

# **COMPARATIVE STUDY ON THE ANTIBACTERIAL ACTIVITY OF AYURVEDA RASAYANAM ON VARIOUS STRAINS OF BACTERIA**



Project Work By

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Fulfilment of requirement for the degree of Bachelor of Science

In zoology

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## **DECLARATION**

I hereby declare that the project work entitled “study of antibacterial effect of allopathic cough syrups on different strains of bacteria” submitted to Mahatma Gandhi University in partial fulfillment of the requirement for the award of Bachelor of Science degree, is a record of original project work done by me, under the guidance and supervision of Dr.Reema Kuriakose, Department of Zoology, St.Teresa’s College, Ernakulam

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# CERTIFICATE

This is to certify that the project report entitled “**STUDY ON ANTIBACTERIAL EFFECT OF ALLOPATHIC COUGH SYRUPS ON VARIOUS STRAINS OF BACTERIA**” submitted by **Ms. THANYA HELEN M D**, Reg No. AB15ZOO010 in partial fulfillment of the requirements of Bachelor of Science degree of Mahatma Gandhi University, Kottayam, is a bonafide work done under my guidance and Supervision and to the best of my knowledge, this is her original effort.

Smt. Dr. Reema Kuriakose  
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Ernakulum.

EXAMINERS:

- 1)
- 2)

# ACKNOWLEDGEMENT

I am very much indebted to Dr.Reema Kuriakose, HOD, Department of Zoology, and my project guide, for her uncompromised support, scientific guidance, affectionate nature and inspiration without which this project work would not have materialized.

I am also obliged to all my teachers, non-teaching staffs, classmates and friends for their co-operation and help for the successful completion of this work.

I am also thankful to my parents who are my pillar of strength.

Above all, I would like to thank God Almighty for his blessings that he has showered upon me.

# SYNOPSIS

The project entitled study on the antibacterial activity of Ayurveda Rasayanams on various strains of bacteria focused on five ayurvedic rasayana's, i.e, Agastya Rasyanam, Kushmanda Rasayanam, Punarnava Mandur, Dasamoolarasyanam, and Sukumara Leham. The five strains of bacteria E.coli, staphylococci, pseudomonas, salmonella, klebsiella were used for the study.

Results indicates that staphylococci exhibited the highest inhibition against Kushmandarasyanam Staphylococcus aureus is a gram-positive cocci, and they have the capacity to catalase and coagulase positive bacterium

The lowest inhibition was shown by Staphylococci aureus by Dasasmoolarasyanam where as Kushmamandarasyanam did not show any inhibition in all five strains of bacteria

# INTRODUCTION

## RASAYANAM

Rasāyana, is a Sanskrit word, with the literal meaning: *Path (Ayana) of essence (rasa)*. It is a term that in early ayurvedic medicine means the science of lengthening lifespan, and in later (post 8th-century) works sometimes refers to Indian alchemy.

The name of the science of Indian alchemy or proto-chemistry, is more generally "The Science of Mercury", in Sanskrit, Nepali, Marathi, Hindi, Kannada and several other languages. Early Indian alchemical texts discuss the use of prepared forms of mercury or cinnabar (see samskaras). However, there is also ample mention of the preparation of medical tinctures in the early science of Indian alchemy. Asayana therapy enriches rasa with nutrients to help one attain longevity, memory, intelligence, health, youthfulness, and excellence of luster, complexion and voice, optimum development of physique and sense organs, mastery over phonetics, respectability and brilliance.

These classical Rasayan formulas, contain a large number of ingredients, including minerals, pearl, coral and gems, and include a specially processed (samskara) mercury. Because of negative publicity and cost factor, the use of the classical rasayana formulas has declined considerably, and most of the preparations available now have herbal ingredients with a couple of mineral and animal products. The non-availability and wild life protection act has made the use of musk, amber, and parts of wild-life animals nearly impossible.

### AGASTYA RAYASANAM

Agastya Rasayanams (Agastya Rasayana) is a good tonic for the lungs. Its main action appears in diseases of upper respiratory tract. It is helpful in common cold, allergic rhinitis, chronic sinus infection (sinusitis), cough due all underlying causes, asthma and hiccup. In addition to these, it is also helpful in abdominal diseases including loss of desire to eat, irritable bowel syndrome, and hemorrhoids.

