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ANTIMICROBIAL STUDIES OF THE LEAF OF *CANNABIS SATIVA L:*

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ABSTRACT

In vitro antimicrobial studies *were* conducted with aqueous, ethanolic and Petroleum ether extracts of the leaves of Cannabis *sativa* L. The acidic fraction was obtained from the ethanolic extract and 2% Sodium Hydroxide extract. Ethanolic extract, petroleum ether extract and the acidic fraction exhibited activity both against Gram-positive and Gram-negative bacteria and also against the fungi used in the study. The aqueous extract however, did not show any antimicrobial activity.

Introduction

Cannabis sativa L. (Hemp, Marihuana, Hashish) is widely grown in Pakistan. The drug is known all over the world for its euphoric properties but it also possesses good medicinal properties. The plant and its preparations have been used for its sedative, narcotic, antispasmodic, analgesic and many other properties including its use for Photophabia, migrain, asthma and piles.[1,2]

Antibacterial studies on the hemp grown in different parts of the world have been carried out by many researchers.

Krejci, in the course of a systematic investigation of Central European plants for Antibacterial components demonstrated that substances in hemp possess high Antibacterial activity. He isolated an amorphous, resinous substance soluble in ethanol, petroleum ether and benzene from acidic fraction of petroleum ether extract by treating it with Sodium hydroxide and then precipitating the water soluble salt with HCl. The substance was shown to possess good antimicrobial activity against Mycobacterium tuberculosis but the Gram-negative bacterial of the Coli-typhus group, Pseudomous aerogenes and Proteus Vulgaris were not affected.[3] Later on activity against many other bacteria was also demonstrated by Krejci and Frantisek.[4] Schultz and Haffiner also isolated antibacterial substance from German hemp.[5] Antibacterial sub-stance has also

been isolated by other researchers and its activity has been shown against many bacteria especially Gram-positive.[6,7]

The present work is planned to observe the antimicrobial activity of the extracts the Cannabis grown in Pakistan. It is planned to include Gram-positive and Gram negative bacteria, as well as the fungi in the study.

Experimental Procedures

Collection of Plant Material:

The plant material Cannabis *sativa* L. was collected from different areas of P tan; Lahore, Jehlam and Hilly areas. It was identified and authenticated by Dr. F Khan, Head of the Pharmacognosy Section, Department of Pharmacy, University the Punjab, Lahore. The plant was dried at room temperature under shade in Pharmacognosy Laboratory. The studies were carried out with *Cannabis sativa* L. grown in Lahore.

Extraction of Plant Material:

The extraction of the active principles was carried out with different solvents as water, ethanol, chloroform and petroleum ether.

i) Aqueous Extract
The leaves were extracted with water at 50-60°C for eight hours with shaking.

ii) Ethanolic Extract
The leaves were macerated in ethanol at a temperature of 40°C on a water bath.

iii) Petroleum Ether Extract
The leaves were extracted in a soxhlet extractor till final extraction was colourless.

Evaporation of the solvent from the above mentioned extracts was made reduced pressure.

Isolation of Acidic Fractions:

Many workers have obtained acidic fraction from the leaves of hemp grown at different areas of the world[6,7] and its antimicrobial activity has been reported. The acidic fraction was isolated following the procedure as specified in the literature and also using

an alternate procedure. Its antimicrobial activity was demonstrated against Gram positive and Gram negative bacteria as well as against certain fungi.

In the present work the following procedures were used for isolation of the above mentioned compounds.

1. Usual Method

The residue obtained after the evaporation of ethanolic extract of the dried leaves of *Cannabis sative* was dissolved in petroleum ether (b.p. 60-80°C). The petroleum ether solution was shaken with 2% NaOH (3 x 50 ml) and the aqueous layer was acidified with HO to give voluminous precipitates which were collected on sintered funnel and washed with *water. The* residue was dissolved in diethyl ether and on evaporation a brownish residue was obtained, *which* was acidic in nature and gave maxima at 262nm and 400nm when scanned at wave length 200,650nm (Fig. 1).

2. Alternative Method

The leaves were extracted with 2% NaOH at 50°C for eight hours. The liquid was passed through cotton plug and was acidified with HCl which produced voluminous precipitates. The precipitates were washed with water and dissolved in ether. Ether was evaporated and a brownish residue was obtained which was dissolved in ethanol. The ethanol soluble portion was collected and evaporated to dryness under reduce pressure. It was scanned at wave length from 200-650nm. and maxima were the same as above (Fig.2).

Concentrations of Extracts and Acidic Fraction:

Following concentrations in ethanol were used.

a) 1 mg/ml
b) 5 mg/ml
c) 10 mg/ml

Organisms Used

The following organisms were used to study the antimicrobial activity.

Gram positive

1. Baccilus subtilus
2. Baccilus pumilus
3. Staphlococcus aureus
4. Micrococcus flavus

Gram negative
1. Proteus vlugaris
2 Bordetella bronchioseptica

Fungi
1. Candida albicans
2. Aspergillus niger

All the organisms were procured from Drug Testing Laboratory, Government Punjab, Lahore.

