracted as described below and then subjected to antimicrobial activity screening.

Preparation of crude extract: ⁽¹⁹⁾

Each of the coarsely powdered plant material (100g) was extracted for 4 hours with petroleum ether in soxhlet apparatus. The petroleum ether extract was filtered and evaporated under reduced pressure using Rota- vapor. The extracted plant material was then air- dried, repacked in the soxhlet and exhaustively extracted with methanol. The methodic extract was filtered and evaporated under reduced pressure again using Rota- vapor. Each residue was weighted and the yield percentage was determined. The petroleum ether residue (1.6g) was dissolved or suspended in petroleum (16ml) to a final volume (16ml) conc. (10mg/ml). The methanol residue (1.6g) was dissolved in methanol (16ml) conc. (10mg/ml), and kept in refrigerator until used.

Aqueous extract for each dried ground plant (10g) was prepared by infusion using boiled distilled water. It was allowed to soak for 2 hours, then, it was filtered, and 1 ml taken from the residue was then dried and weighted and the yield percent age was obtained. The final volume of the residue was used immediately.

Preparation of bacterial suspensions:

The test organisms were aseptically inoculated in broth media and incubated at 37°C for 24 hours. From this media, 1ml was taken and distributed

on nutrient agar slopes, and incubated again at 37°C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8 - 10^9 colony forming units per ml (C.F.U/ml). The suspension was stored in the refrigerator at 4°C.

In vitro testing for antibacterial Activity:

The cup- plate agar diffusion method ⁽¹⁶⁾ was adopted to assess the antibacterial activity of the prepared extracts. One ml of the standardized bacterial stock suspension 10^8 - 10^9 (C. F. U/ml) was thoroughly mixed with 100ml of Mullor hintton agar which was maintained at 45, 20 ml aliquots of the inoculated Mullor hintton agar. They were distributed into sterile plates.

The agar 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 agar discs were removed. Alternate cups were filled with 0.1 ml samples of each of the extracts using automatic microlitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 hours.

Two replicates were carried out for each extract against each of the test organisms. After incubation, the diameters of the result growth inhibition zones were measured, averaged and the mean values were tabulated.

Results:

Screening for antimicrobial activity of *Psidium guajava* leaves and *Carthamus tinctorius* seeds:

In the preliminary screening for antibacterial activity of two Sudanese medicinal plants, belonging to two families, the total number of extracts examined against the tested organism (standard) was six; four of these extracts exhibited inhibitory activity against one or more of four tested bacteria. The two other extracts were devoid of any activity.

Four extracts exhibited inhibitory effects against both gram positive organisms (*S. aureus* and *S. pyogenes*) and gram negative organisms (*P. aeruginosa* and *K. pneumoniae*), but *S. aureus* was the most sensitive organism being inhibited by four extracts, also *Pseudomonas aeruginosa* was more susceptible whereby *Streptococcus pyogenes* and *Klebsiella pneumoniae* were less susceptible (table 1). One of two extracts of petroleum ether showed no activity against all tested organisms. Whereas, all methanol extracts exhibited inhibitory effect against all tested organisms. Meanwhile, one out of two aqueous extracts examined for antibacterial activity was inhibitory to all tested organisms. It is evident that methanolic extract exhibited the highest level of inhibitory effect against the bacteria tested, it could be due to the presence of polar compounds,

two methanolic extract exhibited inhibitory effect against the four tested bacteria (table 1).

The *Psidium guajava* leaves extract provided high activity against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *S. aureus*. Whereby, this extracts showed moderate activity against *S. pyogenes* (table 2). Figure 1 shows activity of petroleum ether extract of *Psidium guajava* leaves extracts against standard organisms. In contrast, *Carthamus tinctorius* seeds extracts provided no activity against all standard organisms used in this study (table 3). Whereas, reference antibacterial drugs used against standard organisms provided various results. Ciprofloxacin produced activity against all standard organisms, whilst ampicillin provided no activity against any one of all standard organisms (table 4).

