

Antimicrobial Activity of *Cannabis sativa* L.

Esra M. M. Ali¹, Aisha Z. I. Almagboul², Salwa M. E. Khogali¹, Umelkheir M. A. Gergeir²

¹Department of Biochemistry, Nutrition and Toxicology, Central Veterinary Research Laboratories, Khartoum, Sudan

²Department of Microbiology and Parasitology, Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum, Sudan

Email: aalmagboul@hotmail.com

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ABSTRACT

The oil of the seeds, petroleum ether and methanol extracts of the whole plant of *Cannabis sativa* belonging to the family Cannabinaceae were screened for their antimicrobial activity against two Gram positive organisms (*Bacillus subtilis*, *Staphylococcus aureus*), two Gram negative organisms (*Escherichia coli*, *Pseudomonas aeruginosa*) and two fungi namely *Aspergillus niger* and *Candida albicans* using the cup plate agar diffusion method. The oil of the seeds of *Cannabis sativa* exerted pronounced antibacterial activity (21 - 28 mm) against *Bacillus subtilis* and *Staphylococcus aureus*, moderate activity (15 mm) against *Escherichia coli* and high activity (16 mm) against *Pseudomonas aeruginosa* and inactive against the two fungi tested. The petroleum ether extract of the whole plant exhibited pronounced antibacterial activity (23 - 28 mm) against both *Bacillus subtilis* and *Staphylococcus aureus* organisms, high activity (16 mm) against *Escherichia coli* and inactive against *Pseudomonas aeruginosa* and both fungi. The methanol extract of the whole plant showed also pronounced antibacterial activity (29 mm) against *Bacillus subtilis*, low activity (12 mm) against *Staphylococcus aureus* and high activity (16 - 18 mm) against both Gram negative organisms, inactive against *Aspergillus niger* and low activity (13 mm) against *Candida albicans*. The minimum inhibitory concentrations of *Cannabis sativa* methanol extracts of the seeds and the whole plant against the standard organisms were determined using the agar plate dilution method. The standard organisms were tested against reference antibacterial and antifungal drugs and the results were compared with the activity of the extracts.

Keywords: Antimicrobial; *Cannabis sativa* Seeds; Whole Plant; Methanol Extract; Petroleum Ether Extract

1. Introduction

Cannabis sativa is a member of the family Cannabinaceae. *Cannabis sativa* preparation is known by various names worldwide. It is called Marijuana in America; Bhang, Ganja and Charas in India; Kif in North Africa; Dogga in South Africa; Krori in Tunisia, Habak in Turkey; Hashish in Middle East; Djomba or Liamba in Central Africa and Brazil; Sodom, Tampl, Gum, Gauge and stuff in Kinshasa, Swala and Whiskt in Ghana; Grifa in Mexico and Macohna in some parts of South America [1].

In Sudan, the most famous names of *Cannabis* preparations are Bango and Hashish. The name Bango in Sudan may be derived from the Indian name Bhang.

The chemical definition encompasses a variety of distinct chemical classes. The classical cannabinoids are structurally related to Tetrahydrocannabinol commonly abbreviated as THC, the nonclassical cannabinoids, the aminoalkylindoles, the eicosanoids related to the endocannabinoids, 1,5-diarylpyrazoles, quinolines and arylsulphonamides and additional compounds that do not fall into these standard classes but bind to cannabinoid receptors. The term cannabinoids also refers to a unique

group of secondary metabolites found in the cannabis plant, which are responsible for the plant's peculiar pharmacological effects. Currently, there are three general types of cannabinoids; phytocannabinoids occur uniquely in the cannabis plant; endogenous cannabinoids are produced in the bodies of humans and animals and synthetic cannabinoids which are similar compounds produced in the laboratory [2].

Cannabis was used in the twentieth century B.C in Egypt to treat sore eyes. In India, prior to the tenth century B.C., bhang was used as an anesthetic and anti-phlegmatic [1]. It is also used in both the Hindu and Moslem systems of drugs as spasmolytic, hypnotic, analgesic in mental conditions and to increase resistance to severe physical stress [3]. *Cannabis* is administered to patients suffering from rabies, cholera, rheumatism, epilepsy and tetanus. Also observation on the analgesic, anticonvulsive and muscle relaxant. In northeastern India, some of the plants species including *Cannabis sativa* have been used for the treatment of specific human ailments such as allergies, burns, cuts and wounds, inflammation, leprosy, leucoderma, scabies, smallpox and sexually transmitted diseases [4].

The plant was used as an anesthetic in surgery in ancient China. Cannabis in Europe and America became the drug of choice in the prevention of migraine attacks. Cases of uterine hemorrhage after birth, asthma, post-partum psychoses, various neuralgias and epilepsy were also successfully treated with Cannabis [3].

Kabelik *et al.*, [5] reported that Cannabis extract was used for irrigation in diseases of the anus, applied to sore toe nails and the seed pulp was a favorite dish. Edible, industrial and medicinal oils were obtained from the seeds.

Cannabis has an ancient tradition of usage as a medicine in obstetrics and gynecology. Cannabis extracts may represent an efficacious and safe alternative for treatment of a wide range of conditions in women including dysmenorrhea, dysuria, hyperemesis gravidarum and menopausal symptoms [6].

