

Antibacterial activity of *Hibiscus sabdariffa*, *Acacia seyal* var. *seyal* and *Sphaeranthus suaveolens* var. *suaveolens* against upper respiratory tract pathogens

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Abstract

Introduction:

Medicinal plants constitute an effective source of both traditional and modern medicines. The emergence of drug resistant bacteria mandates the need for newer antibiotics.

Objective

To evaluate the antibiotic effect of *Hibiscus sabdariffa*

Methods

Three medicinal plants have been selected for investigation of their antibacterial activities; *Hibiscus sabdariffa*. (Dried calyces), *Acacia seyal* var. *seyal* (fresh stem bark) and *Sphaeranthus suaveolens* var. *suaveolens* (herbs) The extracts of these plants at concentration of 100mg/ml have been tested against seven strains of bacteria.; qualitative tests using the Agar Well Diffusion Method have been carried out.

Results

E. coli showed higher resistance to the plant extracts whereas *Pseudomonas aeruginosa* is more sensitive.

Conclusion

Hibiscus sabdariffa extracts showed extensive inhibition zone and were, therefore, effective as antibacterial ingredient.

Key words: *Hibiscus sabdariffa*; *Acacia seyal* var. *seyal*; *Sphaeranthus suaveolens* var. *suaveolens*; antibacterial activities; respiratory tract pathogens.

Medicinal plants constitute to be an effective source of both traditional and modern medicines. Herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as first line curative medicine¹. The screening of extracts for antibacterial activity has revealed the potential of higher plants as a source of new anti-infective agents². Acute respiratory tract infections (ARTI) are a leading cause of childhood morbidity and mortality worldwide. Those at high risk in developing countries have limited access to modern medical service. Almost all ARTI deaths are due to pneumonia. The organisms isolated from the sputum included *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae* as well as anaerobic bacteria like *Bacillus spp.*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*³. *aeruginosa*³.

The objectives of this work are to determine the presence and effectiveness of

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antibacterial activity in the crude extracts of some of the traditionally used medicinal plants that are used to treat respiratory tract infections

Methods:

Plant material:

Hibiscus sabdariffa "karkadeh", *Acacia seyal* var. *seyal* "Taleh" and *Sphaeranthus suaveolens* var. *suaveolens* (Forsk) DC. "Zir El-Fitna".

Screening of plant extracts for their antibacterial activity:

Screening of crude plant extracts for their antibacterial activity involved two components: (a) Extraction of plant material with different polar and non-polar organic solvents. (b) Determination of sensitivity of pure bacterial culture to plant extract. Protocols for preparation of plant extracts⁴ are graphically presented as a flow chart in Fig 1.

Tested material:

Ethanol extract and fractions prepared from this extract (ethyl acetate and methanol) and aqueous extract prepared by 100grams air-dried powder plant material per litre distilled water and left for 24 hours at room temperature.

Bioassay:

a) Microorganisms:

Collection of seven standard bacterial strains associated with respiratory tract infections have been chosen for this study, including gram-