### KUSHMANDA RASAYANAM

KushmantaRasayanams (also called KushmantaAvaleha) is an ayurvedic supplement that provides nourishment and strength to the body. It is an aphrodisiac, strengthening, nervine tonic, and hemostatic medicine. It provides relief from hemorrhages, phthisis, fever, dry mouth (xerostomia), excessive thirst (polydipsia), vomiting, bronchitis, asthma, and physical weakness or debility in old age.

### PUNARNAVA MANDUR

PunarnavaMandur (also spelled as PunarnavaMandoor) is an ayurvedic medicine containing iron content and useful in diseases such as anemia, breathing troubles due to anemia, phthisis, hepatitis, jaundice, abdominal distension, piles, chronic colitis, gout, Hyperuricemia, worm infestation and skin diseases. PunarnavaMandur (also spelled as PunarnavaMandoor) is an ayurvedic medicine containing iron content and useful in diseases such as anemia, breathing troubles due to anemia, phthisis, hepatitis, jaundice, abdominal distension, piles, chronic colitis, gout, Hyperuricemia, worm infestation and skin diseases.

## DASAMoola RASAYANAM

DasamoolaRasayanams (DashmoolAvaleha) is an ayurvedic medicine used to treat a cough, bronchitis, breathing troubles, asthma, common cold, rhinitis, persistent hiccup, phthisis, hoarseness (hoarse voice), chronic fever, anorexia, abdominal lump, bloating, and flatulence. DasamoolaRasayanams is also spelled as DasamoolaRasayana or DashmoolRasayana and called as DashmoolAvaleha and DashamoolaLehyam. These all are the same thing.

## SUKUMARA LEHAM

SukumaraLeham (also called SukumaramLehyam and SukumaramRasayana) nourishes the body and improves the complexion of the skin. It is used for the treatment of the abnormal growths or tumors, hernia, abscesses, abdominal distension, hemorrhoids, disorders related to male and female reproductive system disorders, edema, abdominal diseases, gout, spleen disorders and constipation.



## ESCHERICHIA COLI

Escherichia coli (abbreviated as *E. coli*) are bacteria found in the environment, foods, and intestines of people and animals. *E. coli* are a large and diverse group of bacteria.

Although most strains of *E. coli* are harmless, others can make you sick. Some kinds of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses.

***Escherichia coli***<sup>1</sup> also known as ***E.***

***coli*** is a Gram-negative, anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination.<sup>[4][5]</sup> The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K<sub>2</sub>, and preventing colonization of the intestine with pathogenic bacteria, having a symbiotic relationship. *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards.

*E. coli* and other facultative anaerobes constitute about 0.1% of gut flora, and fecal–oral transmission is the major route through which pathogenic

### SCIENTIFIC CLASSIFICATION

Domain:	Bacteria
Phylum:	Proteobacteria
Class:	Gammaproteobacteria
Order:	Enterobacteriales
Family:	Enterobacteriaceae
Genus:	Escherichia
Species:	<i>E. coli</i>

strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for fecal contamination.<sup>1</sup> A growing body of research, though, has examined environmentally persistent *E. coli* which can survive for extended periods outside a host. The bacterium can be grown and cultured easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. *E. coli* is a chemoheterotroph whose chemically defined medium must include a source of carbon and energy.<sup>1</sup> *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. Under favorable conditions, it takes up to 20 minutes to reproduce.

## STAPHYLOCOCCUS AUREUS

Staphylococcus aureus(also known as *golden staph*) is a Gram-positive, round-shaped bacterium that is a member of the Firmicutes, and it is a member of the normal flora of the body, frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen.<sup>[1]</sup> Although *S. aureus* is

### SCIENTIFIC CLASSIFICATION

DOMAIN:	<u>BACTERIA</u>
KINGDOM:	<u>EUBACTERIA</u>
PHYLUM:	<u>FIRMICUTES</u>
CLASS:	<u>BACILLI</u>
ORDER:	<u>BACILLALES</u>
FAMILY:	<u>STAPHYLOCOCCACEAE</u>
GENUS:	<u>STAPHYLOCOCCUS</u>
SPECIES:	<b><i>S. AUREUS</i></b>

not always pathogenic (and can commonly be found existing as a commensal), it is a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. Despite much research and development there is no approved vaccine for *S. aureus*.

