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Antibacterial and antifungal activity of *Cannabis sativa* L., *Mentha longifolia* L. and *Ricinus communis* L.

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Key words: Antibacterial, Zone of inhibition, Antifungal, *Cannabis sativa, Mentha longifolia*, *Ricinus communis.*

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Abstract

Bacterial and Fungal diseases still possesses a serious threat to public health. In this study the antibacterial and antifungal potential of crude methanolic extracts of *Cannabis sativa* (*C. sativa*), *Mentha longifolia* (*M. longifolia*) and *Ricinus communis* (*R. communis*) was investigated. The antibacterial activity was determined using agar well diffusion method against two gram positive i.e. *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*) and gram negative bacterial strains i.e. *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). The antifungal activity was invistigated by using agar tube dilution method aginst two fungal strains i.e. *Aspergillus niger* (*A. niger*) and *Aspergillus flavous* (*A. flavous*). In antibacterial activity, all the plants extracts showed higher bacterial inhibition against gram negative strains compared to gram positive strains. *E. coli* was recorded being the most susceptible strain while, *B. subtilis* and *P. aeruginosa* was the most resistant strains. *R. communis* showed significant antibacterial potential with maximum zone of inhibition (ZOI) of 28mm against *E. coli* and minimum ZOI of 8mm against *B. subtilis* and *P. aeruginsa*. For *C. sativa*, maximum and minimum ZOI of 26mm and 8mm was recorded against *E.coli, B. subtilis* and *P. aeruginsa*. *M. longifolia* showed maximum of 24mm ZOI against *E. coli* while, 8mm minimum ZOI was recorded against *B. subtilis* and *P. aeruginsa* strains. In antifungal activity, *R. communis* was the most active plants extract with 26.72% and 25.40% fungal inhibition against *A. niger* and *A. flavous*. Furthermore, *C. sativa* showed 18.10% and 18.96% fungal inhibition against *A. niger* and *A. flavous*, while for *M. longifolia* 16.37% and 19.82% fungal inhibition was recorded against *A. niger* and *A. flavous* respectively. These results showed that the methanolic leaves extract of *R. communis* possessed a good antibacterial and antifungal potential as compared to *C. sativa* and *M. longifolia* and can be consider in the bacterial and fungal control strategy.

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Introduction

Infectious diseases are the leading cause of total deaths worldwide. The clinical efficacy of many antibiotics is being threatened by the emergence of multidrug resistance pathogenic microbes (Bandow *et al*., 2003). Bacterial and fungal pathogens responsible for various infections evolved many defense mechanisms against antimicrobial drugs thus resistance to antimicrobial agents is on the rise.

The increasing failure of chemotherapeutics and antibiotics resistance has led to the medicinal plants screening for their potential antimicrobial potential (Scazzocchio *et al*., 2001). There are a number of reports regarding the antimicrobial potential of plants crude extracts (Rojas *et al*., 2003; Parekh and Chanda, 2007a).

Nature has been a source of antimicrobial agents for many years and a number of antimicrobial drugs have been isolated from plants mostly based on their use as folk medicine. Different medicinal plants have been used as a source of medicine for years to treat many microbial infections all over the world (Farombi, 2003). Efforts have been made to discover new antimicrobial agents from plants and the increasing prevalence of multidrug resistant strains raises the specter of untreatable bacterial infections thus adds urgency to the search for new infection controlling strategies (Sieradzki *et al*., 1999; Tomoko *et al*., 2002).

Cannabis sativa L. (*C. Sativa*) belongs to family Cannabaceae locally known as Bhang (Pashto) in Pakistan. It is annual herbaceous plant and its aerial parts are used as a tonic, against rheumatism and as narcotic. Juice of leaves is used for treating malaria and to relieve pain. Leaves are used for male impotency (Ahmad *et al*., 2011; Ahmad *et al*., 2013).

