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A Comparative study on the Antibacterial Properties of Traditional Oils produced from Umwai village, Mawlai town and Lyngkyrdem Village of the State of Meghalaya

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ABSTRACT: The search for new drugs derived from plants is becoming a recent focus for research among ethno pharmacologists, botanists and microbiologists. Folk medicine, essential oils or compounds like alkaloids, flavonoids, etc. isolated, are being studied for anti-microbial properties to be used as alternatives to commercial antibiotics against various ailments. The state of Meghalaya, India, is well known for its production of traditional medicines. Their uses have been circulated among the local people as cures against skin infections, fever, arthritis, body aches etc. In this study, six oils collected from different parts of the state were tested for anti-bacterial properties against eight bacterial species obtained from MTCC, Chandigarh and MCC, Pune using Disc Diffusion and Agar Diffusion Methods. The results of this study showed that only the oil from Lyngkyrdem village showed zones of inhibition against three bacteria and against all bacteria in the disc diffusion and agar diffusion test respectively.

Key words: Traditional oil, Lyngkyrdem village, Anti-microbial properties, Disc Diffusion, Agar Diffusion, Zone of inhibition.

INTRODUCTION

Health is of major importance in today's world. Since 1950s, the use of antibiotics has led to the nonexistence of the use of plant derivatives as anti-microbial agents (Cowan, 1999). But with the increase in number of microbial diseases in humans, which is attributed to the ability of microbes to develop or evolve specific features within themselves enabling them to adapt in any environmental condition, has made antibiotics once used to treat these disease causing microbes, to become ineffective. This is due to the microbes' ability to gain resistance to the prescribed antibiotic which is causing great health concerns to the public consumers. With this high degree of resistance in pathogenic microbes to the present day antibiotics, there is a growing challenge towards discovering new sources for the development of effective drugs.

Plants have a wide range of bioactive molecules. Dating back to ancient times, the uses of plant extracts for healing different kind of maladies have been widely used (Farombi, 2003). These extracts are now being incorporated in the development of today's drugs due to the side effects caused by synthetic drugs produced. According to the World Health Organization (WHO), it states that plants are the best source to obtain novel compounds for the development of drugs (Nascimento *et al*., 2000). Scientists have developed means of screening plant extracts against microbial activities, fungal activities and in the treatment of various diseases (Oka *et al*., 2000; Sokmen *et al*., 2000). The extracts isolated from plants are aromatic in nature and called essential oils or volatile oils. They are called volatile oils because when they are exposed to air at ordinary temperatures, they evaporate to a substantial amount. They are also called essential oils due to the fact that they represent the characteristic "essence" of a plant (Aridogan *et al*., 2002). These oils are extracted from different parts of the plant such as buds, leaves, flowers, barks, etc. by either steam or hydro-distillation. The anti-microbial or anti-fungal activities attributed to these extracts are due their composition such as flavonoids, terpenoids, carotenoids, curcumines, etc. (Cao and Prior, 1998; Koleva, *et al*., 2001; Mantle *et al*., 1998; Soler-Rivas, *et al*., 2000) which has been proven *in vitro* (Cowan, 1999). Trial and error experiments have showed that these essential oils are more effective towards gram positive bacteria than gram negative bacteria (Seow, *et al*., 2013).

Traditional plants have been used for the extraction of essential oils to determine their efficacy against pathogenic bacteria. Many countries such as India, Brazil, Africa, Cuba, etc. have initiated research programs to screen out bioactive plant molecules for anti-microbial properties (Sartoratto, *et al*., 2004). Essential oils extracted from *Chrysanthemum indicum*, *Foenculum vulfare* Mill, *Mentha piperita*, *M. spicata*, *Thymus vulgaris*, *Origanum vulgare*, *O. applii*, *Aloysia triphylla*, *Ocimum gratissimum*, *O. basilicum* have displayed anti-microbial activites (Aridogan, *et al*., 2002; Beaux *et al*., 1997; Coelho, *et al*., 2003; Marotti and Pieeaglia, 1992; Muckensturm, *et al*., 1997; Singh, *et al*., 2002; Tanira *et al*., 1996: Sartoratto, *et al*., 2004). Thus, with the increase in pathogenicity and virulence of different species of infectious bacteria, researchers are exploiting the flexibility of plant derivatives of different species to be tested against these pathogenic bacteria thereby deriving cures of natural origin for the treatment of various diseases.