*Staphylococcus* was first identified in 1880 in Aberdeen, Scotland, by surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint.<sup>[2]</sup> This

name was later amended to *Staphylococcus aureus* by Friedrich Julius Rosenbach, who was credited by the official system of nomenclature at the time. An estimated 20% to 30% of the human population are long-term carriers of *S. aureus*<sup>[4][5]</sup> which can be found as part of the normal skin flora, in the nostrils,<sup>[4][6]</sup> and as a normal inhabitant of the lower reproductive tract of women.<sup>[7][8]</sup> *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples,<sup>[9]</sup> impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis. It is still one of the five most common causes of hospital-acquired infections and is often the cause of wound infections following surgery. Each year, around 500,000 patients in hospitals of the United States contract a staphylococcal infection, chiefly by *S. aureus*.<sup>[10]</sup> Up to 50,000 deaths each year in the USA are linked with *S. aureus* infections

## PSEUDOMONAS

**Pseudomonas** is a genus of Gram-

Negative, Gammaproteobacteria, belonging to the family Pseudomonadaceae and containing 191 validly described species. The members of the genus demonstrate a great deal of metabolic diversity and consequently are able to colonize a wide range of niches. Their ease of culture *in vitro* and availability of an increasing number of *Pseudomonas* strain genome sequences has made the genus an excellent focus for

scientific research; the best studied species include *P. aeruginosa* in its role as an opportunistic human pathogen, the plant pathogen *P. syringae*, the soil bacterium *P. putida*, and the plant growth-promoting *P. fluorescens*.

Because of their widespread occurrence in water and plant seeds such as dicots, the pseudomonads were observed early in the history of microbiology. The generic name *Pseudomonas* created for these organisms was defined in rather vague terms by Walter Migula in 1894 and 1900 as a genus of Gram-negative, rod-shaped and polar-flagellated bacteria with some sporulating species, the latter statement was later proved incorrect and was due to refractive granules of reserve materials. Despite the vague description, the type species, *Pseudomonas pyocyanea* (basonym of *Pseudomonas aeruginosa*), proved the best descriptor.

### Scientific classification

Domain: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Pseudomonadales

Family: Pseudomonadaceae

Genus: ***Pseudomonas***

## SALMONELLA

**Salmonella** is a genus of rod-shaped (bacillus) gram

-negative bacteria of the family Enterobacteriaceae. The two species

of Salmonella are Salmonella

enterica and Salmonella bongori. Salmonella

enterica is the type species and is further

divided into six subspecies <sup>[1]</sup> that include over 2,500 serotypes.

Salmonella species are non-spore-forming,

predominantly motile enterobacteria with cell

diameters between about 0.7 and 1.5  $\mu\text{m}$ ,

lengths from 2 to 5  $\mu\text{m}$ , and

peritrichous flagella (all around the cell

body). They are chemotrophs, obtaining their

energy from oxidation and reduction

reactions using organic sources. They are

also facultative anaerobes, capable of generating ATP with oxygen

("aerobically") when it is available; or when oxygen is not available, using other

electron acceptors or fermentation ("anaerobically"). *S. enterica* subspecies are

found worldwide in all warm-blooded animals and in the environment. *S.*

*bongori* is restricted to cold-blooded animals, particularly reptiles.

*Salmonella* species are intracellular pathogens: certain serotypes cause

illness. *Nontyphoidal serotypes* can be transferred from animal-to-human and

from human-to-human. They usually invade only the gastrointestinal tract and

cause *Salmonella* food poisoning; symptoms resolve without antibiotics.

### Scientific Classification

Domain: Bacteria

Kingdom : Eubacteria

Phylum: Proteobactria

Class : Gammaproteobacteria

Order : Entrobacteriales

Family : Enterobacteriaceae

Genus: Salmonella

However, in sub-Saharan Africa they can be invasive and cause paratyphoid fever, which requires immediate treatment with antibiotics. *Typhoidal serotypes* can

Only be transferred from human-to-human, and can cause *Salmonella* food poisoning, typhoid fever and paratyphoid fever. Typhoid fever occurs when *Salmonella* invades the bloodstream—the *typhoidal form*; or in addition spreads throughout the body, invades organs, and secretes endotoxins—the *septic form*. This can lead to life-threatening hypovolemic shock and septic shock and requires intensive care including antibiotics.

## KLEBSIELLA

*Klebsiella* is

a genus of nonmotile, Gram-negative, oxidase-

Negative, rod-shaped bacteria with a prominent polysaccharide-based capsule.