Cannabis is grown and processed for many uses. Many plant parts are used as medicine for humans and livestock, whole seeds and seed oil are eaten by human, seeds and leaves are fed to animals, seeds oil and stalks are burned for fuel. Whole plants, leaves and wood have environmental uses, bark, fiber and seeds are also of ritual importance [7].

The antimicrobial activity from ethanol and petroleum ether extracts against multiple microorganisms was reported [8]. Essential oils extracted from five fiber varieties of *C. sativa* had antimicrobial activity with the degree of antimicrobial activity varying between cultivars [9]. *C. sativa* has been reported to have pharmacological and also antimicrobial effects [10-14]. Cannabidiol has been identified as a component of hemp oil effective against Gram positive bacteria and yeast [15]. The *C. sativa* chemotypes grown in northern latitudes are reported to have a higher ratio of cannabidiol to tetrahydrocannabinol resulting in stronger antimicrobial activity [15]. *Cannabis sativa* grown in Pakistan has strong antimicrobial activity against all strains of bacteria and fungi [8].

The objective of this work is to evaluate the antimicrobial activity of the extracts of *Cannabis sativa* grown in Sudan against standard bacteria and the fungi.

2. Materials and Methods

2.1. Plant Material

The seeds and the whole plant of *Cannabis sativa* were obtained from Niala, South Darfur, Sudan in 2008, cleaned off from the impurities and dried. It was identified by Dr. Mohammed Abd Elraheem Jah Alnabi (Institute for Forensic Sciences).

2.2. Preparation of the Crude Extract

50 grams of the air-dried and coarsely powdered plant material of *Cannabis sativa* was exhaustively extracted for 20 hours with petroleum ether (40°C - 60°C) in a Soxhlet apparatus. The petroleum ether extract was filtered

and evaporated under reduced pressure. The extracted plant material was then air dried and repacked in the Soxhlet and exhaustively extracted with methanol. The methanol extract was filtered and evaporated under reduced pressure. Each residue was weighed and the percentage yield was determined.

2.3. Test Organisms

The extracts were tested against two Gram-positive organisms, *Bacillus subtilis* (NCTC8236) and *Staphylococcus aureus* (ATCC 25923), two Gram negative bacteria, *Escherichia coli* (ATCC25922), and *Pseudomonas aeruginosa* (27853) and two fungi *Aspergillus niger* (ATCC9763) and *Candida albicans* (ATCC7596). The bacterial cultures were maintained on nutrient agar slopes. They were grown in nutrient agar plate and incubated at 37°C for 18 hrs before being used for the tests. All microorganisms were obtained from the stock cultures of the Department of Microbiology, Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum, Sudan.

2.4. Testing for Antibacterial Activity

The cup-plate agar diffusion method [16] was adopted to assess the antibacterial activity of prepared extracts.

One ml of the standardized bacterial stock suspension (10^8 - 10^9) C.F.U/ ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45°C. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petridishes.

The agars were left to set and in each of these plates 4 cups (10 mm in diameter) were 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. Simultaneously positive controls involving the addition of the respective solvents instead of the extracts were carried out separately. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

2.5. Testing for Antifungal Activity

The same method as for bacteria was adopted instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25°C for one day for the yeast and three to five days for *Aspergillus niger*.

The minimum inhibitory concentrations (MIC) of the methanol extracts against the standard organisms were determined using the agar dilution method [17].

3. Results and Discussion

The oil of the seeds, petroleum ether and methanol extracts of the whole plant of *Cannabis sativa* belonging to the family Cannabinaceae were screened for their antimicrobial activity against two Gram positive organisms (*Bacillus subtilis*, *Staphylococcus aureus*), two Gram negative organisms (*Escherichia coli*, *Pseudomonas aeruginosa*) and two fungi namely *Aspergillus niger* and *Candida albicans* (Table 1).

The oil of the seeds of *Cannabis sativa* exerted pronounced antibacterial activity (21 - 28 mm) against *Bacillus subtilis* and *Staphylococcus aureus*, moderate activity (15 mm) against *Escherichia coli* and high activity (16 mm) against *Pseudomonas aeruginosa* and inactive against the two fungi tested. This result is similar to that reported by Novak *et al.* [9] who found that the oil extracted from *C.sativa* had antimicrobial activity and this could be due to the presence of sesquiterpenes or to the presence of cannabidiol [15]). The petroleum ether extract of the whole plant exhibited pronounced antibacterial activity (23 - 28 mm) against both Gram positive organisms, high activity (16 mm) against *Escherichia coli* and inactive against *Pseudomonas aeruginosa* and both fungi. The methanol extract of the whole plant showed also pronounced antibacterial activity (29 mm) against *Bacillus subtilis*, low activity (12 mm) against *Staphylococcus aureus* and high activity (16 - 18 mm) against both Gram negative organisms, inactive against *Aspergillus niger* and low activity (13 mm) against *Candida albicans*. This result is different from that produced by Borchardt *et al.* [18] who found that the leaves and stems extract was only active against *Staphylococcus aureus* and also different from that reported by Wasim *et al.* [8] who reported that *C.sativa* grown in Pakistan has strong

Table 1. Antimicrobial activity of *Cannabis sativa* extracts against the standard organisms.

