Research Article

Evaluation of antimicrobial activity and ethnobotanical study of *Cannabis sativa* L.

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Abstract

Plants are a great source of antibacterial components. Many plant extracts are being used in daily life to fight fungal and bacterial infections. In the present study *in vitro*, test confirmed the activity of antimicrobial of *Cannabis sativa* L. extracts against different bacterial and fungal strains. Antimicrobial efficiency of *C. sativa* a therapeutic plant (extracts of leaf) was screened using acetone, chloroform, water and ethanol, as solvents and tested against three Bacteria: *E. coli*, *Staphylococcus aureus* and *P. aeruginosa*, Fungi: *A.niger* and *Fusarium spp.* by disk diffusion and agar well diffusion method. All the plant extracts exhibited significant effects on antimicrobes, but the ethanolic extract and acetone extracts of *C. sativa* exposed highest zone of inhibition against most of the microorganisms that are under study. The minimum zone of inhibition was determined in distilled water and chloroform extract of *C. sativa* exhibited minimum activity of antimicrobial against some the tested strains. To find out the ethno botanical uses of *C. sativa* a survey from the rural people of study area (Bhimber Rajani) was carried out, by interviewing the local inhabitants through a questionnaire. It was noticed that it is used assedative, narcotic, intoxicant and analgesic. As a green manure leaves are used. Hashish or Charas is also prepared from it. The powder is used for bronchitis, cough and chest problems.

Keywords: Antibacterial activity; Ethnobotany; Medicinal plants

Introduction

The utilization of plants for treating diseases, to flavor and conserve food is as ancient as the human history. According to WHO, it was claimed that indigenous plants have been employed since long time ago and according to consideration it has been concluded that in the region of Africa, 80% inhabitants utilized therapeutic plants via extraction for curing

principle [1]. Above than 50,000 herbal plants has employed in pharmaceuticals by The Food Agriculture Organization. Plant products have potential to control microbial growth in diverse situations and to treat many diseases [2].

For the human health drug resistance of microorganisms is a big risk. To reduce this problem, herbal medicines were used as

alternative potential treatment. For treating many diseases plants used traditionally and no sign of drug resistance has been reported till now. In our medical exercise hundreds of plants are used just like *Cannabis sativa* L. is a plant that has chemical elements and effects. In the past *Cannabis* is the great controversial plant [3]. Now a days *C. sativa* would be exposed, rising in some portions of the world, its nature would be healed; that plant has the potential to cure the disease from headaches to cancer to nervous disorders.

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

Materials and methods

Plant material

Leaves of *C. sativa* were collected from Bhimber AJK. The leaves were shadow dried and crushed into powder using an electrical mixer. For further use powder was kept in air tight containers.

Test organisms

Clinical isolates of three bacteria (*S. aureus*, *E. coli*, *P. aeruginosa*) fungal strains (*A. niger* and *Fusarium spp.*) were selected on their pharmacological and clinical importance. The fungal and bacterial stock cultures were incubated for 37 hours at 24°C on potato dextrose agar (PDA) and nutrient agar medium respectively, next kept in refrigerator at 4°C.

Preparation of plant extracts

Leaf extraction of *C. sativa* was completed with acetone, chloroform, 60% Ethanol and distilled water. For preparing extracts, 10 g of dry leaf powder of *C. sativa* was taken and mixed with 100 ml of each solvent. The mixtures of acetone and chloroform were kept for 48 h and mixtures of ethanol and water were kept for 5 days, to ensure maximum metabolite extraction. The filtered extracts were stored at 4°C [4].

Antibacterial screening

Paper disc diffusion method

Nutrient Agar plates and potato dextrose agar (PDA) were prepared and by the spread plate method experimental microorganisms were inoculated. Previously prepared agar plates used for the antimicrobial activity. On that plates filter paper discs about 6mm in diameter were soaked with the plant extract was used. The agar plates were then incubated at 37°C. After the incubation diameters of the zone of inhibition were measured in millimeters. All these experiments performed at least three times. Average values ($\pm$ standard deviation) are reported in this study. Standard discs of Ampiciline (2 μ g) served as positive controls for antimicrobial activity [5].

Agar-well diffusion method

Nutrient Agar plates and potato dextrose agar (PDA) were prepared and the assessment microorganisms were inoculated by the spread plate method. Petri plates containing 20ml nutrient agar and potato dextrose agar (PDA) were seeded with 24hr culture of bacterial and fungal strains respectively. On these plates, wells (10mm diameter and about 2 cm a part) were dug with the help of a sterile borer. Each plate had six wells at equal distance. For each pathogen as well as for plant sample, separate plates were prepared. By the 20 μ l plant extracts were filled the wells (namely distilled water, methanol, acetone and chloroform extracts). The plates were then incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for 48 hours fungal pathogens. Antibacterial or antifungal activity of each extract was expressed in terms of zone of inhibition (diameter in mm) formed around the well. Ampicillin disc and distilled water were used as a positive control [6].