*Klebsiella* species are found everywhere in nature. This is thought to be due to distinct sub lineages developing specific niche adaptations, with associated biochemical adaptations which make them better suited to a particular

environment. They can be found in water, soil, plants, insects, animals, and humans.

The species of *Klebsiella* are all gram-negative and non-motile. They tend to be shorter and thicker when compared to others in the Enterobacteriaceae family.

The cells are rods in shape and generally measures 0.3 to 1.5 µm wide by 0.5 to 5.0 µm long. They can be found singly, in pairs, in chains or linked end to end. *Klebsiella* can grow on ordinary lab medium and do not have special growth requirements, like the other members of Enterobacteriaceae. The species are aerobic but facultatively anaerobic.

### Scientific classification

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: *Klebsiella*



## REVIEW OF LITRATURE

Ayurveda deals with preventive, promotive as well as curative aspects. The latest definition of ' Health' as brought out by WHO is ' Total Physical, mental, social & spiritual well - being & not just absence of a disease' which Ayurveda has been preaching over the centuries. As the time changes, the lifestyle of people is changing day by day. Therefore some diseases, which are troublesome to the people since the ancient time, are becoming major health hazards of the society

Immunity is an important of contemporary developments in the field of medicine. Diseases like AIDS and Cancer are being explored from immunity angle in the current researches. Mechanism of Natural Killer Cells – a special type of lymphocytes is offering a new way to contain the cancer. AIDS itself is a disease complex caused by deficiency of immunity in the body. Further, “Health to all by 2000 A.D.” has become “a sour grape fruit” by the advent of resistant malaria and tuberculosis bacilli. It is less known that what happens with smallpox and other infectious disease if various synthetic substances and styles of living degrade the immunity of human beings further. At this juncture it is a need to probe in to the natural substances, which can promote immunity. Extensive researches are conducted all over the world focusing on natural herbs influencing the immunity. RASAYANA is a unique branch of Ayurveda. It promotes the inner healing power of an individual and helps him in over taking

the pain and disease while promoting the immunity (SVASTASYA ORJASKARAM).

The project was on antibacterial effect on Rasayanams on various strains of bacteria like E.coli, staphylococci, pseudomonas, salmonella, klebsiella and the rasayanams used were Agastya Rasayanams, Kushmanda Rasayanams, Punarna Mandooram, and Sukumara Leham. Among all the rasayanams the highest inhibition was shown by Agastya Rasayanams against Staphylococcus aureus and the least inhibition was shown by Dasamoolarasayanam by the same bacteria. Kushmandarasayanam did not show any inhibition in all the bacteria

Jagdevsingh ;(2016) Agastya Haritaki Rasayana (AHR) is an important Ayurveda confectionary formulation (*Avaleha*) containing *Dashamoola, Pippali, Bharangi, Kapikacchu, Haritaki* etc. as main ingredients. Though A H R is very effective for disorders of Respiratory tract, but till date no work has been carried out to standardize this formulation. Hence the present study was undertaken to standardize the Poly Herbal formulation (AHR) through pharmacognostical and pharmaceutical evaluations. The sample was subjected for various physicochemical parameters like water soluble extractive (71.10% w/w), alcohol soluble extractive (44.93% w/w), ash value (2.95% w/w), acid insoluble ash (0.39% w/w), loss on drying (9.07% w/w), the pH(5), dextrose value, HPTLC. Thus, the physicochemical and microscopic characters achieved may provide guideline for standardization of formulation A H R. Agastyarasayanam improves the respiratory strength and it is also useful in many acute and chronic disorders. This is also excellent in all types of asthma and tuberculosis. It develops immunity and can be used suitably in hiccups, chronic fever, and gulma, piles, grihani, and heart ailments

Studies conducted by Pt. Ambika Dutt Shastri, said that the reference of kushmanda in Vedic literature is found as kushmanda, is used for animal in Yagyana for sacrifice. In Brihatrayee, application of kushmanda is mentioned in detail. Acharya Charaka has described kushmanda as Aharadravya in the chapter ‘Annapanvidhi 6 of Sutrasthana1. Total 6 references are available in Sushruta Samhita. Acharya Sushruta has described Kushmanda in the class of madhura rasa dravya in the chapter rasa – visheshavijnaniya of Sutrasthana2. Kushmanda is described under tailavarga. Talia of this fruit is madhur in rasa and vipaka3. Kushmanda is again described under Shakavarga. Unripe kushmanda is pitta nashak, half ripe is kaphakarak, while ripen is laghu, ushna, kshar, and bastishodhak4. Kushmandasneha is indicated in mutrsanga5. The multiple benefits of Kushmanda (Benincasahispida) made it a true miracle of nature. It has several effects like antioxidant, antifibrinolytic, anti-inflammatory, hepatoprotective effects. A detailed and systematic study is required for identification, cataloguing and documentation of plants, which may provide a meaningful way for the promotion of the traditional knowledge of the herbal medicinal plants. In view of the nature of the plant, more research work can be done on humans so that a drug with multifarious effects will be available in the future.