Figure 1: activity of petroleum ether extract of *psidium guajava* leaves extracts against standard organisms:

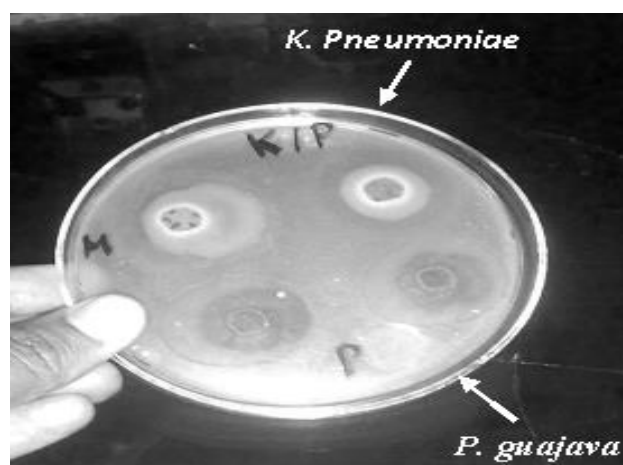


Table 1: Antibacterial activity of two plants extracts against the standard organisms:

Family/ Botanical/ vernacular names	Part used	Yield %	Solvent	Test organisms used MDIZ mm			
				<i>S.aureus</i>	<i>S.pyogenes</i>	<i>K.pneumoniae</i>	<i>P.aeruginosa</i>
Myrtaceae <i>Psidium guajava</i> (guava)	leaves	2.099	CHCl ₃	22	14	22.5	22.5
		15.258	MeOH	25.5	14.5	18.5	22.5
		0.05	H ₂ O	12.5	14.5	18.5	20
Asteraceae <i>Carthamus tinctorius</i> <i>Osfer</i>	Seeds	7.273	CH Cl ₃	-	-	-	-
		21.578	MeOH	16	11.5	14.5	16.5
		0.035	H ₂ O	-	-	-	-

CHCl₃ = chloroform

MeOH = Methanol

H₂O = water

MDIZ = Mean diameter of growth inhibition zone in (mm) average of (2) replicates.

MDIZ > 18 Sensitive, 14- 18 Moderate, 14 resistant, (-) No activity

Table 2: The activity of *Psidium guajava* leaves extract against standard organisms:

Standard organism	Solvent	No. of standard organism		
		Sensitive	Intermediate	Resistant
<i>S. aureus</i>	CHCl ₃	1	0	0
	MeOH	1	0	0
	H ₂ O	0	0	1
<i>S. Pyogenes</i>	CHCl ₃	0	1	0
	MeOH	0	1	0
	H ₂ O	0	1	0
<i>K. pneumoniae</i>	CHCl ₃	1	0	0
	MeOH	1	0	0
	H ₂ O	1	0	0
<i>P. aeruginosa</i>	CHCl ₃	1	0	0
	MeOH	1	0	0
	H ₂ O	1	0	0

Sensitive organism exhibit inhibition zone > 18 mm

Concentration used = 10 mg/ml at 0.1 ml/cup

MeOH = Methanol

H₂O = waterCHCl₃ = chloroform**Table 3:** The activity of *Carthamus tinctorius* seeds extract against standard organisms:

Standard organism	Solvent	No. of standard organism		
		Sensitive	Intermediate	Resistant
<i>S. aureus</i>	CHCl ₃	0	0	1
	MeOH	0	1	0
	H ₂ O	0	0	1
<i>S. Pyogenes</i>	CHCl ₃	0	0	1
	MeOH	0	0	1
	H ₂ O	0	0	1
<i>K. pneumoniae</i>	CHCl ₃	0	0	1
	MeOH	0	1	0
	H ₂ O	0	0	1
<i>P. aeruginosa</i>	CHCl ₃	0	0	1
	MeOH	0	1	0
	H ₂ O	0	0	1

Sensitive organism exhibit inhibition zone > 18 mm

Concentration used = 10 mg/ml at 0.1 ml/cup

MeOH = Methanol

H₂O = waterCHCl₃ = chloroform**Table 4:** Antibacterial activity of reference drugs against standard organisms

Drugs	Concentration used mg/ ml	Standard organisms used MDIZ mm			
		<i>S.aureus</i>	<i>S.pyogenes</i>	<i>K.pneumoniae</i>	<i>P.aeruginosa</i>
Ampicillin	40	-	-	-	-
	20	-	-	-	-
	10	-	-	-	-
	5	-	-	-	-
Ciprofloxacin	40	24	35	25	34
	20	21	35	25	32
	10	20	30	24	26
	5	19	30	20	20
Erythromycin	40	-	11	-	-
	20	-	11	-	-
	10	-	11	-	-
	5	-	11	-	-
Amoclan	40	13	11	14	15
	20	-	-	13	14
	10	-	-	12	-
	5	-	-	11	-
Gentamycin	40	15	25	17	26
	20	12	23	15	23
	10	-	15	12	12
	5	-	15	11	-

Discussion:

In this study, the petroleum ether leaves extract of *Psidium guajava* gave high activity against *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, and Moderate activity against *Streptococcus pyogenes*. This study agreed with Abdelraheem et al ⁽⁹⁾ and Almagboul et al ⁽⁵⁾.

