INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 4, p. 65-70, 2014

RESEARCH PAPER OPEN ACCESS

In vitro antibacterial activity of *Cannabis sativa* leaf extracts to some selective pathogenicbacterial strains

Muhammad Naveed*, Tahir Ali Khan, Izhar Ali, Adil Hassan, Hamid Ali, Zaheer Ud Din, Zohaib Hassan, Shumaila Tabassum, Saqib, Abdul Majid, Mujaddad Ur Rehman

Department of Microbiology, Hazara University, Mansehra 21300, Pakistan

Key words: Antimicrobial, well diffusion method,*Cannabis sativa*, zone of inhibition.

http://dx.doi.org/10.12692/ijb/4.4.65-70 Article published on February 20, 2014

Abstract

Plantmaterials are important for animaland human health care and also important for microbial controlling program. This present study has been attempt to determine the antibacterial activity of *Cannabis sativa* leaf extract to some selective pathogenicbacterial strains such as *Staphylococcus aureus*, *Escherichia coli,Pseudomunas aeruginosa*, *Enterococcusfaecalis*, *Salmonella typhi* and *Klebsiella* by using leaf Ethanol extract and Hot water extract. Antibacterial activity of Cannabis Sativa was evaluated by well diffusion methods.The highest zone of inhibition produced by Ethanol extract. The leaf of Cannabis Sativa exerted pronounced antibacterial activity (24.1mm) against *Staphylococcus aureus*, (10.3mm) against *Pseudomonas aeruginosa*, (22.2mm) against *Escherichia coli*, (18.1mm) against Enterococcus faecalis respectively and inactive against the two strains*Salmonella typhi* and *Klebsiella*.The minimum inhibitory effect of *C. sativa* leaf extract is due to certain compounds present in the *C.sativa*.Further research should be done to identify the compounds responsible for its activity which can be used as medicines to control a wide range of disease in the world.

* **Corresponding Author:** Muhammad Naveed ✉ microbiologistkhan@yahoo.com

Introduction

Antimicrobial activity of therapeutic plants has turn out to be a global concern. This problem is one of great issue particularly in 3rd world countries because are one of the major causes of mortality in these countries is due to these infectious diseases. There is a continuous and serious need to discover new antibacterial and anti-fungal compounds for new infectious diseases (Majid *et al.*,2013).

Cannabis sativa is a dioecious, annual and herbaceous plant belongs to family Cannabinaceae. *Cannabis sativa* grows well at low temperature, and well-adjusted to moderate climates. The most essential*Cannabis sativa* products in the food and drug trade are whole hemp seed, hulled hemp seed, hemp seed oil, marijuana, and hashish (Adams and Martin, 1996). *Cannabis sativa* are commonly known as marijuana that grows freely throughout the universe. This plant most commonly is known today as a powerful psychoactive substance, but for many years it was cultured primarily for its fibers and these hemp fibers were used in the production of rope, clothes and ship sails (Maisto*et al.,* 1999).This plant is one of the most insufferable, maligned and detested anywhere in the universe and huge sums of money and efforts are being used to thrash its production, supply, marketing and consumption (Ayenigbara, 2012).

Cannabis sativa leaves are best, astringent, tonic, aphrodisiac, alterative, intoxicating, stomachic, analgesic and abortifacient. They are used in convulsions, otalgia, abdominal disorders, malarial fever, dysentery, diarrhoea, skin diseases, hysteria, insomnia, gonorrhoea, colic, tetanus and hydrophobia. Its extreme use causes dyspepsia, cough, impotence, melancholy, dropsy, restlessness and insanity. The bark istonic, and is useful in inflammations, haemorrhoids and hydrocele. The resin is smoked to allay hiccough and bronchitis. It is useful in insomnia, sick headaches, neuralgia, rnigrain, mania, whooping cough, asthma, dysuria and in relieving pain in dysmenorrhoea and menorrhagia (Merzouki *et al.,* 2000; Nath *et al.,* 1997; Anonymous, 1996). Therapeutically, Indian hemp has been used in the treatment of diseases and health problems such as HIV/AIDS, glaucoma, eye problems, cachexia, treatment of pain, muscle spasticity, convulsion, insomnia, asthma, hypertension, depression etc. Cannabis is being used as a shampoo and for other cosmetic purposes (Maisto *et al.,* 1999).

