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EVALUATION OF ANTIBACTERIAL ACTIVITY OF *MYRISTICA FRAGRANS* SEED EXTRACT USING DISK DIFFUSION METHOD

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ABSTRACT

Myristica fragrans Houtt is mostly cultivated for spices in most of Asian regions as well as Penang Island, Malaysia. It is also used in food preparations for its aromatic flavor. This study was designed to evaluate the antibacterial activity of hydro methanolic *Myristica fragrans* seed extract tested against some Gram-negative and Gram-positive bacteria such as *Bacillus subtilis, Bacillus cereus, Bacillus frimicutes, Escherichia coli, Entrobacter, Klebsiella, Escherichia coli*. The antimicrobial activities were evaluated using agar disc diffusion method. The results indicated that the antimicrobial of *Myristica fragrans* Linn inhibit the growth of microorganism's as dose dependently manner. The appear results confirm that the antibacterial activity of *Myristica fragrans* seed extract in present test system. It is concluded that this plant can be indispensible source for secondary metabolites.

KEYWORDS

Myristica fragrans, Hydro methanolic, Bacteria, *E.Coli*, Seeds and Antibiotics.

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INTRODUCTON

The medicinal uses of any plant in which one or more of its organ, contain substances that can be used for therapeutic purposes or which is a precursor for the synthesis of useful drugs (WHO, 2011)[1]. Wild range from plants which are used in the production of mainstream pharmaceutical products to plants used in herbal medicine preparation which could either be in the form of infusion or decoction. In Indian culture the spice is a dried seed, fruit, root, bark, or flower of a plant or an herb used as food additives, for flavor, colour or as preservative, many spices are also used for other purposes, such as medicine, religious rituals,

cosmetics and perfumery (Linda, 2007)[2]. Today as throughout history, spices are used for their fragrance, flavor, preservation, colour and medicinal properties, they serve a very important biological function. They are extensively used, particularly in many Asian, African and other countries. Recently, on the basis of beneficial effects, use of spices has been gradually increasing in developed countries also. Spices have been shown to be indispensible for daily human health. It has been added flavor and taste to dishes, they help prevent and alleviate various health problems. As per the research articles on last few years several bio-ingrained have been isolated from spices, providing a scientific basis for the use of spices in our diet (Suekawa *et al*, 2006)[3]. Every spices have a unique aroma and flavor which are derived from compounds known as phytochemicals or secondary metabolites (Avato *et al*, 2000)[4].

In India, *Myristica fragrans* commonly known as Jaiphal and Javitri, belongs to the family Myristicaceae. It produces spice, nutmeg. All the Indian spices are used in folk medicine to treat several diseases. Depending on its origins, it contains the aroma compounds as like monoterpenes (87.5%), monoterpene alcohols (5.5%), and other aromatics (7%) (Pooja *et al*, 2012)[5]. The oil of nutmeg has also been used for medicinal purposes and it is this fraction of the nutmeg which contains the pharmacologically active components (Kaushik and Singh, 2012)[6]. The active component of *Myristica fragrans* as trimyristin, an active compound obtained from seed, also exhibited good antibacterial properties against Gram-positive and Gram negative bacteria (Narasimhan and Dhake, 2006)[7]. Several chemical structures of secondary plant metabolites continue to provide new and important leads against several pharmacological targets (Porto, 2009)[8]. Several studies have showed the great importance of natural products, both plant extracts and isolated compounds, as natural antibacterial agents in oral care products (Haffajee *et al*, 2008)[9]. Dental caries is a localized infectious disease that leads to loss of teeth. Individuals heavily colonized by cariogenic bacteria are considered to be at high risk for dental carie (Deshpande and Kadam, 2012)[10]. Presently, there are high interests in oral care products that are incorporated with medicinal plant extracts and are used extensively by the consumers due to low toxicity compared to oral care products (Shafiei *et al*, 2012)[11]. The current study investigates the anti-microbial potential of *Myristica fragrans* seed extract against some gram positive and gram negative bacterias.