Mentha longifolia L, (*M. longifolia*) also known locally as Villaney (Pashto) belongs to family Lamiaceae. It is Perennial plant with a musty or aromatic smell.

Traditionally whole plant is used in stomach diseases, dysentery and diarrhea and as a stimulant. Leaves are used as antispasmodic, carminative, and to relieve abdominal pain. (Ahmad *et al*., 2011; Ahmad *et al*., 2013).

Ricinus communis L. (*R. communis*) is a member of Euphorbiaceae family. It is locally known as Arhanda (Pashto) in Pakistan. It is a large monoecious annual plant having single stem or a branched shrub or tree upto 5 m in length (Radcliffe-Smith, 1986). Its seeds are known as the castor bean and are used as laxative, purgative, emetic and to relieve swellings (Ahmad *et al*., 2013; Ahmad *et al*., 2011).

This study provides detailed information on the *in vitro* antibacterial and antifungal potential of the methanolic extracts of *C. sativa*, *M. longifolia* and *R. communis* leaves against different bacterial and fungal strains.

Material and methods

Extraction

The leaves of *C. sativa*, *M. longifolia* and *R. communis* were collected from different parts of Lower Dir, Khyber Pakhtunkhwa, Pakistan. The collected plants were identified and voucher specimens were deposited in Herbarium of Quaid-i-Azam University, Islamabad.

The shade-dried leaves of plants were chopped into fine powder. Methanolic extract of each plant sample was prepared by soaking it in methanol for 3 days with vigorous agitation. The extracts were then filtered twice, using Whatman-41 filter paper and solvents were completely evaporated using rotary evaporator and stored at 4°C. This process of extraction was repeated 3 times to get maximum extraction.

Sample preparation

Initially 15mg of Plant extracts were dissolved in 10ml of Dimethyl sulfoxide (DMSO) to make stock solution of extracts.

Stock solution was further diluted to prepared 8 concentrations (1mg/ml - 15mg/ml). Doxycyclin (2mg/ml) and DMSO were used as positive and negative control.

Test microorganisms

Four bacterial i.e. *Bacillus subtilis* (*B. subtilis*): ATCC 6059, *Staphylococcus aureus* (*S. aureus*): ATCC 6538, *Escherichia coli* (*E. coli*): ATCC 25922 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 7221 and two fungal clinical strains i.e. *Aspergillus niger* (*A. niger*) and *Aspergillus flavous* (*A. flavous*) used in the study were collected from Microbiology laboratory, Quaid-i-azam University, Islamabad, Pakistan. The bacterial cultures were inoculated individually on nutrient agar and incubated at 37°C and were maintained at 4°C. The bacterial cell number was adjusted to 0.5 Mc Farland standard. The fungal strains were grow on sabouraud agar for 72 h at 25°C.

Agar well diffusion method

The antibacterial activity of crude methanolic extracts of *C. sativa, M. longifolia* and *R. communis* was determined by agar well diffusion method (Naz and Bano, 2012). The antibacterial potential of plant extracts were recorded by measuring the diameters of inhibition zone in millimeter (mm).

Antifungal activity

The antifungal activity of *C. sativa*, *M. longifolia* and *R. communis* leaves methanol extract was determined by using agar tube dilution method previously used by Choudhary *et al.*, 1995. The tubes containing solidified media and test compound were inoculated with 4mm diameter piece of inoculum taken from a 7 days old culture of fungus used. Test tubes containing extract and extract free were used as positive and negative controls respectively. The tubes were incubated at 28°C for 7 days. Cultures were examined daily during the incubation. Percentage inhibition of fungal growth for each extract concentration was determined by using the following formula:

$$\% \text{ inhibition} = 100 - \frac{\text{Linear growth in test (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

Statistical analysis

Each of the experiment was carried out in triplicates. The results are presented with their means, standard deviation and standard error using Microsoft Office Excel 2007.

Results and discussion

Recently, much attention has been directed towards plants extracts to isolate biologically active compounds from it. The use of plants as a medicine plays a vital role in covering the basic health needs in developing countries.