Marijuana (*Cannabis sativa*) has been known tocontain antibacterial cannabinoids which arecannabidiol, cannabichromene, cannabigerol, Δ9-tetrahydrocannabinol and cannabinol. All these compounds showed effectiveactivity against a range of methicillin-resistant*Staphylococcus aureus* (MRSA) strains (Appendino *et al.,*2008). Thisplant is known throughout the globe for its good excited and medicinal properties and also its preparations have been used for its good antibacterial studies (Kreji,1958). The leaf of this plant possess good antimicrobial activity against *Mycobacterium tuberculosis*, Gram-negative bacteria of the Escherichia coli, *PseudomounasAeruginosa, Proteus Vulgar, Enterococccus facalis,*acid-fast bacteria, yeast like fungi, filamentous fungi and dermatophyt (Turner *et al.,* 1981; Wasim *et al.,* 1995). Cannanbinoids havestrong antileishmanial activity and effective to killing *Candida albicans* (Whittakar *et al.,* 2004). Thecontact of both herpes simplex virus type 1 and herpes simplex virus type 2to various absorptions of delta-9- tetrahydrocannabinol present a plaque assay utilizing confluent monkey cells that have possible mechanisms for antiviral activity and that this activity is modified by the presence of serum proteins (Lancz *et al.,* 1991; Blevein *et al.,* 1980).The antibactericidal acitvity of delta9-tetrahydrocannabinol and cannabidiol for *Staphylococci* and *Streptococci* in broth are in the range of 1-5 µg/ml (Klingeren and Ham, 1976).

The present study was conducted to investigate the antibacterial activity of Cannabis Sativa leaf extracts against gram positive ATCC (Amrican type cell Culture) bacteria S.aerious ATCC®6538, and gram negative bacteria Escherichia coli ATCC®25922,

Pseudomonas aeruginosa ATCC®74303 and *Enterococcus faecalis* ATCC®35824.

Materials and methods

This research work was conducted at the research laboratory of Microbiology Department, Hazara University Mansehra, Pakistan.

Collection of Plant Material

Healthy, disease free, mature *Cannabis Sativa* leaves were collected directly from the back side of vice chancellor office garden campus Hazara University Mansehra Pakistan and brought to the research laboratory of Microbiology Department, Hazara University Mansehra. The leaves were cleaned with tap water. After cutting the leaf into small pieces, they were air dried in room temperature for 5-7 days, and then dried leaves were crushed into a fine powder by blender machine.

Preparation of Hot Water and Ethanol extracts

Five grams powdered samples of leaf was soaked in 50ml cold water in 250ml sterile flask and rotated on shaker at 150 rpm for 24 hours at room temperature. The extract was filterd through a muslin cloth and then centrifuge at 4400 rpm for 7 minutes. The supernatant were collected and the pellet was discarded. These steps were repeated three times. The coming supernatant was considered as 100% concentration of extract. The Hot water extracts were evaporated to dryness using a rotary evaporates (Stuart, Barloworld and Model RE 300). Their crude extracts were evaporated in a water bath to give gummy solid residue.

Media Sterilization

All Media were sterilized by using automatic autoclave (SANYO) at 121°C for 15 minutes.

Media Pouring and Drying

Media was poured in pre-sterilized glass Petri plates of 90mm in Laminar Flow Hood which was sterilized by overnight exposure of UV light and disinfected with 70% ethanol solution. Media plates were kept open for half an hour in the Laminar Flow Hood for drying and solidifying media.