Reference Standard

To study the antibacterial activity following refernce standard was used.

Celphalexin (USP Ref. Std.)	0.02 mg/ml in buffer pH-8

To study antifungal activity following reference standard was used.

Nystatin (USE Ref. Std.)	0.1 mg/ml in dimethylformamide

Testing of Antimicrobial Activity:

The following method mentioned in British Pharmacopeoca[8] was used to mine the antimicrobial activity.

Petri dishes were f$_i$lled with molten nutrient agar medium that had previous been inoculated with test organism and were solidified for 30 minutes before use. The solidified medium was bored aseptically at five points to a depth of 8 mm. Extracts, fraction and reference standard were introduced into separate boles with the help micro pippet. As ethanol was used as solvent for non aqueous extracts, it was used control and one of the hole for every organism was filled with ethanol. The petri were left for two hours at room temperature and then incubated at 37°C for 18 hours.

Results

The plant extracts which included aqueous, ethanolic and petroleum ether tracts and the isolated acidic fraction were studied for antimicrobial a Cephalexin and Nystatin were used as standard drugs.

1. Aqueous extract did not show any activity for Gram positive, Gram negative or fungi as shown in Table 1.

2. Petroleum ether extract exhibited activity against both Gram positive, Gram negative bacteria and also against *Aspergillus niger* and Candida with all concentration used i.e. 10 mg/ml, 5 mg/ml, l mg/ml. Activity even with 1 mg/ml concentration is fairly high. Cephalexin and Nystatin reference standards were used for comparison (Table 1).

3. Ethanohc extract similarly showed activity against all micro-organisms used in the study. The results of antimicrobial activity of petroleum ether and ethanolic extracts have been given in Table-1.

4. Acidic fraction isolated from the ethanolic extract exhibited good antimicrobial activity against Gram positive, Gram-negative bacteria and also against fungi used in the study (Table-2).

5. Acidic fraction isolated from sodium hydroxide extract exhibited similar activity as in case of above mentioned acidic fraction. The results of antimicrobial activity of acidic fraction isolated from ethanolic extract and from sodium hydroxide ex-tract have been given in Table-2.

Scanning at a wave length range 200-650nm showed maxima of the acidic fraction isolated from ethanolic extract or sodium hydroxide extract at 262nm and 400nm (Fig. 1 & 2).

Discussion

Aqueous extracts did not show any activity against any Gram positive or Gram negative bacteria. Also the extract was without effect on Candida albicans and Aspergillus.

For demonstrating the antimicrobial effect of ethanolic and petroleum ether extract the dilutions of the extract were made in ethanol, which was, therefore, used as control for each strain. The results recorded in Table-land Table-2 are shown after deducting sensitivity for ethanol if any.

Both ethanolic and petroleum ether extracts showed marked activity against all the strains of Gram positive and Gram negative bacteria. The activity is fair also at low concentration (1 mg/ml) and is comparable with standards used. It is evident that the

extracts can produce antibacterial effect at very low concentrations. Also the extracts showed marked activity against the fungi *Canidada albicans* and *Aspergillus niger*. The activity against these fungi has not been reported earlier. It is evident the Cannabis saliva grown in Pakistan has strong antimicrobial activity against bacteria and fungi.

The acidic fraction produced from the ethanol extract and sodium hydroxide extract showed activity similar to the crude extracts. Probably the antimicrobial activity of the crude extracts is due to this acidic fraction. The same acidic fraction, however, was also isolated from sodium hydroxide extract, which shortens the procedure to obtain the acidic fraction. The acidic fraction so isolated from ethanolic or sodium hydroxide extract exhibited maxima at about 262nm and 400nm. It showed marked activity against all of the strains of bacteria used, the fungi Aspergillus niger and Candida. The activity against fungi is *very* high even at low concentrations as compar with Nystatin at the concentration of 1 mg/ml used. Candida is a very common ca of the fungal infections and the antifungal substance with potent action against Candidiasis can be prepared from it.

Conclusions

It is evident from the results that the plant *Cannabis sativa* L. grown in Pakistan has strong antimicrobial activity against all the strains of bacteria and the fungi (Aspergillus niger and Candida). The activity against fungi is very high even with low concentrations when compared with Nystatin. Candida is very common cause of fungal infections. After clinical trials on humans, this antifungal acidic fraction with potent action against Candida can be produced on commercial scale.