Table 1. Antifungal activity of methanolic leaves extract of *R. communis*.

Fungal strain	Linear growth (mm)	Percentage inhibition (%)
A. niger	85	26.72
control	116	-
A. flavous	91	21.55
control	116	-

Table 2. Antifungal activity of methanolic leaves extract of *C. sativa*.

Fungal strain	Linear growth (mm)	Percentage inhibition (%)
A. niger	95	18.10%
control	116	-
A. flavous	94	18.96%
control	116	-

These plants can offer a new source of antibacterial, antifungal agents. We studied the methanol extracts of *C. sativa, M. longifolia* and *R. communis* to explore its antibacterial and antifungal potential against different bacterial and fungal strains.

It have been reported that methanol extract of plants exhibited high antimicrobial activity compared to other solvents (Coelho de Souza *et al.*, 2003; Duraipandiyan *et al.*, 2006).

Table 3. Antifungal activity of methanolic leaves extract of *M. longifolia*.

Fungal strain	Linear growth (mm)	Percentage inhibition (%)
A. niger	97	16.37%
control	116	-
A. flavous	93	19.82%
control	116	-

Antibacterial potential of methanolic extracts

During antibacterial activity, *R. communis* showed maximum antibacterial activity against *E.coli* strain with zone of inhibition (ZOI) from 28mm to 15mm at different concentrations; while minimum activity was recorded against *B. subtilis* and *P .aeruginosa* with ZOI from 14mm to 8mm and 15mm to 8mm respectively as shown in figure 1. Parekh and Chanda, 2007 also reported the antimicrobial potential of *R. communis*.

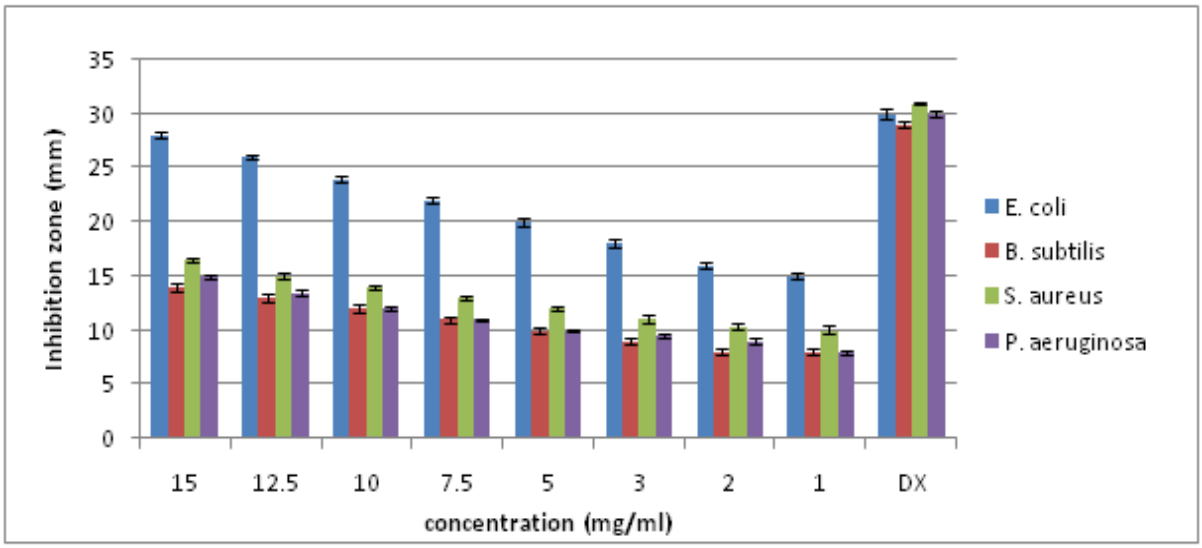

Fig. 1. Zone of Inhibition (mm) against different bacterial strains by the methanolic extracts of *R. communis* after 24h incubation. Data represent as mean ±standard error. (n=3).

