

## **Antibacterial Screening of Plant Extracts against Biochemically Characterized Bacteria isolated from Different Public Sites**

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### **ABSTRACT**

With the growing population, public health is an issue of major concern. Environment and surroundings play a critical role in the health of an individual. Environment plays an important role in transmission of microorganisms that cause diseases and infections. Public sites like ATM machine, door latches and currency notes are potential reservoirs of pathogenic microorganisms. Medicinal plants can be utilized for their therapeutic value. *Ficus religiosa* (Peepal), *Cannabis sativa* (Marijuana), *Ricinus communis* (Castor) have been considered as plants with medicinal values for a long time. This has led to the investigation of antimicrobial activity of these plants against bacterial strains isolated from various public sites. Plant extracts were made using three solvents - methanol, chloroform and acetone. Acetone extract of all the plants showed effective results for most of the bacterial isolates used in the current research. Out of the three plants *Ricinus communis* (Castor) showed the most effective results over the others.

### **Keywords:**

Bacterial contamination, currency notes, ATM, Door latches, Antimicrobial, Plants: *Ficus religiosa* (Peepal), *Cannabis sativa* (Marijuana), *Ricinus communis* (Castor), Solvents: Acetone, Chloroform, Methanol

### **1. INTRODUCTION**

We live in the age of bacteria, which were the first living organisms and probably constitute

the largest of the earth's biomass [1]. Microbes are living things ordinarily too small to be seen without magnification, in terms of numbers and range of distribution and are the dominant organisms on earth [2]. The environment plays a critical role in transmission of microorganisms to humans with many environmental materials. These microbial contaminants may be transmitted either directly, through hand-to-hand contact, or indirectly via food or other inanimate objects [3]. Public sites are reservoirs of highly harmful pathogenic microbes. Scientific research has shown that commonly used surfaces such as computers, telephones, headsets, desks and ATM machines are potential sources of infectious bacteria and viruses leading to the spread of colds, flu, sickness and diarrhea [4-5]. Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. Plants produce a wide variety of secondary metabolites which are used directly as precursors [6]. The use of crude extracts of plants parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. Their systematic screening results in the discovery of novel active compounds and has forced scientists to search for new antimicrobial substances from various sources like medicinal plants [7].

## **2. MATERIAL AND METHODS**

### **2.1 Collection of microorganisms:**

The study was undertaken from January, 2015 to April, 2015 in the city of Phagwara, Punjab, India. A total of three samples were taken for the collection of microorganisms. The first sample was of paper currency notes. Three currency notes of an Indian denomination 100 were collected from butcher shops, vegetable sellers, transporters (bus conductors). The collection of microorganisms (from both surfaces) of currency notes was done with the help of swabbing. The second sample site was an ATM. Collection of microorganism from the ATM was done by swabbing areas of pin pad, LCD display and keyboards of three different ATMs (SBI ATM, Axis Bank ATM and Punjab National Bank ATM) The third sample was from the door latch. This study took place in Lovely Professional University (LPU) Phagwara, Punjab, India. Collection of microorganisms was done by swabbing the door latch areas of LPU's Mall door handle, classroom door handles and the washroom door handle

### **2.2 Collection of Plant material:**

Fresh leaves of three different plants such as *Ficus religiosa*, *Cannabis sativa*, *Ricinus communis* free from disease were collected from Phagwara, Punjab, India.

### **2.3 Solvent extraction:**

Fresh leaves of three different plants *Ficus religiosa*, *Cannabis sativa* and *Ricinus communis* were washed thoroughly 2-3 times with running water and once with sterile distilled water. The leaves of all three plants were then dried in a hot air oven at 60°C for 1 hour. Dried samples were crushed by using mortar and pestle. Powdered material was packed in Soxhlet apparatus and subjected to continuous percolation using three different solvents methanol, acetone and chloroform, for each plant thus running the Soxhlet nine times. The prepared extracts were concentrated by evaporation in waterbath[8].

### **2.4 Isolation of Bacteria**

Isolation of various bacterial strains from the currency notes was performed via standard technique. The method involved direct inoculation of currency notes in nutrient broth and was allowed to incubate for 24 hrs at 36 °C on a rotary shaker set at 120 rpm. After 24 hrs, 1ml of the broth from each samples were taken and serial dilution was performed up to 10<sup>-11</sup>, 10<sup>-12</sup> folds and inoculated on nutrient agar plates and incubated at 36°C for 24 hrs. Similarly isolation of various bacterial strains from ATM and door's latch samples were performed by other method which described the use of a sterile cotton-tipped swab (cotton bud) moistened with sterile physiological saline (0.9%) which was used to swab areas of the pin pad, LCD display, keyboard of ATM and door's latch areas. The swabs were then dipped in 1% peptone to isolate the microorganisms from the swab into the solution. This peptone solution was then diluted (10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup> folds) to prepare the inoculums [9].