Test Microorganisms

The in-vitro activity of the extracts was assayed against the bacterial strains. All the ATCC (MicroBioLogics) against gram positive bacteria *S. aerious* ATCC®6538, and gram negative bacteria *Escherichia coli* ATCC®25922, *Pseudomonas aeruginosa* ATCC 74303 and *Enterococcus faecalis* ATCC 35824.

Inoculation of Test Organisms

100µl of 1McFarland bacterial suspensions were aseptically introduced and spread using pre-sterilized cotton swabs on surface of MHA plates.

Wells Preparation by Cork Borer

Agar well diffusion techniques as described by Adeniyi *et al.,* (1996). Wells of 6mm diameter with sterile cork borer were aseptically punched in the 90mm MHA agar plates.

Evaluation of Antimicrobial Activity

Antimicrobial activity of *Cannabis Sativa* leaf extract was tested using agar well diffusion method. With the help of sterile micropipette tips *Cannabis Sativa* leaf extract (Hot water) 100µl were poured into the wells. The plates were incubated at 370C for 24 hours. After incubation, the diameter of the resulting zone of inhibition was measured with the help of Digital Vernier Caliper (Mitutoyo) and the average values were recorded. Each antimicrobial assay was performed three times. Mean values were reported in this report.

Data Analysis

All data were measured average value of three replicates and standard error (±). Results were subjected to Microsoft excel 2010.

Results

In the present study, the antimicrobial activity of the Hot water extracts and Ethanol extracts against two gram negative and one gram positive bacterial strains and their potential activity were qualitatively and

quantitatively assessed by the presence or absence of inhibition zones and MIC values.

Antibacterial activity

The extracts of the investigated plant species showed antimicrobial activities against all tested bacterial strains. Results of the antimicrobial activity obtained using the well diffusion assay is summarized in Table1 and figure 1, 2, 3 and 4.

Table 1. Activity of Hot water and Ethanol extract of Cannabis Sativa leaf against bacterial strains.

Zone of inhibition of *Cannabis Sativa leaf* Hot water and ethanol extract to bacterial strains					
S.N	bacterial strains	1st replica	2nd replica	3rd replica	Average (±)
1	*Pseudomonas aeruginosa*	25.7mm	24.9mm	25.3mm	25.3mm
2	*Escherichia coli*	21.9mm	22.2mm	22.5mm	22.2mm
3	*S. aerious*	11.5mm	10.3mm	9.2mm	10.3mm

Discussion

The goal of this research was to find out the antibacterial activity of Cannabis sativa leaf extracts to some selective bacterial strains. The activity of this plant leaf extract is due the presence of phenyl moiety of cannabinoids which act as a good antimicrobial agent (Appendino, *et al* 2008). The acidic fraction from the ethanolic extract of Cannabis *sativa* leaf showed activity against both Gram-positive and Gram-negative bacteria (Wasim *et al.,* 1999; Radwan *et al.*,2009). These reports and presence of Cannabinoid in different extract of *Cannabis sativa* confirm its potential against all selected pathogenic bacterial strains.

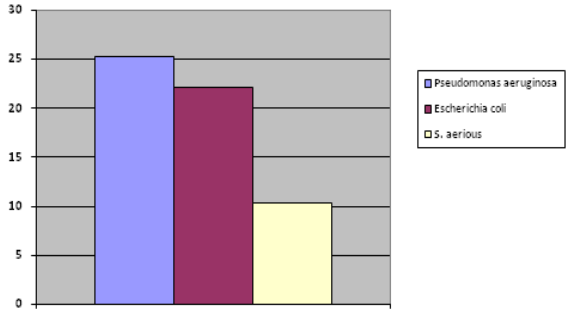

Fig. 1. Activity of Hot water and Ethanol extract of Cannabis Sativa leaf against bacterial strains.

In the present study, the antimicrobial activity of the Hot water extracts and Ethanol extracts against three gram negative and one gram positive bacterial strains and their potential activity were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and MIC values.

The extracts of the investigated plant species showed antimicrobial activities against all tested bacterial strains. Results of the antimicrobial activity obtained using the well diffusion assay is summarized in Table.