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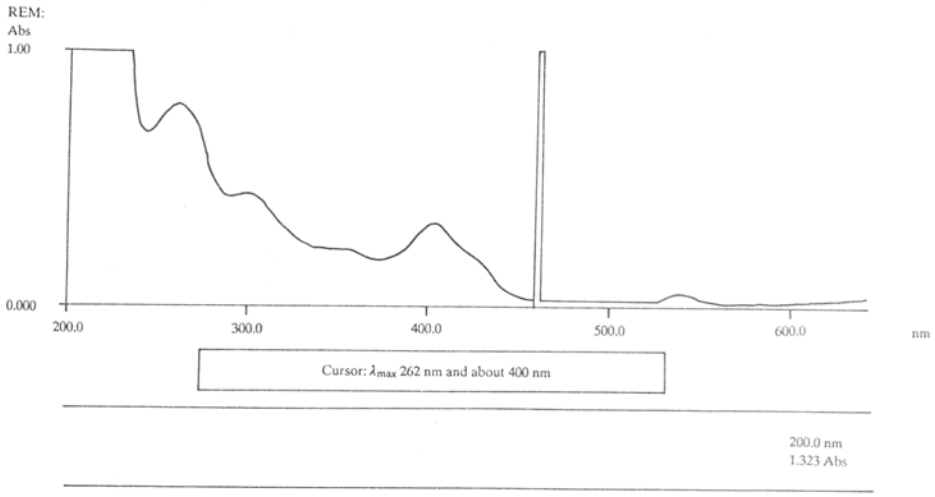

Fig. 1: Spectrophotometric scan of the acidic fraction obtained from Ethanolic extract of *Cannabis saliva* leaves.

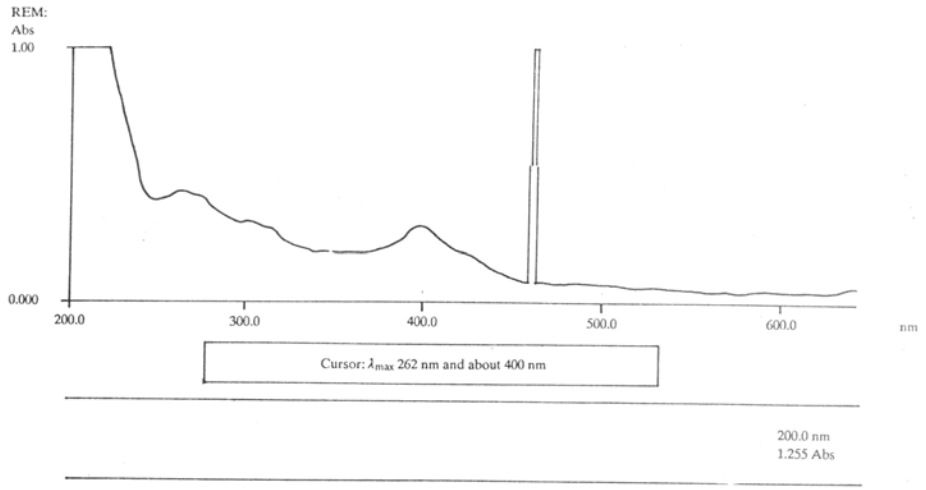

Fig. 2: Spectrophotometric scan of the acidic fraction obtained from Sodium Hydroxide extract of *Cannabis saliva* leaves.

Table 1
Antimicrobial studies on the extract of leaves of *Cannabis sativa L.*

Extract/Drug	Concen-tration mg/ml	Zone of Inhibitation (mm) Gram + IVE Bacteria			Gram - IVE			Fungi	
		B. subtilus	E. pumilus	S. aureus	M. flavus	B. bronchio-septics	P. vulgaris	A. Niger	C. albicans
Cephalexin	0.02	24.65	27.00	25.85	21.25	22.5	24.15	*	*
Nystatin	0.1	*	*	*	*	*	*	15.70	12.50
Petroleum Ether Extract	1	20.15	20.35	18.75	19.75	19.50	19.25	19.70	20.45
	5	24.20	25.50	22.50	25.90	20.75	24.25	24.50	24.75
	10	32.45	27.60	35.76	34.74	34.35	30.25	25.75	32.74
Ethanolic Extract	1	17.20	18.50	21.75	19.70	18.75	18.80	26.70	18.50
	5	24.90	23.25	24.25	24.90	22.10	26.75	26.35	23.15
	10	34.45	38.70	32.37	36.20	33.90	34.50	33.80	32.40
Aqueous Extract	100	-	-	-	-	-	-	-	-

*Not applied
- No senstivity

Table 2
Antimicrobial studies on the acidic fraction obtained from leaves of *Cannabis sativa* L.

Isolated/ Drug	Concen-tration mg/ml	Zone of Inhibitation (mm) Gram + IVE Bacteria			Gram - IVE			Fungi	
	B. subtilus	B. pumilus	E. aureus	S. flavus	M. septics	B. bronchio-vulgaris	P.	A. Niger	C. albicans
Cephalexin	0.02	22.45	24.54	22.45	25.40	24.60	22.75	*	*
Nystatin	0.1	*	*	*	*	*	*	18.70	15.35
Acidic	1	27.65	24.57	23.75	23.55	24.67	27.10	24.45	25.50
fraction from	5	29.75	26.56	28.20	27.75	26.57	28.50	26.54	32.50
organic solvent	10	35.54	33.45	34.75	34.10	37.00	32.50	36.70	40.10
Acidic	1	24.10	22.45	24.25	19.65	22.45	24.15	16.75	18.75
fraction obtained	5	27.75	30.60	26.50	28.75	27.70	27.25	25.42	26.75
from 2% Hydr. Extract	10	32.54	34.45	28.45	32.74	36.57	34.45	30.57	34.55

* Ref. standard not applied.

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