MATERIAL AND METHODS

Plant Materials

The *Myristica fragrans seed* were collected from local market of Raipur, Chhattisgarh and dried for few days in shade, which were then powdered and preserved in airtight bottles for further studies.

Extract Preparation

Myristica fragrans seed (20g) was extracted in 50% of methanol and Millipore water solvent the supernatant was collected and concentrated in water bath at 40-50 C .The dried powder was kept in air tied box.

Microorganisms

The tested microorganisms included the Gram positive bacteria; *Bacillus subtilis, Bacillus cereus, Bacillus frimicutes* and Gram negative bacteria; *Escherichia coli, Entrobacter, Klebsiella, Escherichia coli.* These bacteria's strains were procured from National Chemical Laboratory (NCL), Pune, India. The bacteria were grown in the nutrient broth at 37ºC and maintained on nutrient agar slants at 4ºC.

Antibacterial Assay

Antibacterial activity of *Myristica fragrans seed* extract was determined by agar disk diffusion method (Nair, *et al*, 2005)[12] at four concentrations i.e., 100, 75, 50 and 25mg/ml. Muller Hinton agar was prepared according to the manufacturer's instructions and the plates were seeded with appropriate microorganisms (Gram positive bacteria; *Bacillus subtilis, Bacillus cereus, Bacillus frimicutes* and Gram negative bacteria; *Escherichia coli, Entrobacter, Klebsiella, Escherichia coli*). Discs of 6mm diameter were prepared from Whatmann filter paper No.24 and sterilized. The discs were then impregnated with the extracts and solvent DMSO. Antibiotics for Gram positive (NX - Norfloxacin, OF- Ofloxacin, E-Erythromycin, CFM- Cefixime) and Gram Negative (NX - Norfloxacin, OF- Ofloxacin, E-Erythromycin,

CFM- Cefixime). Bacteria were used as standard. The plates were incubate at 37ºC for 24 hrs and the zones of inhibition were measured with a measuring scale. Above experiment was carried out in triplicate for their confirmation.

RESULTS AND DISCUSSION

The result of microbial growth was considered as zero hour and further accordingly reading was taken. Our present study show that antimicrobial activity of 50% methanolic extract of *Myristica fragrans* against B. subtilis is best in 100% concentration after 12 hrs. 8.33mm zone of inhibition. Although 75% concentration is having mild effect as 6.99mm zone of inhibition. In *B.cereus* is best in 100% concentration of extract is 8.11mm and in 50% concentration is 6.33mm zone of inhibition. In *B.frimicute* the 100% concentration is having 7.88mm zone of inhibition. In *E. coli* 100% concentration show maximum activity of 7.00mm and in 75% also show a good zone of inhibition 6.77mm. In Klebsiella 100% concentration show minimum 5.33 zone of inhibition. In Entrobacter 100% and 75% show activity of 4.22mm zone of inhibition.

The above observation suggested that the different concentration (50%, 75%, 100%) were having good anti-bacterial activity against some gram positive (+) bacteria *B. subtilis, B. cereus, B. frimicute* and some gram negative bacteria *E. Coli, Klebsiella, Entrobacter*. The selected plant extract is showing great activity against all microorganisms. On comparing the zone of inhibition of extract to that standard antibiotic extract showed better activity than Ciprofloxacin (CIP), Doripenem (DOR), Ofloxacin (OF), Maxifloxacin (OM) in these conditions.