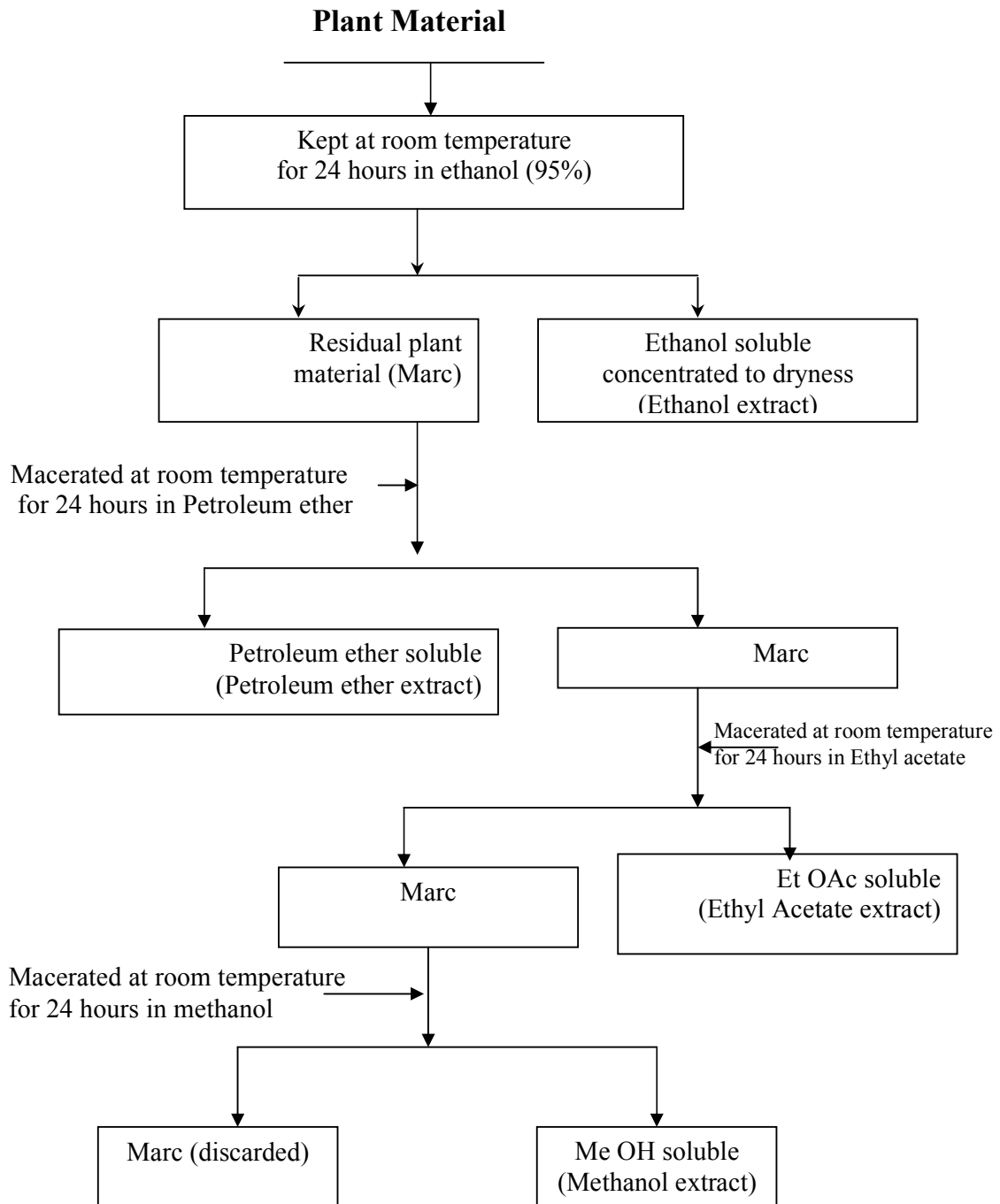


Figure 1. Flow chart for plant extraction

positive and gram-negative strains, were used: (1) gram-positive: *Streptococcus pyogenes* (ATCC 12344), *Streptococcus pneumoniae* (ATCC 6305), *Staphylococcus aureus* (ATCC 25923), (2) gram-negative: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 15380) and *Haemophilus influenzae* (ATCC 10211).

The organisms were activated and cloned three successive times in nutrient agar and stored on nutrient agar slants at 4°C. Subsequent cultivation and testes were done on nutrient agar medium.

b) Antibacterial testing:

The antibacterial activity was tested by Well-Agar Diffusion method⁴. 250 ml of sterilized nutrient agar was used for testing. The inoculum size of each tested organism was adjusted to suspension of 10⁶ cells. 2 ml of 24 hours old culture of bacteria were added to 250 ml of melted cooled test agar and after through mixing, approximately 20 ml of this seeded agar were poured into 10 cm diameter presterilized petri dishes and allowed to solidify. Three wells (10 mm in diameter) were bored in the agar using a sterile cork borer and the agar discs were removed. 0.1 ml aliquot of the reconstituted extract was placed into a well with a pipette and the plate was held for 2 hours at room temperature for diffusion of extract into agar. Subsequently, the plate was incubated at 37°C for 24 hours. After incubation, the diameters of the zones of inhibition were measured to the nearest mm⁵.