In an effort to expand the spectrum of anti-microbial agents prepared from natural resources, six traditional medicines have been selected from the state of Meghalaya which are known to be used among the common people against various ailments. Two of these oils was obtained from the village of Umwai located in Shella Bholaganj CD Block of East Khasi Hills District, three other oils were obtained from the town of Mawlai, Mylliem Block, East Khasi Hills District and one oil was obtained from the village of Lyngkyrdem, East Khasi Hills District. The composition of these oils is however, a trade secret of the families that produce them and so the components of the oil which are plant extracts, mixed in mustard oil, are not known. These oils, in their final produced state, have been selected and anti-microbial tests have been carried out against eight species of bacteria namely *Alcaligenes faecalis, Staphylococcus epidermidis, Enterobacter aerogenes, Escherichia coli, Bacillus subtilis, Micrococcus luteus, Serratia marcescenes* and *Bacillus megaterium.*

MATERIALS AND METHODS

A. Microbial Culture Revival

8 (eight) bacteria of Biosafety Level Class I were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India and Microbial Culture Collection (MCC), Pune, India for testing their susceptibility against the traditional oils in study. These bacteria namely *Alcaligenes faecalis* (MTCC 3134), *Staphylococcus epidermidis* (MTCC 7919), *Enterobacter aerogenes* (MTCC 2822), *Escherichia coli* (MCC 2089), *Bacillus subtilis* (MCC 2511), *Micrococcus luteus* (MCC 2155), *Serratia marcescenes* (MCC 2689), *Bacillus megaterium* (MCC 2197) were revived from their lyophilized state in Lurai Bertani (LB) broth (HiMedia, India) and incubated overnight at 37ºC.

B. Disc diffusion method

Sterile Luria Bertani agar (HiMedia, India) plates were inoculated with the 8 bacteria by spread plating. Sterile discs made from Whatman’s filter paper no. 1 with the help of a paper puncher were impregnated with the 6 traditional oils and placed on the inoculated LB plates. The plates were then incubated at 37 ºC overnight. This test was done in triplicates.

C. *Agar diffusion method*

In this test, another batch of sterile LB plates were taken and wells were punched in the agar with the help of 1 ml micropipette tips using the broad end of the tips. The eight bacterial species were then spread plated on the LB plates. The traditional oils in study were then added into the wells making sure that they did not spill out of the wells. The plates were incubated at 37ºC overnight.

RESULTS AND DISCUSSION

A. Disc diffusion method

The six oils, three from Mawlai, two from Umwai village and one from Lyngkyrdem Village, were tested, in triplicates, for anti microbial activity against eight different bacterial species, *Alcaligenes faecalis*, *Staphylococcus epidermidis*, *Enterobacter aerogenes*, *Escherichia coli, Bacillus subtilis, Micrococcus luteus*, *Serratia marcescenes* and *Bacillus megaterium.* Out of the six oils, only the oil obtained from Lyngkyrdem village displayed trace amounts of anti-microbial activity against three bacteria, namely, *Alcaligenes faecalis*, *Enterobacter aerogenes* and *Staphylococcus epidermidis* (Fig. 1). The zone of inhibition of microbial growth was measured and tabulated in Table 1. This test indicated that the oil obtained from Lyngkyrdem village has certain components that displayed properties of preventing the growth of three bacterial species around its vicinity. According to the zone of inhibition measured, *Staphylococcus epidermidis* seems to be most susceptible to the anti-microbial properties of the oil. The other five bacterial species showed no zone of inhibition. Also, none of the other five oils showed any anti-microbial activity against any of the eight microorganisms.

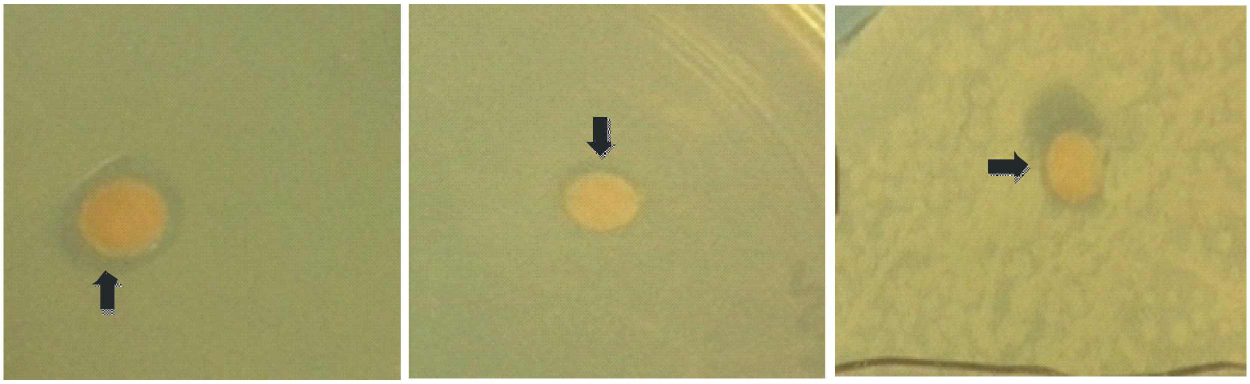

Fig. 1. (A). Zone of inhibition for *Alcaligenes faecalis,* (B) Zone of inhibition for *Enterobacter aerogenes,* (C) Zone of Inhibition for *Staphylococcus epidermidis.* Test for anti-microbial property of Lyngkyrdem oil using Disc Diffusion method. Arrows indicate the zone of inhibition.