Their mode of antimicrobial action is related to their ability to inactivate microbial adhesion, enzymes and cell envelope proteins (Ibrahim, *et al*, 2013)[13]. It is known that medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. Over the past few years, medicinal plants have regained a wide recognition due to an escalating faith in herbal medicine. In view of its lesser side effects compared to allopathic medicine in addition, the necessity of meeting the requirements of medicine for an increasing human population (Akrayi, 2012)[14]. In conclusion, it can be concluded from the results that methanol extract of *M. fragrans* possess significant antibacterial activity against tested oral bacteria. However, the active components responsible for the antimicrobial activities need to be evaluated further. The antibacterial that are currently used suffer from several drawbacks such as high toxicity, low absorption, and high cost of treatment. The area of antibacterial drug discovery is at a nascent stage. The challenge in antibacterial drug discovery is its high toxicity in humans. *Myristica fragrans* is an evergreen tree native of the *E. moluccas* and cultivated throughout the Malaya. It is found only as a specimen tree in Botanical gardens. The seed of plant is known as “nutmeg” and the arillus of seed is called “mace”. Both nutmeg and mace contain many volatile oils. These oil constituents have variety of individual pharmacological effect, some of which oppose other (Jellin *et al*, 2005). The fruit contain ethereal oil cells often with phenolic and myristicin; the seed and the aril are used for flavouring food.

The most common plant as *Myristica fragrans* is one of the aromatic plants that are endowed with alluring properties of fragrance and flavours. The plants produce odoriferous secondary metabolites in their fruits. The nutmeg and mace of the fruits have been popular for several hundreds of years. However the structure and organization of the aromatic parts and the specific cells or tissue which possess the fragrant compound have not been studied fully. The seed of *Myristica fragrans* is so complex that is worthwhile to make detailed study of the seed. In our experiment the microbial growth was considered as zero hour and further accordingly a reading was taken. Our present day shows the antibacterial activity of extract of *Myristica fragrans* against *B. subtilis* is best in 100% concentration after 12 hrs of 8.33mm zone of inhibition. Although 75% concentration is having mild effect as 6.99mm zone of inhibition. In *B.cereus* is best in 100% concentration of extract is 8.11mm and in 50% concentration is 6.33mm zone of inhibition. In *B.frimicute* the 100% concentration is having 7.88mm zone of inhibition. In *E. coli* 100% concentration show maximum activity of

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The combined effect of tannins, glycosides and flavonoids may be responsible for the antimicrobial effect of the extract seen in this study. The result of present investigation clearly indicates that the antibacterial activity with the species of the plants and plant material used. The study most useful for ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs.

SUMMERY

Plants have been used as healers and health rejuvenators since time immemorial. Even now, WHO recognizes that medicinal plants play important role in the health care of about 80% World population in developing countries and depend largely on traditional medicines, of which herbal medicine constitutes the most prominent part (Farnsworth *et al*, 1988)[15].

In our experiment the microbial growth was considered as zero hour and further accordingly a reading was taken. Our present day shows the antibacterial activity of extract of *Myristica fragrans* against B. subtilis is best in 100% concentration after 12 hrs. Of 8.33mm zone of inhibition. Although 75% concentration is having mild effect as 6.99mm zone of inhibition. In *B.cereus* is best in 100% concentration of extract is 8.11mm and in 50% concentration is 6.33mm zone of inhibition. In *B. frimicute* the 100% concentration is having 7.88mm zone of inhibition. In E. coli 100% concentration show maximum activity of 7.00mm and in 75% also show a good zone of inhibition 6.77mm. In Klebsiella 100% concentration show minimum 5.33 zone of inhibition. In Entrobacter 100% and 75% show activity of 4.22mm zone of inhibition.

The information collected above on the use of *Myristica fragrans* across the world having similarity with available literature. In the last few year, ethanol - botanical and traditional applications of natural compounds, especially of herbal origin received most of attention as they are well tested for their efficacy and generally believed to be safe and effective for human.

Experiment for antibacterial study was also done which show a better antibacterial activity against all the six test gram-positive and gram-negative bacteria species used and show antibacterial susceptibility to *Myristica fragrans* extract with clear zone of inhibition. So, in future it can be used as alternate to synthetic antibiotics. Much effort has needed to increase *Myristica fragrans* as dietary supplement in food so as to acquire harm generated by free radicals and resist the human pathogenic bacterial disease.