Kushmanda Rasayanams is a polyhedral Ayurveda Medicine which is used to treat various diseases. It is malleable, semi-solid, sticky preparation and dark brown in color. It has a spicy odor as well as pungent and a sweeter taste.

Benefits of this rasayanam is that it is a Rejuvenator

helps in cases of bleeding disorders, Excess heat, and Pitta in the body.

Extremely nutritious, Provides immunity to our Body, Relieves from respiratory problems, it also cures dryness (internal), Improves Immunity.

Facilitates Weightgain, Gives Luster and encourages Digestion, Gives relief

from constipation. Strengthens our Brain, Eyes, Nerves and other working organs.

And its uses are it is used for Cough, Dyspnea, Chronic fever, Sinusitis Chest wound, Bleeding disorder, Chronic respiratory Chardi, Thirst, Deficiency of semen, Emaciation, Pthisis (pulmonary tuberculosis), Svarabheda

Sengupta S et .al;(2002) PunarnavadiMandura is an Ayurveda herbo-mineral preparation primarily used as an anemia correcting agent while its role in other clinical conditions has also been studied. The role of PunarnavadiMandura as an anemia correcting agent has been studied by different authorities in India.1-4 the classical description of PunarnavadiMandura is found in one of the major classics of Ayurveda, Charak Samhita. Acharya Charak has described the role of PunarnavadiMandura as an anemia correcting agent in his classical treatise, CharakSamaita, in Chikitsa Stana.5 the ingredients described in Charak Samhita for the preparation of PunarnavadiMandura have been delineated in Table 1. Furthermore a brief description of the preparation of PunarnavadiMandura is described in Table 2. PunarnavadiMandura act as an anemia correcting agent possibly by its effect on Srotosa (micro channels) and Agni (digestive fire mechanism) and thereby enhancing digestive capacity owing to Deepana (appetizer), Pachana (digestive) properties.6 In addition to anemia its role in other clinical conditions has been studied as well7 PunarnavadiMandura is being used for many other clinical conditions which include malabsorption syndrome, inflammation, splenic disease, intermittent fever, haemorrhoids, diseases of skin, and helminthiasis/ worm infestation.5,8 Furthermore PunarnavadiMandura is currently a part of national health programme in India. It has been included in the ASHA drugs kit for the management of iron deficiency anemia (IDA) at community level in India. This drug has been

included with the initiation of National Rural Health Mission (NRHM) in India under the aegis of “Mainstreaming of AYUSH and Revitalization of Local health Traditions” in India.<sup>9,10</sup> With this background a review was carried out in order to understand the role of PunarnavadiMandura in anemia and other pathological conditions.

It can be used as a source of Iron, since it contains Iron oxide as ingredient.

It is used in Ayurveda treatment of anemia, splenomegaly, hemorrhoids, chronic fever, inflammatory conditions, malabsorption syndrome, infective skin diseases, dermatitis, and helminthiasis.

Jagdevsingh ;(2017) SukumaraLeham nourishes the body and improves the complexion of the skin. It is used for the treatment of the abnormal growths or tumors, hernia, abscesses, abdominal distension, hemorrhoids, disorders related to male and female reproductive system, Vata disorders, edema, abdominal diseases, gout, spleen disorders and constipation. Furthermore, SukumaraLeham provides relief from abdominal pain, menstrual disorders, polycystic ovarian disease (ovarian cysts), uterine fibroids, and polycystic kidney disease. The three months regular course with SukumaramLehyam helps to cure these problems.

In Ayurveda, SukumaraLeham is used during pregnancy for nourishing the mother and developing a baby. It is also used to relieve intestinal gas, abdominal pain, and constipation occurring in pregnant women. However, the safety of SukumaraLeham is not fully clear. Consult an ayurvedic physician before using SukumaramLehyam during pregnancy. SukumaraLeham is likely to be safe to consume by lactating mothers. There are no adverse effects reported with the use of SukumaramRasayana in lactating mothers and breastfeeding babies.