### **2.5 Identification of Bacteria**

On the basis of different colony morphology appearance, 7 bacterial colonies were isolated and subculture for the further characterization. The 7 isolated bacteria was then assessed for colony characteristics and Gram reaction, and by conducting biochemical tests with standard protocols.

### **2.6 Anti-bacterial activity**

The leaf extract of three different plants were tested by Agar well diffusion method. The Mueller Hinton Agar (MHA) plates were inoculated with each bacterial culture. The plates were allowed to solidify for 10 minutes. A volume of 100 µl inoculums suspension was spread over entire MHA plate to prepare lawn culture. After incubation of one day, wells (10 mm diameter and about 2 cm a part) were made in each of these plates using sterile pipette tip. Ciproflaxin disc was placed as positive control and 30 µl of solvents (methanol, acetone and chloroform) were poured into each well as negative control without plant extract. The plates were incubated at 37 °C for 18-24 hr. The diameter of the inhibition

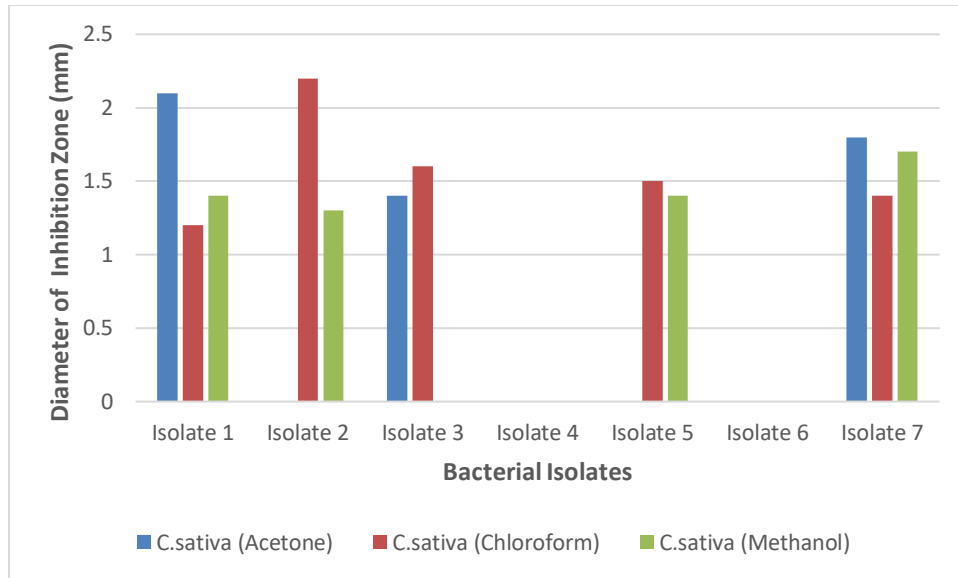
zone (mm) was measured.

### 3. RESULTS

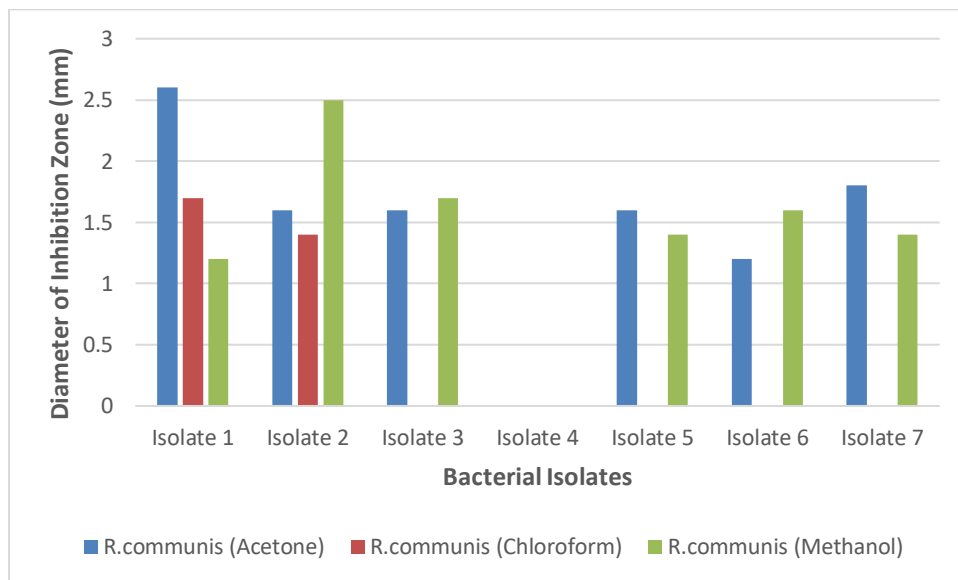
The study was done on three Indian currencies (paper currencies), three different ATM and on three different door latch's areas. Colonies in currency sample showed two different kinds of morphology. A round and pin point shaped colonies were observed at dilution levels of  $10^{-11}$ ,  $10^{-12}$  in currency while in ATM sample and door latch sample only round shaped colonies were observed. The isolated organisms were characterized using biochemical characteristics. After analysis from Bergey's Manual of Systematic Bacteriology we predict different species of bacteria from all isolates which is tabulated below in table 1. The results obtained in the present study of anti-bacterial activity relieved that all the extracts of different plants showed antimicrobial activity against all bacterial isolates which is shown in the following Fig 1,2 and 3.