Family Botanical/ Vernacular /names	Pat used	Yield %	Solvent	Standard organisms used MDIZ* (mm)					
				Bacteria*			Fungi**		
				<i>B.s</i>	<i>S.a</i>	<i>E.c</i>	<i>Ps.a</i>	<i>As.n</i>	<i>C.a</i>
Cannabinaceae <i>Cannabis sativa</i>	Seeds	30	MeOH	21	28	16	15	-	-
	Whole plant	50	Pet. ether	28	23	14	-	-	-
	Whole plant	50	MeOH	29	12	16	18	-	13

Standard organisms: *B.s** = *Bacillus subtilis*, *S.a* = *Staphylococcus aureus*, *E.c* = *Escherichia coli* and *Ps.a* = *Pseudomonas aeruginosa*; *As.n*** = *Aspergillus niger*, *C.a* = *Candida albicans*; MeOH = Methanol, Pet.ether = Petroleum ether, Concentration used 100 mg/ml at 0.1 ml/cup. Interpretation of results: M.D.I.Z. = Mean diameter of growth inhibition zones in (mm). Average of 2 replicates; M.D.I.Z. = >15 Sensitive; 14 - 15 = intermediate; <14 = resistant; (-) = No activity.

antimicrobial activity against all strains of bacteria and fungi. Also our result is similar to that produced by Wasim *et al.* [8] who reported antimicrobial activity from ethanol and petroleum ether extracts against multiple microorganisms. This shows in our results that the Gram positive organisms are more active than the Gram negative organisms and the fungi (Table 1).

The oil of *Cannabis sativa* seeds exerted high antibacterial activity against *Bacillus subtilis* which is similar to that produced by 20 µg/ml Gentamicin. It inhibited *Staphylococcus aureus* which is similar to 10 µg/ml Benzyl penicillin and 40 µg/ml Cloxacillin. It also inhibited *Escherichia coli* similar to 10 µg/ml Gentamicin and inhibited *Pseudomonas aeruginosa* similar to 20 µg/ml Gentamicin (Table 2).

The petroleum ether extract of the whole plant of *Cannabis sativa* inhibited *Bacillus subtilis* which is almost similar to 40 µg/ml Gentamicin and inhibited *Staphylococcus aureus* almost similar to 5 µg/ml Benzyl penicillin and 10 µg/ml Cloxacillin. It inhibited *Escherichia coli* almost similar to 10 µg/ml Gentamicin and inhibited *Pseudomonas aeruginosa* similar to 20 µg/ml Gentamicin (Table 2).

The methanol extract of the whole plant *Cannabis sativa* inhibited *Bacillus subtilis* and *Pseudomonas aeruginosa* almost similar to 40 µg/ml Gentamicin, *Staphylococcus aureus* similar to 5 µg/ml Gentamicin and inhibited *Escherichia coli* similar to 10 µg/ml Gentamicin (Table 2).

The activity of the methanol extract is very low even when compared with Nystatin (Table 3).

Table 2. Antibacterial activity of reference drugs against standard organisms.

Drugs	Concentration used µg/ml				
		<i>B.s</i>	<i>S.a</i>	<i>E.c</i>	<i>Ps.a</i>
Ampicillin	40	16	-	-	-
	20	14	-	-	-
	10	13	-	-	-
	5	12	-	-	-
Benzyl penicillin	40	-	38	-	-
	20	-	33	-	-
	10	-	28	-	-
	5	-	24	-	-
Cloxacillin	40	-	29	-	-
	20	-	27	-	-
	10	-	22	-	-
	5	-	18	-	-
Gentamicin	40	30	20	22	18
	20	20	16	18	16
	10	16	14	15	12
	5	16	12	11	-

Table 3. Antifungal activity of reference drugs against the standard organisms.

Drugs	Concentration used (µg/ml)	MDIZ (mm)	
		<i>Aspergillus niger</i>	<i>Candida albicans</i>
Clotrimazole	20	24	43
	10	19	33
	5	16	30
Nystatin	50	17	28
	25	14	28
	12.5	-	23

Table 4. Minimum Inhibitory Concentrations (MIC) of *Cannabis sativa* extracts against the standard organisms.

Part Used	Solvents system	Standard organisms/Minimum Inhibitory Concentrations (MIC) µg/ml		
		<i>S. a</i>	<i>E. c</i>	<i>Ps. a</i>
Seeds	MeOH	25	25	50
Whole plant	MeOH	50	25	12.5
Seeds	MeOH	25	25	50

The minimum inhibitory concentrations of *Cannabis sativa* methanol extracts of the seeds and the whole plant against the standard organisms showed that the methanol extract of the seeds against *Staphylococcus aureus* and *Escherichia coli* is lower than that of *Pseudomonas aeruginos* i.e. it is active against *S.aureus* and *E.coli* and the (MIC) of the whole plant showed that both Gram negative organisms exhibited lower (MIC) than the Gram positive organism (Table 4).

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