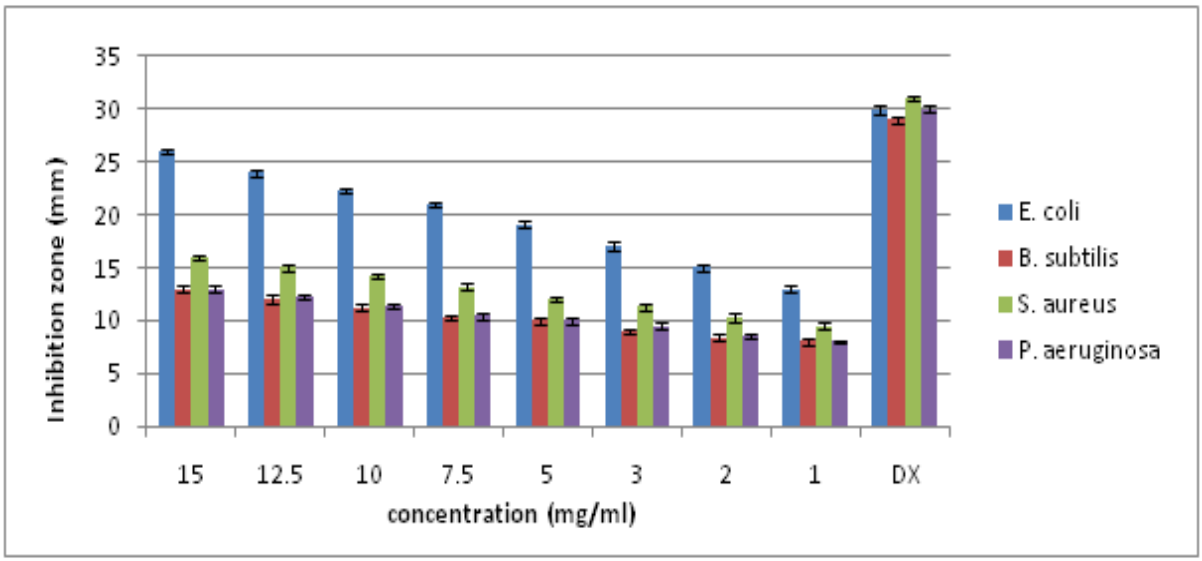

Fig. 2. Zone of Inhibition (mm) against different bacterial strains by the methanolic extracts of *C. sativa* after 24h incubation. Data represent as mean ±standard error. (n=3).

Parameswari and Tulasilatha, 2001 studied different solvents extract of *R. communis* leaves for antibacterial potential and concluded that methanol extract was more active against *E.coli*, *K. pneumoniae*, *P. aeruginsa* and *B. subtilis* compared to other solvents used. For *C. sativa*, maximum antibacterial activity was recorded with ZOI from 26mm to 14mm while, minimum bacterial inhibition was measured against *B. subtilis* and *P. aeruginosa* with ZOI from 13mm to 8mm as shown in figure 2. Appendino *et al.*, 2008 reported that *C. sativa* contains cannabinoids responsible for antibacterial potential.

Wasim *et al.*, 1995 also reported the antibacterial potential of *C. sativa* leaves extracts of different solvents. Furthermore, *M. longifolia* also exhibited higher inhibition against *E. coli* ZOI from 24mm to 12mm and minimum inhibition was recorded against *B. subtilis* with ZOI from 13mm to 7mm respectively as shown in figure 3. Mimica-Dukic *et al.*, 2003 recorded a very high antibacterial potential of essential oil of *M. longifolia* particularly against *E. coli*. In antibacterial assay, DMSO has no antibacterial effect and also a concentration dependent bacterial inhibition was recorded for all the extracts used.

