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ANTIBACTERIAL ACTIVITY OF *JATIPHALA*(*Myristica Fragrans Houtt.)* WITH SPECIAL REFERENCE TO BACTERIA CAUSING RESPIRATORY TRACT INFECTIONS – AN IN VITRO STUDY

*[1] T. S.Akshatha [2]N .Rajashekhara., [3]Kotigadde Subbannayya
[1]PG Scholar, [2]Professor & HOD, Department of Dravyaguna, KVG Ayurveda Medical College Sullia (D.K.)
[3]Professor & HOD, Department of Microbiology KVG Medical College Sullia (D.K.) Karnataka

ABSTRACT

According to W. H.O. respiratory infections stand first among the top ten causes of death in low income countries. About 1.05 million people die annually which account for 11.3% of total mortality. Most of these infections are caused by organisms like, *Staphylococcus aureus, Klebsiella pneumoniae, E.coli* etc. Irrational usage of antibiotics has promoted the development of antibiotic resistance among organisms. Management of these infections is of great difficulty for the clinicians. Therefore there is an increasing need for the introduction of herbal remedy to overcome this problem. Many herbs are enlisted to overcome this problem, *Jatiphala* is one among them. It has qualities like *ushna, teekshna, shwasahara, kasahara* etc.So present study was designed to screen antimicrobial activity of the *Jatiphala* on *Staphylococcus aureus and Klebsiella pneumoniae* keeping *Escherichia coli* and *staphylococcus aureus* as standards.

Keywords: *Jatiphala*, *Myristica fragrans* Houtt., In-vitro, respiratory tract infections

INTRODUCTION: Research on the drugs acting on respiratory system is needed to overcome mortality rate due to respiratory tract infections. Scientists who are trying to develop newer drugs from natural resources are looking towards Ayurveda. Several drugs of plant, mineral and animal origin are mentioned in Ayurveda for respiratory tract infections under the terms *kasa hara, shwasa hara*. Most of these drugs are plant origin only. *Jatiphala* (*Myristica fragrans* Houtt.)[1] is such a drug which is known to people since vedic period. Its well known spices used in many food articles. It is also used as a medicine, as explained in many Nighantus of Ayurveda for to treat different ailments.

Jatiphala[1] an evergreen moderate sized aromatic tree with greyish black bark having lenticular spots on the outside and red juice on the inner side. **Leaves:**Leaves elliptic or oblong-lanceolate, acuminate, shiny above, dull beneath. **Flowers:** Flowers creamy yellow, fragrant, in umbellate cymes, stamina column of female flowers stalked. **Fruits:** Fruit yellow, globose or pyriform, pericarp fleshy. **Seed:** Seeds oblong, obtuse, testa shiny, aril yellowish red, irregularly lobed.

Jatiphala has got many therapeutic importance explained in many Ayurveda classics. It possesses the properties like, *ushna*, teekshana[2].As *Jatiphala* is indicated in infectious diseases *like kasa, shwasa, jwara*[2]. By considering its immense qualities it can be inferred to its antibacterial action on *Staphylococcus aureus and Klebsiella pneumoniae* keeping *Escherichia coli* and *staphylococcus aureus* as standards[3]. This

action was analyzed through in-vitro microbiological studies.

AIM AND OBJECTIVES:

- Antibacterial activity of Jatiphala (*Myristica fragrans* Houtt.)
- Collection of Jatiphala (*Myristica fragrans* Houtt.) from natural habitat.

MATERIALS AND METHODS:

Collection of the fruit of the plant *Myristica fragrans* Houtt from the surrounding areas of Sullia township of Dakshina kannada, Karnataka. Fully matured fruit was collected and was dried and used for further study. Alcoholic extract was prepared in *Dravyaguna* lab. In-vitro study was carried out in Microbiology laboratory of KVG Medical College Sullia-Karnataka.