The methanolic leaves extract of *Psidium guajava* gave high activity against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, and moderate activity against *Streptococcus pyogenes*. This result was in line with Abdelraheem et al ⁽⁹⁾, Almagboul et al ⁽⁵⁾ and Verma et al ⁽¹⁰⁾.

The aqueous leaves extract of *Psidium guajava* exhibited high activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, and moderate activity against *Streptococcus pyogenes* and low activity against *Staphylococcus aureus*. This finding agreed with the finding of Abdelraheem et al ⁽⁹⁾, Almagboul et al ⁽⁵⁾ and Holets et al ⁽⁴⁾.

The petroleum ether seeds extract of *Carthamus tinctorius* exhibited no activity against all four organisms. Meanwhile, the methanolic seeds extract of *Carthamus tinctorius* exhibited moderate activity against *staphylococcus aureus*, *klebsiella pneumoniae* and *pseudomonas aeruginosa*, and low activity against *streptococcus pyogenes*.

The aqueous seeds extract of *Carthamus tinctorius* showed no activity against all standard organisms.

The petroleum ether extract of *Psidium guajava* leaves inhibited *staphylococcus aureus* higher than 20 mg/ml of ciprofloxacin, and inhibited *Klebsiella pneumoniae* higher than 5 mg/ml of ciprofloxacin and *Pseudomonas aeruginosa* inhibited higher than 5mg/ml of ciprofloxacin. In contrast, it inhibited *Streptococcus pyogenes* lower than 5 mg/ml of ciprofloxacin.

The petroleum ether extract of *Psidium guajava* leaves inhibited all four standard organisms higher than 40 mg/ml of amoclan. Moreover, this extract of *Psidium guajava* leaves inhibited *Staphylococcus aureus* higher than 40mg/ml of gentamycin, adding to that it inhibited *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* higher than 10mg/ml of gentamycin, whereby, it inhibited *Streptococcus pyogenes* lower than 5ml/ml of gentamycin.

On the other side, methanolic extract of *Psidium guajava* leaves inhibited *Staphylococcus aureus* higher than 40mg/ml of ciprofoloxacin, amoclan and gentamycin, inhibited *Streptococcus pyogenes* higher than 40 mg/ml of amoclan and lower than 5 mg/ml of gentamycin and ciprofloxacin. Also, this extract inhibited *Klebsiella pneumoniae* higher than 40 mg/ml of amoclan and gentamycin, and lower than 5 mg/ml of ciprofloxacin. Moreover, it inhibited *Pseudomonas acerugionsa* higher than 40 mg/ml of amoclan, higher than 10mg/ml of gentamycin, and lower than 5 mg/ml of ciprofloxacin.

The aqueous extract of *Psidium guajava* leaves inhibited *Staphylococcus aureus* higher than 10 mg/ml of gentamycin and amoclan, and lower than 5 mg/ml of ciprofloxacin, inhibited *Streptococcus pyogenes* higher than 10 mg/ml of amoclan, and lower than 10 mg/ml of gentamycin and ciprofloxacin and inhibited *Klebsiella pneumoniae* higher than 10 mg/ml of gentamycin and amoclan, and lower than 5mg/ml of ciprofloxacin. This extract inhibited *Pseudomonas aerugionsa* equal to 5 mg/ml of ciprofloxacin, and higher than 10 mg/ml of amoclan and gentamycin.

The petroleum ether extract of *Carthamus tinctorius* seeds inhibited all tested standard organisms lower than 5 mg/ml of ciprofloxacin, amoclan and gentamycin. Whereas, methanolic extract of *Carthamus tinctorius* seeds inhibited *Staphylococcus aureus* higher than 40 mg/ml of amoclan and gentomycin, and lower than 5 mg/ml of Ciprofloxacin, inhibited *Streptococcus pyogenes* higher than 40 mg/ml of amoclan, lower than 10 mg/ml of gentamycin and lower than 5 mg/ml of ciprofloxacin, inhibited *Klebsiella pneumoniae* higher than 40 mg/ml of amoclan, and gentamycin, and lower than 5 mg/ml of ciprofloxacin, inhibited *Pseudomonas aeruginosa* higher than 40mg/ml of amoclan, higher than 10 mg/ml of gentamycin and lower than 5 mg/ml of ciprofloxacin.

The aqueous extract of *Carthamus tinctorius* seeds inhibited all tested standard organisms lower than 5mg/ml of ciprofloxacin, amoclan and gentamycin.

In this study, the ampicillin and erythramycin had no activity against four standard organisms.

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