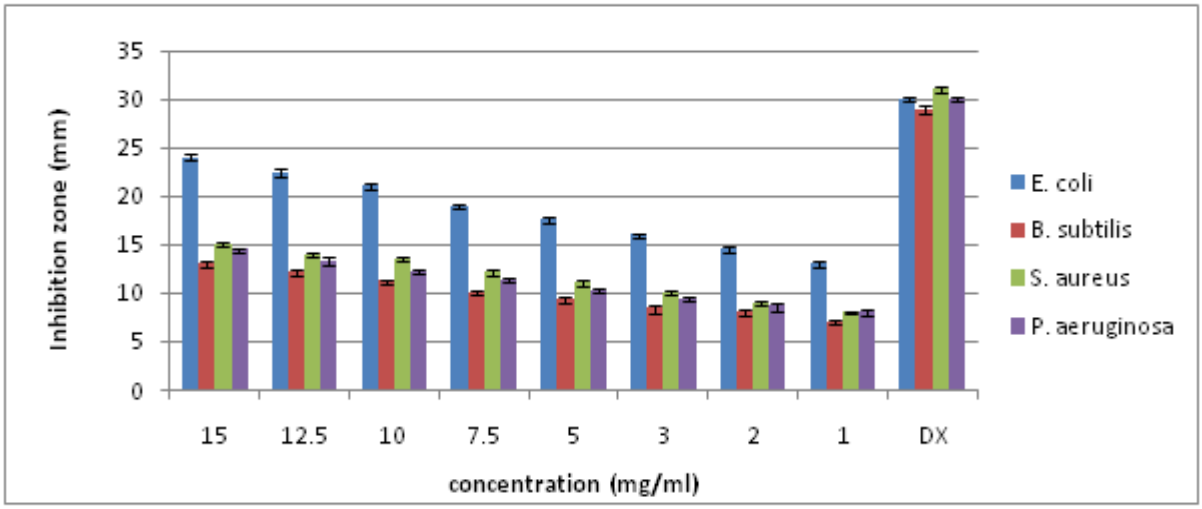

Fig. 3. Zone of Inhibition (mm) against different bacterial strains by the methanolic extracts of *M. longifolia* after 24h incubation. Data represent as mean ±standard error. (n=3).

Antifungal activity of methanolic extracts

In antifungal assay, the methanolic extracts of *R. communis* leaves showed high fungal inhibition. It showed 26.72% inhibition against *A. niger* while against *A. flavous*, 21.55% inhibition at 15mg/ml concentration was recorded as shown in table 1. The antifungal potential of plant extracts has been reported by different studies. For *C. sativa*, fungal inhibition of 18.10% and 18.96% were recorded against *A. niger* and *A. flavous* as shown in table 2. The ethanolic, petroleum and ether extracts of *C. sativa* leaves exhibited antifungal activity against *C. albicans* and *A. niger* (Wasim *et al.*, 1995). *M. longifolia* showed 16.37% and 19.82% fungal inhibition against *A. niger* and *A. flavous* respectively as shown in table 3.

Yigit *et al.*, 2008 reported the antifungal potential of methanolic extracts of *M. longifolia* and *M. piperita* against 99 human pathogenic clinical isolates belongs to 35 *C. albicans*, 33 *C. tropicalis* and 31 *C. glabrata* species. They concluded that *M. piperita* showed high antifungal potential compared to *M. longifolia*. Mimica-Dukic and Bozin, 2008 reported that the antimicrobial potential of Mentha species is attributed to the presence of two classes of secondary metabolites; one called monoterpenoids in essential oils and different structural phenolic compounds types.

Taken together, we reported the antibacterial and antifungal potential of methanolic extracts of *C. sativa, M. longifolia* and *R. communis* against different bacterial and fungal strains.

These findings showed that *R. communis* possessed good antibacterial and antifungal potential as compared to *C. sativa* and *M. longifolia* and there is a need for further study in order to be used in control strategies against different bacterial and fungal infections.

Conflict of interest

The authors have no conflict of interest to disclose.

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