Fig. 2. Activity of Hot water and Ethanol extract of Cannabis Sativa leaf against S.aereous strain.

The current study suggests that the Ethanol leaf extract of *Cannabis Sativa* have a broad range of antimicrobial activity, although the degree of susceptibility could different between different microorganisms. The antimicrobial activity found in this present conducted study may be recognized to the presence of secondary metabolites either individually or in combination of various types of chemical composition present in the plant material.

Antibacterial agents currently available in the market are limited due to their toxicity, low effectiveness and prove expensive in case of prolonged treatment. The discovery of a potent therapy from plant origin will be

a great advancement in microbial infection therapies. Therefore, there is needed to develop new antibacterial agents which can satisfy the present demand.

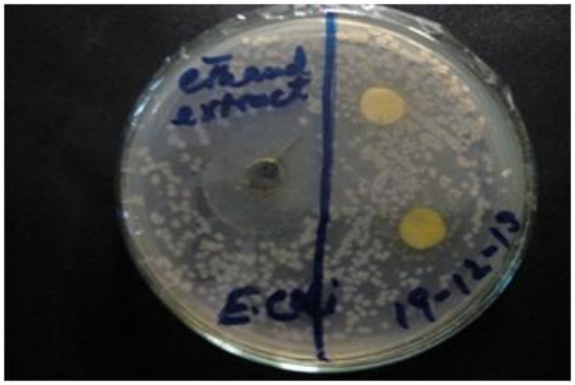

Fig. 3. Activity of Hot water and Ethanol extract of Cannabis Sativa leaf against E.coli strain.

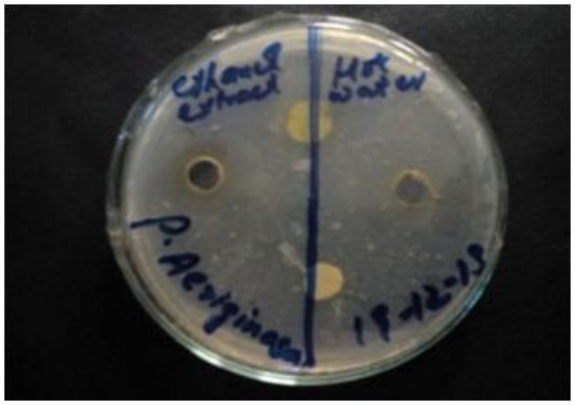

Fig. 4. Activity of Hot water and Ethanol extract of Cannabis Sativa leaf against P.aeroginosa strain.

Conclusion

The minimum inhibitory effect of *C. sativa* leaf extract is due to certain compounds present in the *C.sativa*.Further research should be done to identify the compounds responsible for its activity which can be use as medicines to control a wide range of disease in the world.

Acknowledgment

We are very grateful to Department of Microbiology Hazara University, Mansehra for providing research facilities for this study. We also appreciate Mr. Junaid Ali shah, Mr. Imran Zamin, Mr. Zakir Ullah, Mr. Aftab Ahmad and Shehzad Hassan Microbiology Department, Hazara University, Mansehra for helpful discussions and advice during development of the manuscript.

Competing interest

The author and co-authors of this manuscript do not have any conflict and competing interest.

References

Adams IB, Martin BR. 1996. Cannabis pharmacology and toxicology in animals and Humans. Addiction **91,** 1585-1614. http://dx.doi.org/10.1046/j.13600443.1996.911115852.x

Adeniyi BA, Odelola HA, Oso BA. 1996. Antimicrobial potentials of *Diospyrosmespiliformis* (Ebenaceae). African Journal of Medicine and Medical Sciences **25,** 221–224.

Anonymous. 1996. Pharmacological Investigations of Certain Medicinal Plants and Compound FormulationsUsed in Ayurveda and Siddha, Central Council for Research in Ayurveda and Siddha, Government of India, New Delhi, 58.

