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Studies on Antibacterial Activity of Some Medicinal Plants against Selected Bacterial Strain

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Abstract

The petroleum ether, methanol and chloroform extracts of five plants were evaluated to detect antibacterial activity against five standards bacterial strain viz *Bacillus subtitles* (NCTC 8236), *Klebsiella pneumonia* (ATCC 53657), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), using well-diffusion agar diffusion method. The petroleum ether and chloroform extracts were inactive compared to methanol extracts. The maximum antibacterial activity against the test organisms was found in methanol extract. Methanol extract of *Citrullus colocynthis* had maximum inhibitory activity (32 mm) against *Escherichia coli*. The MIC (minimum inhibitory concentration) of extracts was observed using well diffusion method. Amongst Gram negative bacteria *Escherichia coli* being inhibited at <3.12 mg/ml by *Citrullus colocynthis* root methanolic extract.

Keywords: Antibacterial potential; Medicinal plant extracts; Petroleum ether; Chloroform; Methanol

Introduction

Plants with medicinal properties have been utilized for the treatment of many human diseases since long. A revolution came in the medicinal world with the discovery of antibiotics, for treatment of various bacterial infections. However their indiscriminate use had led to an alarming increase in antibiotics resistance among microorganisms, giving rise to multi resistant strain, which has become global concern [1]. Thus, there is renewed interest in exploring natural resources for such compound. The need of the hour is to screen a number of new medicinal plants for promising biological activity and there in vitro propagation to conserve the biodiversity [2-4]. Infectious diseases account for about half of the death in tropical countries. Many studies indicate that in some plants there are many substances such as peptides, unsaturated long chain aldehydes, alkaloids constituents, some essential oils, phenol and water, ethanol, chloroform, methanol and butanol soluble compounds [5-7]. In Sudan, the use of plant compound for pharmaceutical purposes has gradually increased. The aim of this study was to investigate *in vitro* antibacterial activity of different extracts from five plant species against five standard bacterial strains.

Materials and Methods

Plant materials

The five plants (*Citrullus colocynthis, Grewia tenax, Mentha longifolia, Senna obtusifolia and Zingiber officinale*) were purchased from the local market in Omdurman. The voucher of these plants was deposited at herbarium of the Institute of medicinal and Aromatic plants, Ministry of High Education and Scientific Research. The seeds of *Citrullus colocynthis*, fruits of *Grewia tenax*, leaves of *Mentha longifolia*, and *Senna obtusifolia* and roots of *Zingiber officinale* were air-dried, coarsely powdered and were then extracted.

Preparation of the crude extracts

Different extracts were prepared by modification of the method according to [8].

Bacteriological techniques

The bacteriological techniques followed were those described by [9-11].

Tested bacteria

Five standard strains of bacteria were taken from National Collection Type Culture (NCTC) and American Type Collection Culture (ATCC). They were Gram positive (G +ve): *Bacillus subtitles* (NCTC 8236), *Staphylococcus aureus* (ATCC 25923) and Gram negative (G -ve): *Klebsiella pneumonia* (ATCC 53657), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922). The bacteria were generated and cloned successively for 3 times in nutrient agar and then were stored as nutrient agar slants at 4°C temperature. Subsequent tests and cultivation were performed on nutrient agar medium.

Antibiotics susceptibility testing

The effect of antibiotics on test organisms was obtained from using the same procedure of antibacterial susceptibility test, but instead of plant extracts, antibiotics were introduced into cup plate. The zone of inhibition was measured and recorded. Gentamycin, Tetracycline and

Ampicillin were used at concentration ranging from 40 mg/ml to 5 mg/ml. Antibacterial breakpoints and interpretation were taken from the CLSI standards [12,13].

Antibacterial testing

The well diffusion method was performed according to [14], to measure the antibacterial activity of the prepared extracts. 1 ml of the standardized bacterial stock suspension 108-109 C.F.U/ml were mixed thoroughly with 100 ml of nutrient agar (maintained at 45°C). 20 ml aliquots of the inoculated nutrient agar were poured into sterile Petri dishes.

The agar was allowed to dry and in each of these plates 4 wells (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were them removed. Alternate wells were poured with 0.1 ml of sample of each extracts using automatic micropipette apparatus, and then were allowed to diffuse at 37°C for 2 hours. The plates were incubated in the inclined position at 37°C for 18 hours. Duplicates were performed out for each extracts against each of the test organisms. Subsequently addition of each extracts was carried out like control. After incubation, the diameters and growth inhibition zones were measured, average values were calculated and then the mean values were tabulated.

Results and Discussions

The antibacterial properties of the petroleum ether, chloroform and methanol extracts of some medicinal plants viz (*Citrullus colocynthis* seeds, *Grewia tenax* fruits, *Mentha longifolia*, *Senna obtusifolia* leaves and *Zingiber officinale* roots) at concentration 100 mg/ml were tested against five standard bacterial strains: *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumonia* (ATCC 53657).

The results of diameter of the zone of inhibition are presented in Table 1. Plant extracts resulting in 15 mm or more growth inhibition zones were considered to be active and those resulting in less than 15 mm were inactive [11,15].

The leaves methanol extract of *Senna obtusifolia* was found to be effective similar to that of 40 mg/ml ampicillin against *Pseudomonas aeruginosa*. The roots extract of *Zingiber officinale* was found to be effective similar to that of 40 mg/ml Ampicillin against *Pseudomonas aeruginosa* (Table 1).