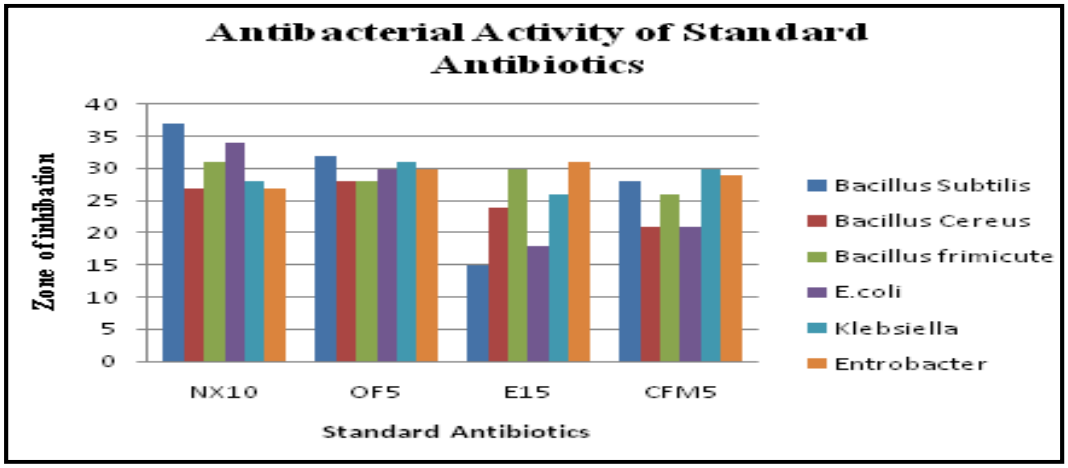
Activity of the spices against pathogens that appear to be resistant to many antibiotics shows that spices have a glowing future in the treatment of illnesses caused by the food pathogen investigated in this study. However it is necessary to determine the toxicity of the active constituents, their side effect and pharmaco-kinetic properties.

Table No.1: The study of anti-bacterial activities of *Myristica fragrans* extracts using disk diffusion method (Mean ± SE)

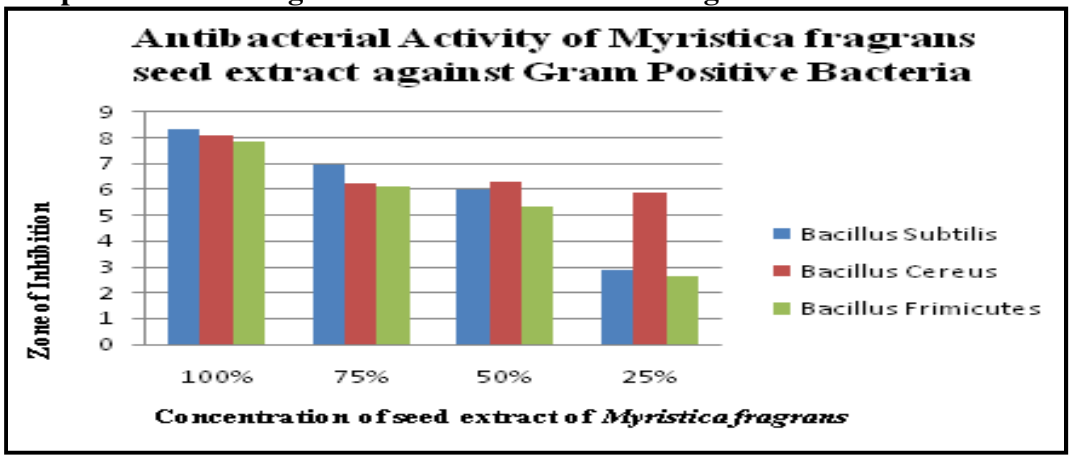
S.No	Bacterial Stain	Bacteria use	Zone of inhibition (In MM)			
			100%	75%	50%	25%
1	Gram Negative (-)	*Bacillus subtilis*	8.33±1.01	6.99±0.33	5.99±0.77	2.88±1.49
		Bacillus cereus	8.11±1.05	6.21±0.29	6.33±0.84	5.88±0.22
		Bacillus frimicute	7.88±0.77	6.10±0.10	5.33±0.69	2.66±0.66
2	Gram positive (+)	*E. coli*	7.00±0.57	6.77±0.69	6.55±0.38	0
		Klebsiella	5.33±1.83	4.33±1.07	3.55±1.22	3.21±1.23
		Enterobacter	4.22±0.58	4.22±0.10	2.99±1.50	1.33±1.33