Gentamycin, tetracycline, ampicillin, penicillin and cephalosporin were used at concentrations ranging from 40 µg/ml to 5 µg/ml, as positive control and Dimethyl Sulphoxide (DMSO) as a negative control.

The sensitivity of tested bacteria to the investigated plant species has been determined by the parameter called the Relative Magnitude Inhibition (RMI):

Results:

Table 1 shows the sensitivity of the bacteria to the various samples tested. The more interesting species was *Hibiscus sabdariffa* for their broad spectrum of activity. Using qualitative test, the following classification could be made from the most susceptible to the least sensitive strains:

(a) Ethanol extract: *Streptococcus pyogenes* followed by *Streptococcus pneumoniae*, *staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Haemophilus influenzae* and then *Escherichia coli*.

(b) Methanol extract: *Pseudomonas aeruginosa* followed by *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus* and *Escherichia coli*.

(c) Aqueous extract: *Pseudomonas aeruginosa* followed by *Escherichia coli*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* and then *Streptococcus pneumoniae*.

The results of antibacterial activity of standard antibiotics against selected bacteria presented in Table 2.

Values were taken as the diameters of inhibition zone (IZ) (mm) and the means of three replicates; (-) = no inhibition. DMSO did not show any inhibitory activity.

Discussion:

Various solvent extracts (except ethyl acetate) of investigated plant species exhibited varying degrees of antibacterial activity. The antibacterial activity exhibited by the aqueous and ethanol extracts of *H. sabdariffa* (except ethanol extract against *E. coli* and *P. aeruginosa*) against all tested bacteria tend to corroborate folklore claims that this plant species are efficacious in the treatment of respiratory tract infections.

Previously it had been demonstrated that the polyphenolic nature of the flavonoid gossypetin isolated from the flowers of *Hibiscus sabdariffa* possesses a potent antibacterial activity against *Escherichia coli*, *Bacillus pumilus* and *Pseudomonas aeruginosa*⁶.

Compared to reference antibiotics, candidate plant species exhibited broader spectrum of antibacterial activity and were found to be clearly superior in case of *Hibiscus sabdariffa* and *Acacia seyal* var. *seyal*.

Some strains of bacteria, especially of the *Streptococcus pyogenes* and *Streptococcus pneumoniae* are becoming resistant to commonly used antibiotics, this study may be worthy of exploring newer antibacterial agents effective against these resistant bacteria.

Conclusion:

The aqueous and ethanol extracts were found to be effective against tested microorganisms. The ethyl acetate extract of three candidate plant species (except against *H. influenzae*) did not exhibited any antibacterial activity against the organisms under investigations. In general, maximum activity was observed against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The plant with most antibacterial activity was found to be *Hibiscus sabdariffa* where as the least active one was

Table 1. Antibacterial activity: Inhibition zone (mm) using the extracts from investigated plant species