Botanic Names	Family	Solvents	Zone of inhibition (mm)				
			B. subtitles	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. pneumonia*
C. colocynthis	Cucurbitaceae	Petroleum ether	-	-	-	-	-
		Chloroform	-	-	-	-	27
		Methanol	-	27	32	26	-
G. tenax	Malvaceae	Petroleum ether	-	-	-	-	-
		Chloroform	-	-	-	-	-
		Methanol	16	16	23	-	18
M. longifolia	Lamiaceae	Petroleum ether	-	15	-	14	-
		Chloroform	20	20	20	18	20
		Methanol	17	17	15	17	16
S. obtusifolia	Fabaceae	Petroleum ether	-	-	15	-	-
		Chloroform	15	-	14	-	-
		Methanol	19	18	-	18	-
Z. officinale	Zingiberaceae	Petroleum ether	-	-	-	-	-
		Chloroform	18	15	20	18	14
		Methanol	16	16	17	19	14

Table 1: Antibacterial activity of chloroform, petroleum ether, and methanol extracts of selected medicinal plants against the bacterial strains.

Methanol extract of *Citrullus colocynthis* seeds showed high bacterial activity against *Staphylococcus aureus* (27 mm), *Escherichia coli* (32 mm) and *Klebsiella pneumonia* (26 mm) and chloroform extract against also *Klebsiella pneumonia* (27 mm). These might be due to the presence of alkaloids, steroid, glycosides and flavonoids. These results were similar to those reported by [16]. But petroleum ether extract of this plant seeds did not show any activity against all tested bacteria (Gram positive and Gram negative).

Methanol extract of *Grewia tenax* fruits showed high antibacterial activity against *Escherichia coli* (23 mm), whereas, petroleum ether and chloroform extracts of this plant fruits did not show any activity against all tested bacteria. The highest activity of the plant might be

due to the presence of β-carboline alkaloids this results was similar to that reported by [17].

Chloroform extract of *Mentha longifolia* leaves showed high antibacterial activity against *Bacillus subtitles*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* (20 mm), methanol extract was found moderately active against all tested (15-17 mm). This might be due to some active compounds in *Mentha longifolia* leaves. These results in agreement with [18], who reported that *Mentha longifolia* leaves showed the presence of important ones, were: 1.8- cineole (5.6-10.8%), methone (20.7-28.8%), terpineol-4 (3.1-4.9%), menthol (19.4-32.5%), pulegone (7.8-17.8%) and piperitone (2.2-3.3%).

Methanol extract of *Senna obtusifolia* leaves showed high antibacterial activity (19 mm) against *Bacillus subtitles* and (18 mm) against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Petroleum ether extract did not show any activity against all tested bacteria except *Escherichia coli* (15 mm). Chloroform extract was found moderately active against *Bacillus subtitles* (15 mm) *Escherichia coli* (14 mm).

Chloroform extract of *Zingiber officinale* roots showed high bacterial activity against *Escherichia coli* (20 mm) and methanol extract against *Pseudomonas aeruginosa* (19 mm), but petroleum ether extract did not show any activity against all tested bacteria. This might be due to the presence of bioactive components in the plant. This antibacterial activity supported by [19], who mentioned that this plant chemically include main constituents are sesquiterpenoids, with (–) zingiberene as main component with smaller amounts of their sesquiterpenoids (β-sequiphellandrene, bisabolene and farnesene) and small monoterpenoid fraction (β-phelladrene, cineol and citral).

The seeds methanol extract of *Citrullus colocynthis* was found to be effective similar to that of 40 mg/ml ampicillin against *Klebsiella pneumonia*. The fruits methanol extract of *Grewia tenax* was found as effective as 40 mg/ml gentamycin against *Escherichia coli*. The leaves chloroform extract of *Mentha longifolia* was found to be effective similar to that of 40 mg/ml tetracycline against *Klebsiella pneumonia* and 20 mg/ml tetracycline against *Bacillus subtitles*.

The MIC value shows that the methanolic extract of *Citrullus colocynthis* is significant against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The results were in agreement with the initial antimicrobial screening test result. *Escherichia coli* was more sensitive to the antimicrobial extracts from among the Gram negative bacteria being inhibited at <3.12 mg/ml (Table 2).

Antibiotics	Concentration	Zone of inhibition (mm)				
		B. subtitles	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. pneumonia*
Gentamycin	40	19	16	22	15	20
	20	15	12	18	13	18
	10	12	-	15	-	13
	5	-	-	12	-	-
Tetracycline	40	22	15	16	16	20
	20	20	12	15	13	18
	10	18	-	14	-	16
	5	16	-	12	-	15
Ampicillin	40	-	-	15	18	26
	20	-	-	-	15	23
	10	-	-	-	-	20
	5	-	-	-	-	19

Table 2: Antibacterial activity of antibiotic against different standard bacterial strain.

Conclusion

The antibacterial activity of the plants under the study (*Citrullus colocynthis* seeds, *Grewia tenax* fruits, *Mentha longifolia, Senna obtusifolia* leaves and *Zingiber officinale* roots), have shown good, and low activity, the antibacterial screening proved the significance of these plants and the frequent use by the healers as traditional medicines and indicated a good guiding for further research in these plants.

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