Preparation Of Alcoholic Extract[4]

Five grams of air dried drug, coarsely powdered, is macerated with 100 ml of absolute alcohol in a closed flask for twenty-four hours, shaking frequently during first six hours and allowing to stand for eighteen hours. Filter rapidly, taking precaution against loss of the content by spillage. Evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish.

In-Vitro Study[5]:

Minimum Inhibitory Concentration (MIC): One ml each of the undiluted and 1 in 2, 1 in 4, 1 in 8 dilutions of the extract were added 19ml of sterile molten and cooled to 55^0c Mueller -Hinton agar, mixed well and poured into glass petri dishes. The final concentrations made were 2.5mg/ml, 5mg/ml and 10mg/ml. A growth control plate without the plant extract was also included. The agar was allowed to solidify.

Preparation of Mcfarland 0.5 Standard:

McFarland 0.5 standard was prepared by adding 99.5ml of 1% sulfuric acid and 0.5ml of 1.175% barium chloride. The solution was dispensed into glass tubes comparable to those used for inoculum preparation, tubes in thickness sealed tightly and stored in dark at room temperature. McFarland 0.5 standard provides turbidity comparable to that of a bacterial suspension containing 1.5×10^8colony forming units (CFU) per ml.

Inoculum Preparation- Four to 5 colonies of pure culture of each of the test bacteria, *Staphylococcus aureus, Klebsiella pneumoniae* and *E.coli* drawn from stock cultures available at the Department of Microbiology, KVG Medical college and Hospital, Sullia, were inoculated to Mueller-Hinton (MH) broth incubated at 37^0c for 3 to 4 hrs.

To standardize the inoculum, under adequate light, the inoculated broth and McFarland 0.5 standard or tubes were positioned side by side against a white card containing several horizontal black lines. The turbidities were compared by looking at black lines through the suspensions. The inoculum of the test bacteria matching with McFarland 0.5 Standard (1.5×10^8 CFU/ml) were further diluted with MH broth as to get a standard inoculum containing 10^4 CFU/ml.

The standardized inocula of test organisms were spot inoculated on to each plate containing the diluted extract and on growth control plates. After overnight incubation at $37^{0,}$ the MIC was read as the lowest concentration of the extract that inhibited the visible growth of the test bacterium.

Time Kill Assay (TKA)[5]: The alcoholic extract of fruit of *Myristica fragrans*

Houtt. In MH broth containing 20mg/ml concentration was distributed in 1 ml quantities to several test tubes. Each of the tubes containing the above dilutions of the extract was inoculated with inocula of test bacteria namely *Staphylococcus aureus*, *Klebsiella pneumonia* and *E.coli*. The inocula volume of 0.02ml of the log phase of broth culture was adjusted to an initial bacterial concentration of 1.5×10^4CFU/ml. All inoculated tubes were incubated at 37^0c and observed for bactericidal activity after 2,4,6,8 and 24 h of incubation. A growth control of each test bacteria in MH broth without extract was included. Subcultures of tubes showing no turbidity were done on MH agar plates, incubated at 37^0c overnight and observed. The time taken by different concentrations of the extract to kill specific bacteria after a given time of incubation was recorded.

RESULT:

Minimum Inhibitory concentration of alcohol extract of *Myristica fragrans* Houtt. fruit

Concentration (%)	*Staphylococcus aureus*	*Klebsiella pneumonia*	*E Coli*
100%	-	-	-
75%	-	-	-
50%	-	+	-
25%	-	+	-
12.5%	-	+	+
6.25%	-	+	+

Note: + Growth; – No growth

Krimighna activity of 75% concentration (Time Kill Assay) of alcohol extract of *Myristica fragrans* Houtt. fruit

Time in hrs	*Staphylococcus aureus*	*Klebsiella pneumonia*	*E.coli*
2	-	-	-
4	-	-	-
6	-	-	-
8	-	-	-
24	-	-	-
Control group without plant extract	+	+	+

Note: + Growth; – No growth

DISCUSSION: The present in vitro study was under taken to screen the *krimighna* effect of *Myristica fragrans* Houtt on selected microbes. The alcoholic extracts of fruit *Myristica fragrans* Houtt. Was tested against *Staphylococcus aureus* and *Klebsiella pneumonia* keeping *staphylococcus aureus ATCC* strain and *E.coli ATCC* strain as standard.Although both *Klebsiella pneumonia* and *Staphylococcus aureus* can cause lower respiratory infection. *K. pneumonia* is one of the important respiratory pathogen called porins present in the outer membrane of Gram negative bacteria resist the diffusion of larger molecules into bacterial cytoplasm.