<b>Isolate</b>	<b>Bacterial strain</b>
Isolate 1	<i>Micrococcus spp.</i>
Isolate 2	<i>Micrococcus spp.</i>
Isolate 3	<i>Bacillus spp.</i>
Isolate 4	<i>Bacillus spp.</i>
Isolate 5	<i>Bacillus spp.</i>
Isolate 6	<i>Bacillus spp.</i>
Isolate 7	<i>Bacillus spp.</i>

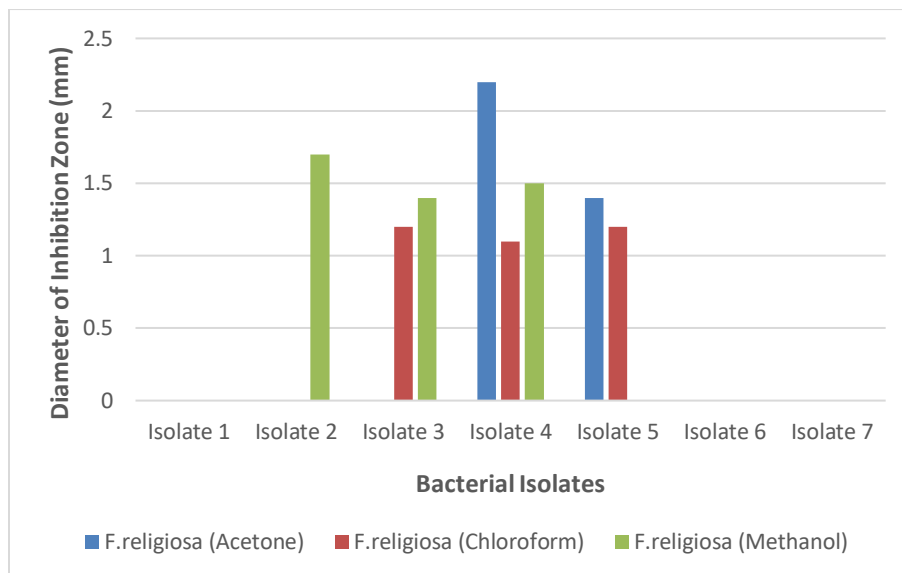
**Table 1: Identification of bacterial strain name on the basis of morphological and biochemical test**



**Fig 1: Antibacterial activity of *C. sativa* with different solvent against the isolates**



**Fig 1: Antibacterial activity of *R. communis* with different solvent against the isolate**



**Fig 3: Antibacterial activity of *F. religiosa* with different solvent against the isolates**

#### 4. DISCUSSION

The present study revealed that different bacteria which were isolated from different sample collected from separate public utility sites belong to *Bacillus* and *Micrococcus* spp category. *Ricinus communis* commonly known as castor gave the best results out of the three plants under consideration. Acetone proved to be the better solvent for *Ricinus communis*. Earlier studies have also revealed that methanol extract of *R. communis* exhibited strong activity comparable to the aqueous extracts sanitizer, hand washes, paper soap containing places. With the availability of primary information, further studies can be carried out like phyto-pharmacology of different extracts, standardization of the extracts, identification and isolation of active principles and pharmacological studies of isolated compound. These may be followed by development of lead molecules as it may serve for the purpose of use of specific extract in specific herbal formulation.

#### 5. CONCLUSION

Public utilities are the commonly used surfaces, consisting of a huge microbial flora which can spread numerous diseases. Prevention from such danger can be done by using hand sanitizers, hand washes, soaps and lotions *etc.* having herbal formulation. The bioactive components present in the formulations prevent the transmission of such harmful microorganisms. India

is sitting on the goldmine of well recorded and traditionally well practiced knowledge of herbal medicine. This country is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world. It is quite evident that medicinal plants contain several important secondary metabolites i.e. bioactive components and some have already shown their potential. Considering these facts the current research was carried out to find the antimicrobial activities of medicinal plants namely *Ficus religiosa*, *Cannabis sativa* and *Ricinus communis* against 7 bacterial strains isolated from public utilities like currency in circulation, ATMs and door latches. Although this study investigated preliminary screening of antimicrobial activity, the results showed that the Acetone extracts of *Ricinus communis* possess better antibacterial activity over others.

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