Table No.2: The study of anti-bacterial activities of standard antibiotics using disk diffusion method

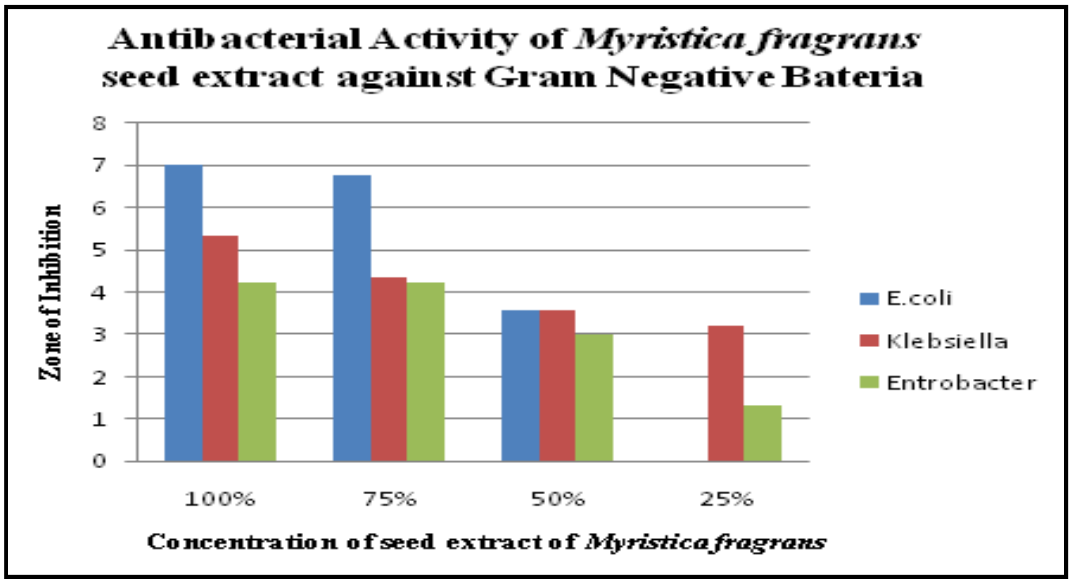
S.No	Bacterial Stain	Bacteria use	Zone of inhibition (In MM)			
			NX10	OF5	E15	CFM5
1	Gram positive (+)	*Bacillus Subtilis*	37.00	32.00	15.00	09.00
		Bacillus cereus	27.00	28.00	24.00	21.00
		Bacillus frimicute	31.00	28.00	30.00	26.00
2	Gram Negative(-)	*E. coli*	34.00	30.00	18.00	21.00
		Klebsiella	28.00	31.00	26.00	29.00
		Enterobacter	27.00	30.00	31.00	29.00



Graph No.1: Showing the Antibacterial Activities against Standard Antibiotic



Graph No.2: Antibacterial Activity of *Myristica fragrans* seed extract against Gram Positive Bacteria



Graph No.3: Antibacterial Activity of *Myristica fragrans* seed extract against Gram Negative Bacteria

CONCLUSION

Plants have been used as healers and health rejuvenators since time immemorial. Even now, WHO recognizes that medicinal plants play important role in the health care of about 80% World population in developing countries and depend largely on traditional medicines, of which herbal medicine constitutes the most prominent part (Farnsworth *et al*., 1988).

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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