Ethnomedicinal/ Folklore therapeutic uses of the plant from study area methodology used for data collection

There is a broad range of potential therapeutic applications of *C. sativa*, due to which it is extensively used for various purposes. The data are gathered by using the questionnaire method from the study area. In order to extract maximum knowledge from people a survey plan was designed and causal interview were taken from the inhabitants. The field survey was conducted during the year 2014 in the months of June, July, August and September. Several visits were made to Tehsil Bhimber of District Bhimber. This field data was first collected and then documented from the selective areas of district Bhimber. During the field trips the information was collected by questionnaire method. In this way a maximum information was collected about the folklore uses of *Cannabis sativa*.

Statistical analysis

All the data that was collected was analyzed by applying statistics on data. Simple arithmetic means of the different extracts and standard error was used to find out the antimicrobial activity of *C. sativa*. Data of the questionnaire was analyzed using DMR. DMR was used to find out the used citation of given plant species based on the data collected from the respondents. The values (average scores) were summed up and ranked.

Results and discussion

The results portrayed that the leaves of *Cannabis sativa* have antibacterial and antifungal activity on selected pathogens. Effectiveness of different extracts is determined by the size of the control organism growth inhibition zone around the well (diameter of zone in mm).

Table 1 and figure 1 showed that the wider zone of inhibition was shown by the acetone

extract, i.e., 24.6 mm while the value decreases to 17.6 mm of aqueous extract when tested against the bacteria. The result showed that there is a need to use a broad range of extractive solvents in the extractions of possibly phytochemicals from medicinal plants.

As compared to disc method wider zones of inhibition obtained by agar well diffusion assay. The zone of inhibition was 29 mm for the extract of ethanol of plant against *Escherichia coli*. For the paper disc assay agar diffusion assay was used against 19.3 mm. According to Omenka and Osuaba [7], for the enhancing contact with the organism agar well diffusion assay permits well diffusion of the extracts into the medium. As a barrier paper discs play a role between the organism and extract, through the discs into the medium avoiding complete dispersion of active components absorbed and also responsible for the experimental variances, shown in (Table 1 & Figure 1 & 2).

Results represented in (Table 3 & Figure 3) showed that best solvent of aqueous and ethanolic extracts for extracting antifungal elements from this plant compared to water and chloroform. The extensive zone of inhibition (24.3 mm) for aqueous and 23 mm for ethanolic extract, was demonstrated while the value dropped to 18.3 mm for chloroform extract, when tested against the fungi.

The larger zone of inhibition was 29.6 mm for the aqueous extracts of *C. sativa* against *Aspergillus niger* when agar diffusion assay was used, it's the maximum value and the smallest zone of inhibition was for 12.6 mm for aqueous extract, shown in (Table 4 & Figure 4).

Table 1. Measurement of antibacterial activity (zone of inhibition, mm) of *Cannabis sativa* Leaf extracts using paper disc method

Experimented Organisms	Acetone extract	Chloroform extract	Ethanol extract	Aqueous extract	Ampicillin
<i>Escherichia coli</i>	24.66±1.52	23±2	19.33±1.15	20.3±0.57	17.3±0.57
<i>Staphylococcus aureus</i>	19.6±2.08	18.6±2.08	18.6±3.05	24.6±4.16	18±1
<i>Pseudomonas aeruginosa</i>	19±2.6	22.3±1.52	20±1	17.6±1.52	19.6±2.5

Table 2. Measurement of antibacterial activity (zone of inhibition, mm) of leaf extracts of *Cannabis sativa* using agar well diffusion method

Test Organisms	Acetone extract	Chloroform extract	Ethanol extract	Aqueous extract	Ampicillin
<i>Escherichia coli</i>	18±1	19.6±2.08	29±1	21.6±1.52	22.6±2.08
<i>Staphylococcus aureus</i>	19.6±2.5	19.3±2.08	23.3±2.08	26.3±3.05	19.6±1.52
<i>Pseudomonas aeruginosa</i>	18.6±2.8	18±1	22±1	25±2.6	19±1.7

Table 3. Measurement of antifungal activity (zone of inhibition, mm) of leaf extracts of *Cannabis sativa* using paper disc method