Appendino G, Gibbons S, Giana A, Pagani A, Grassi G, Stavri M, Smith E, Rahma MM. 2008. Antibacterial Cannabinoids from *Cannabis sativa* A Structure-Activity Study, journal of nature **71,** 127-130. http://dx.doi.org/10.1021/np8002673

Ayenigbara GO. 2012. Medical Utility of Cannabis Sativa.Journal of Pharmacy **2(3),** 460-463.

Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. 1985. Natural plant chemicals: sources of industrial and medicinal materials. Science, **228,** 1154–1160. http://dx.doi.org/10.1126/science.3890182

Blevins RD, Dumic MP. 1980. The effect of delta-9- tetrahydrocannabinol on herpes simplex virus replication. Journal of General Virology **49(2),** 427-431. http://dx.doi.org/10.1080/15287398709531019.

Callaway JC, Summers LK,Bradshaw H, Frayn KN. 2002. Changes in LDL particle composition after the consumption of meals containing different amounts and types of fat. American Society for Clinical Nutrition **76,** 345-350.

Darshan SK, Rudolph IL. 2000. Effect of fatty acids of w-6 and w-3 type on human immune status and role of eicosanoids. Nutrtion **16,** 143-145.

Klingeren VB, Ham TM. 1976. Antibacterial activity of delta9- tetrahydrocannabinol and cannabidiol. Antonie van Leeuwenhoek **42(1-2),** 9-12.

Kreji Z. 1958. Univ-palacky, Olomouc, Czech, hemp cannabis sativaan antibiotic drug II methods and results of bacteriological investigations and preliminary clinical experiences. Journal of Pharmazi, **13,** 155-166.

Lancz G, Specter S, Brown HK. 1991. Suppressive effect of delta-9- tetrahydrocannabinol on herpes simplex virus infectivity in vitro. Proceedings of the Society for Experimental Biology & Medicine **196(4),** 401-404.

Majid A, Rahman MU, Shah JA, Khan K, Ali MA, Zamin I, Ullah Z, Ibrar I, Zaman Q. 2013. In vitro antibacterial activity of *Camellia sinensis* leaf extracts to some selective pathogenic bacterial strains. International Journal of Biosciences **3(9),** 69-75.
http://dx.doi.org/10.12692/ijb/3.9.69-75

MerzoukiA, Ed-derfoufi F, Molero J. 2000. Hemp (Cannabis sativa L.) and abortion, Journal of Ethnopharmacology **73(3),** 501-503.
http://dx.doi.org/10.1016/S0378-8741(00)00323-8

Nath D, Sethim N, Srivastava S, Jain AK, Srivastava R. 1997. Survey on indigenous medicinal plants used for abortion in some districts of Uttar Pradesh, Fitoterapia **68(3),** 223-225.

Radwan MM, Elsohly MA, Slade D, Ahmed SA, Khan IA, Ross SA. 2009. Biologically active cannabinoids from high-potency Cannabis sativa. Journal of Nature Production **72(5),** 906-911.
http://dx.doi.org/10.1021/np900067k

Roth MD, Baldwin GC, Tashkin DP. 2002. Effects of delta-9 tetrahydrocannabinol on human immune function and host defense.journal of Chemistry and Physics of Lipids **121(1-2),** 229-239.
http://dx.doi.org/10.1016/S0009-3084(02)00159-7

TurnerCE, Elsohly MA. 1981. Biological activity of cannabichromene, its homologs and isomers. Journalof Clinical Pharmacology **21(8-9),** 283S-291S.

Wasim K, Ikram H, Ashrafa M. 1995. Antimicrobial studies of the leaf of Cannabis sativa.Pakistan Journal of Pharmaceutical Sciences, **8(1),** 29-38.

Whittaker K, Roth MD, Salehi K, Tashkin DP, Baldwin GC. 2004. Mechanisms for impaired effector function in alveolar macrophages from marijuana and cocaine smokers. Journal of Neuroimmunology **147(1-2),** 82- 86.
http://dx.doi.org/10.1016/j.jneuroim.2003.10.017