In agar dilution method of undiluted alcohol extract of fruit showed inhibition of growth of the two test organisms.

Further various concentrations of the alcohol extracts of *Myristica fragrans* prepared (reduced to ½, ¼, $^1/_8$). Showed inhibition in growth of the *S. Aureus* but it is failed to inhibit *K. Pneumonia* and *E-coli.* In time kill assay 75% concentration of the extraction showed bactericidal action in two hour on *S. aureus* and *K. pneumonia* and control strains. Further dilution of the extracts showed bactericidal action only on *S. aureus*. However, at this concentration the 25% extract failed to inhibit the other organisms *E.coli* and *K. pneumonia*. Since high concentrations of the extract was used, it showed bactericidal action in an hour on further dilution of the extracts showed bactericidal action on *S. aureus* and didn't inhibit the other organisms *E-coli* and *K. pneumonia.*

These observations showed that the drug *Jatiphala* (*Myristica fragrans* Houtt.) is more effective against *S. aureus*. *S. aureus* clinical isolates are always hardier to tackle with anti-microbial agents and they are responsible for nosocomical infections. It is estimated that Gram negative bacteria are more resistant to longer molecules of drugs than Gram positive bacteria. It is also true that the bacteria tested survive and grow in slightly alkaline pH (7.2-7.4). Acidity is chleterious to them. The plant extract used had a pH of 4.5, which may be further responsible for the bacteriacidal activity of the extract.

CONCLUSION:

- Study showed that the fruit *Jatiphala* (*Myristica fragrans* Houtt.) is more effective against S. *aureus*. *S. aureus* is an alkaliphile with an optimum growth pH of 7.6 to 8.5. It can't survive acidic conditions. Unlike *K. pneumonia* and *E-coli* although need a slight alkaline pH of 7.2 to. 7.4, unlike *S. aureus* they endure acidic pH better.
- The drug *Jatiphala* (*Myristica fragrans* Houtt.) has exhibited *krimighna* action probably due to its *tikta rasa, laghu, ruksha guna* along with *katu vipaka*, which holds good according to Acharya Charaka's *chikitsa sutra* of *krimiroga*.

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REFERENCES:

1. K Gopalakrishnabhat, Flora of Udupi, first published 2003, Anipal press limited manipal page no: 542-543
2. *Sri. Bhavamishra*-Bhavaprakasha Nighantu, Commentry by Dr. K.C. Chunekar, Edited by Dr.G.S.Pandey, Published by Chaukhambha Bharathi Acadamy, tenth edition, Reprinted 1995: sloka 95, 96:245.
3. Connie R Mohan, George Manuselis-Text book of Diagnostic Microbilogy, WB Saunders Company, Philadelphia, 2000, Page: 62-95.
4. Dr. Mukharjee .K. Pulok- Quality Control Of herbal Drugs. An Approach To Evaluation Of Botanicals.
5. Hindler JF ,Special procedure in antimicrobial susceptibility testing Ch 13 Anti-microbial susceptibility In. Textbook of Diagnostic Microbiology 3rd ed.MahonCR,Lehman DC and Manuselis G (Editors) Saunders ,An Imprint of Elsevier, Delhi 2007:35

Corresponding Author: Dr. Akshatha T. S. PG Scholar, Department of Dravyaguna, KVG Ayurveda Medical College Sullia (D.K.)E-mail: drakshata22@gmail.com

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