Test Organisms	Acetone extract	Chloroform extract	Ethanol extract	Aqueous extract	Distilled water as control
<i>Aspergillus niger</i>	21.3±2	20.6±1.5	23±2	21±2.6	00
<i>Fusarium spp.</i>	20±2.64	18.3±1.52	21.3±3.21	24.3±3.51	00

Table 4. Measurement of antifungal activity (zone of inhibition, mm) of leaf extracts of *Cannabis sativa* using agar well diffusion method

Test Organisms	Acetone extract	chloroform extract	Ethanol extract	Aqueous extract	Distilled water as control
<i>Aspergillus niger</i>	19.3±1.15	19.6±2.08	18±1	19±1	00
<i>Fusarium spp.</i>	13.6±1.52	16.6±0.57	13±1	12.6±2.08	00

Figure 1. Zone of inhibition of leaf extracts of *Cannabis sativa* determined against bacteria by using Paper Disc Method

Figure 2. Zone of inhibition of leaf extracts of *Cannabis sativa* determined against bacteria by using Agar well diffusion Method

Figure 3. Zone of inhibition of leaf extracts of *Cannabis sativa* determined against fungi by using Paper Disc Method

Figure 4. Zone of inhibition of leaf extracts of *Cannabis sativa* determined against fungi by using Agar well diffusion Method

List of ethnobotanical uses of *cannabis sativa* in study area according to the data collected by using questionnaire

1. Used as a stimulant and tonic.
2. Used for stomach diseases, chest problems, whooping cough & muscle contraction.
3. Seeds are occasionally eaten and much valued for feeding birds.
4. Leaves are used as green manure.
5. Seed oil is massaged on the affected part(s). The treatment is undertaken at bedtime once a day.
6. It is used to cure urogenital diseases.
7. It is also used as firewood.
8. Consumed in food as flavoring agent.

The plant extracts which included aqueous, chloroform, ethanolic and acetone extracts were studied for antimicrobial activity of *Cannabis sativa*. Our initial study displayed that all aqueous, chloroform, ethanol and acetone extracts of *C. sativa* were active against the bacteria including *S. aureus*, *P. aeruginosa* and *E. coli* fungal strains, *Fusarium spp.* and *A.niger*. The ethanol extracts of *C. sativa* portrayed significant activity of antimicrobial against selected microorganisms. For the action of these plant elements is not completely study, different types of solvent used for the, clear and effectiveness study of the plant. As compare to aqueous extracts maximum results exposed in organic extracts. Cowan explained that maximum compound for the antibiotic previously known in plant recorded as saturated organic or aromatic that can simply solubilized in organic solvents. The research also exposed that extract of aqueous and chloroform exposed moderated antimicrobial activity. The results similar with the research work of Esra *et al.* [3] that screened the activity of antimicrobial of *C. sativa*. Whole plant extracts of petroleum ether showed activity of the antibacterial 23-28 mm against both *B. subtilis* and *S. aureus*, maximum potential i.e., 16 mm against *E. coli* and inactive against *P. aeruginosa* and

both fungi. The whole plant of the methanolic extract exposed 29 mm zone of inhibition against *B. subtilis*, low activity i.e., 12 mm against *S. aureus* and maximum activity i.e., 16-18 mm against both Gram negative organisms, inactive against *A. niger* and low activity (13 mm) against *C. albicans*. Naveed *et al.* [8] also worked on antibacterial activity of *C. sativa* and showed similar results that the leaf used definite activity of antibacterial against *S. aureus*, *P. aeruginosa*, *E.coli* and *Enterococcus faecalis* (24.1mm), (10.3mm), (22.2mm), (18.1mm) respectively and inactive against the two strains *Klebsiella* and *Salmonella typhi*.

Conclusion

In conclusion, of the current search *Cannabis sativa* comprises antimicrobial elements for the cure of numerous diseases that may be of excessive use for the progress of pharmaceutical industries as a therapy. Results exposed that extract of leaf showed notable activity of antimicrobial against all the experimental species. Some of the leaf extract of were *Cannabis sativa* acceptable with respect to the standard antibiotics and so can be used as a natural remedy for treatment of numerous fungal and bacterial infections without developing resistance among any fungal/bacterial species. The second aim of the study to enlist the ethno botanical uses of *Cannabis sativa*. It is reported that it is extensively used for various purposes as drug, to cure various diseases etc. Further research is needed on this plants to study other diseases which are not reported by local inhabitants and to utilize it in pharmacology, drugs production, phytochemistry and other fields of science.

Authors' contributions

Conceived and designed the experiments: M Anjum, Performed the experiments: S Azam & J Khadim

Analyzed the data: P Rehman, Contributed materials/ analysis/ tools: J Khadim, Wrote the paper: ZE Arooj & M Anjum.

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