Extracts	Plant species	Plant part	Tested organisms						
			1	2	3	4	5	6	7
Ethanol	<i>Hibiscus sabdariffa</i>	calyces	-	15 (0.5)	27 (1.7)	27 (1.7)	20 (1.0)	29 (1.9)	29 (1.9)
	<i>Acacia seyal</i> var. <i>seyal</i>	Stem bark	16 (0.6)	28 (0.8)	19 (0.9)	13 (0.3)	16 (0.6)	15 (0.5)	16 (0.6)
	<i>Sphaeranthus suaveolens</i> var. <i>suaveolens</i>	Whole plant	20 (1.0)	19 (0.9)	21 (1.1)	11 (0.1)	18 (0.8)	27 (0.7)	27 (1.7)
Ethyl acetate	<i>Hibiscus sabdariffa</i>	calyces	11 (0.1)	-	-	-	-	11 (0.1)	-
	<i>Acacia seyal</i> var. <i>seyal</i>	Stem bark	-	-	-	-	-	-	-
	<i>Sphaeranthus suaveolens</i> var. <i>suaveolens</i>	Whole plant	-	-	-	16 (0.6)	-	-	-
Methanol	<i>Hibiscus sabdariffa</i>	calyces	16 (0.6)	29 (2.9)	28 (1.8)	27 (1.7)	25 (1.5)	34 (2.4)	-
	<i>Acacia seyal</i> var. <i>seyal</i>	Stem bark	12 (0.2)	29 (1.9)	17 (0.7)	20 (1.0)	25 (1.5)	20 (1.0)	17 (1.7)
	<i>Sphaeranthus suaveolens</i> var. <i>suaveolens</i>	Whole plant	20 (1.0)	12 (0.2)	23 (1.3)	11 (0.1)	-	14 (0.4)	-
Aqueous	<i>Hibiscus sabdariffa</i>	calyces	30 (2.0)	31 (2.1)	22 (1.1)	25 (1.5)	23 (1.3)	20 (1.0)	19 (0.9)
	<i>Acacia seyal</i> var. <i>seyal</i>	Stem bark	21 (1.1)	16 (0.6)	22 (1.2)	11 (0.1)	21 (1.1)	-	-
	<i>Sphaeranthus suaveolens</i> var. <i>suaveolens</i>	Whole plant	15 (0.5)	20 (1.5)	21 (1.1)	14 (0.4)	24 (1.4)	11 (0.1)	-

1= *Escherichia coli* (ATCC 25922), 2= *Pseudomonas aeruginosa* (ATCC 27853), 3= *Klebsiella pneumoniae* (ATCC 15380), 4= *Haemophilus influenzae* (ATCC 10211), 5= *Staphylococcus aureus* (ATCC 25923), 6= *Streptococcus pyogenes* (ATCC 12344), 7= *Streptococcus pneumoniae* (ATCC 6305). Values were taken as the diameters of inhibition zone (IZ). (mm) and the means of three replicates; (-) no inhibition; The figures in parenthesis pertains to the parameter called the relative magnitude inhibition (RMI).

Table 2. Antibacterial activity: inhibition zone (mm) using the standard antibiotics

Antibiotics Bacteria	Gentamycin µg/ml				Tetracycline µg/ml				Ampicillin µg/ml				Penicillin µg/ml				Cephalosporin µg/ml			
	40	20	10	5	40	20	10	5	40	20	10	5	40	20	10	5	40	20	10	5
<i>Escherichia coli</i>	20	18	15	12	19	16	14	11	15	-	-	-	11	-	-	-	18	15	13	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	15	12	-	-	-	-	-	-	-	-	-	-	25	15	-	-
<i>Klebsiella pneumoniae</i>	20	18	12	-	20	18	16	15	22	20	18	15	15	-	-	-	28	24	17	15
<i>Haemophilus influenzae</i>	18	17	15	-	16	14	-	-	-	-	-	-	-	-	-	-	17	15	12	-
<i>Staphylococcus aureus</i>	21	-	-	-	17	15	12	-	15	-	-	-	14	12	-	-	25	20	15	-
<i>Streptococcus pyogenes</i>	20	15	12	-	15	-	-	-	-	-	-	-	-	-	-	-	22	19	15	-
<i>Streptococcus pneumoniae</i>	19	17	16	12	-	-	-	-	-	-	-	-	12	-	-	-	19	17	14	-

Values were the diameters of inhibition zone (IZ) (mm) and the means of three replicates; (-) = no inhibition. DMSO did not show any inhibitory activity.

Sphaeranthus suaveolens var. *suaveolens*. The result of this research provides valuable information and high light the potentiality of these plants in drug development. More investigation, however, are required to ascertain that in vitro results are attainable in vivo. For example, no information is available on bioavailability of the active ingredients, especially in extracts administered orally. The plant extracts tested in this study are generally regarded as safe.

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