Table 1: Diameters of Inhibition zones showed by Lyngkyrdem oil against eight test bacterial species.

Bacterial Cultures	Disc Diffusion Method	Agar Diffusion Method
	Zone of Inhibition Diameter	
Alcaligenes faecalis (MTCC 3134)	10 mm	11 mm
Staphylococcus epidermidis (MTCC 7919)	11 mm	22 mm
Enterobacter aerogenes (MTCC 2822)	6 mm	18 mm
Bacillus subtilis (MCC 2511)	-	16 mm
Escherichia coli (MCC 2089)	-	22 mm
Bacillus megaterium (MCC 2197)	-	20 mm
Serratia marcescenes (MCC 2689)	-	19 mm
Micrococcus luteus (MCC 2155)	-	20 mm

B. Agar diffusion method

In order to confirm anti-microbial activity of the six traditional oils, another experiment was conducted, that is, Agar Diffusion Method. Here, all the six oils were again tested, in triplicates, for anti-microbial activity against the eight microorganisms. Again, Lyngkyrdem oil was the only oil out of the six that displayed anti-microbial activity (Fig. 2). This time, zones of inhibition were observed against all the eight bacterial species and carefully measured (Table 1). Results obtained showed maximum zones of inhibition (22 mm) against *Escherichia coli* and *Staphylococcus epidermidis*. *Bacillus megaterium*, *Micrococcus luteus*, *Serratia marcescenes* and *Micrococcus luteus* displayed a zone of inhibition close to the maximum, that is, 20 mm, 20 mm, 19 mm and 18 mm respectively. The zone of inhibition measured for *Bacillus subtilis* was 16 mm and *Alcaligenes faecalis* measured the smallest zone of inhibition of 11 mm. Thus, we can conclude that out of all the six traditional oils studied, only the oil obtained from Lyngkyrdem village displayed anti-microbial activity proven especially by the Agar Diffusion test conducted.

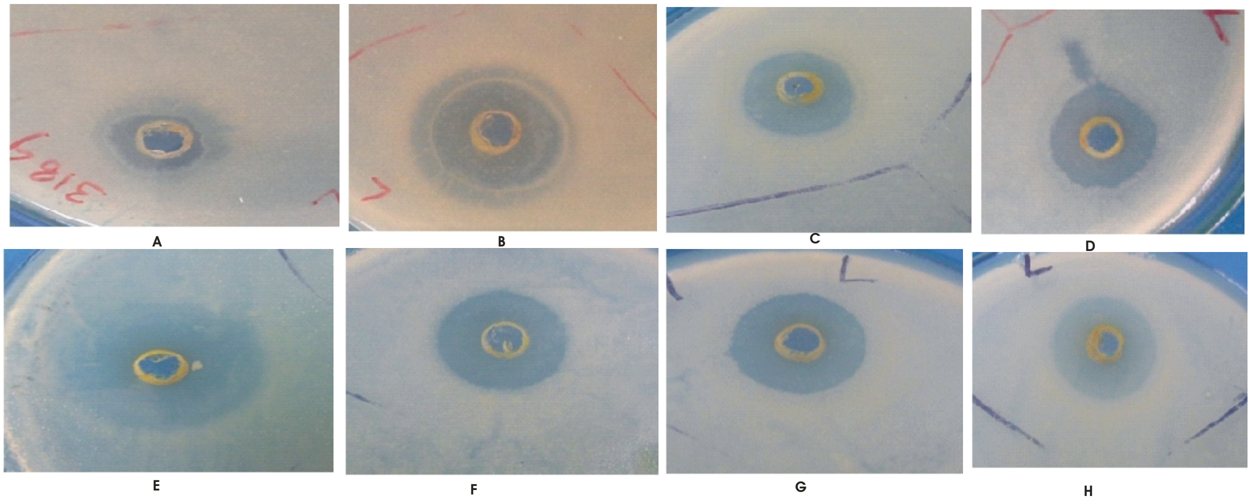

Fig. 2. Agar Diffusion test: Zones of inhibition shown by Lyngkyrdem oil against A) *Alcaligenes faecalis,* B) *Staphylococcus epidermidis,* C) *Enterobacter aerogenes,* D) *Bacillus subtilis*, E) *Escherichia coli,* F) *Bacillus megaterium,* G) *Serratia marcescenes* and H) *Micrococcus luteus.*

Staphylococcus epidermidis seems to be most susceptible to the oil since it displayed zone of inhibitions with both test conducted, i.e. Disc Diffusion Method and Agar Diffusion Method. *Escherichia coli* also showed a similar zone of inhibition reading like that of *Staphylococcus epidermidis* when Agar Diffusion test was carried out. So we can say that there might be certain bioactive molecules in the oil that prevents the growth of these bacteria in its vicinity. As had been stated earlier, the plant components in this oil are not known as the production of this oil is a family secret. Further tests need to be conducted to determine what component/s is in this oil that gives it its anti-microbial properties.

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