J V Habbar.; (2012) DasamoolaRasayanams is an effective Ayurveda medicine for acute and chronic upper respiratory tract infections. This is a traditional Kerala Ayurveda preparation and the reference is in the classical text book Sahasrayoga(Ten hundred Preparations). This is presented as an herbal jam in Jaggery (Sugar Cane) base.

Indications and benefits of DasamoolaRasayana are Productive and nonproductivecough. Bronchitis, Asthma, Fever. Indigestion

The preparation is very effective in respiratory ailments by increasing the digestion, relieving the phlem and clearing the respiratory tract. Its anti-inflammatory and bronchodilator actions are very useful in the treatment.

It is also used in the treatment of following ailments or diseases and Upper respiratory tract infections. Cough. Headache.Sinusitis.Indigestion. Dyspepsia. There are no side effects if taken in prescribed dosage. Over dosage will cause gastric irritation and acidity.

DasamoolaRasayanams (DashmoolAvaleha) is an ayurvedic medicine used to treat a cough, bronchitis, breathing troubles, asthma, common cold, rhinitis, persistent hiccup, phthisis, hoarseness (hoarse voice), chronic fever, anorexia, abdominal lump, bloating, and flatulence. DasamoolaRasayanams is also spelled as DasamoolaRasayana or DashmoolRasayana and called as DashmoolAvaleha and DashamoolaLehyam

The safety profile of DasamoolaRasayanams is not well established for pregnant women and lactating mothers. Consult an ayurvedic physician before using DasamoolaRasayanams during pregnancy or lactation.

# **METHADODOLOGY**

## **MATERIALS REQUIRED**

AYURVEDIC PREPARATIONS (The commercial herbal preparations were purchased from Rose medicals, kacheripadyErnakulum.)

## **BACTERIAL STOCK CULTURE:**

Nutrient agar, conical flask, cotton, distilled water, forceps, inoculating loop, paper discs, petridish watch glass and test tubes

## **PREPARATION OF EXTRACT**

1mg of extract was diluted in 1ml of distilled water. Each extract thus obtained was taken separately in a Petri dish and marked for identification

## **INOCULATING THE BROTH**

Bunsen burner was lightened and the area of work was sterilized using ethanol. The cotton plug of both the stock culture and broth culture to be inoculated was loosened, and then the inoculating loop was flamed to red hot and cooled by waving for few seconds. The cotton plug from the stock culture tube was then removed and then mouth was flamed. The cooled sterilized loop was inserted into the culture tube being careful to not touch the sides of the tube to prevent contamination. A visible amount of the culture was scrapped with the loop and then removed carefully and the mouth of the test tube was plugged back after flaming. By using similar procedure for removing the cap, the loop was inserted into the broth. The loop was then withdrawn; the tube mouth was flamed and recapped. The inoculating loop was then resterilized and the broth culture was gently rotated to mix the contents properly. It was labeled with the

name of the respective microbes on each test tube. After inoculating each broth they were kept for incubation for 24-48 hours for sufficient bacterial growth

## **PREPARATION OF NUTRIENT AGAR (CULTURE MEDIA)**

35 gm of nutrient agar was weighted out and added to 200 ml of distilled water in a 250 ml conical flask. With a gentle stirring 1 gm of agar-agar was slowly added to the media and conical flask was plugged with cotton. Then the flask was sterilized for 15 minutes in an autoclave for 15psi. The medium was allowed to cool. Cooled agar was poured into 50 sterilized petriplate and they were kept upside down for few hours for solidification. After setting, the plate was used for the study

## **PREPARATION OF FILTER PAPER DISC**

Filter paper disc which was about 3 mm thickness was prepared using a punching machine and sterilized using autoclave. The disc was then soaked in the extract for specific time and was used for antimicrobial sensitivity test'

## **METHOD**

The microbial activity against specific pathogens was determined using disc diffusion method. A lawn culture of each bacterium was prepared for the broth by swabbing uniformly across a culture plate using cotton swabs.

A sterile swab dipped into the suspension and then it was moved from side to side down the whole agar plate so that all the area is covered. It was done for each bacterium. Once the lawn culture was prepared, a filter paper disc impregnated with the compound to be tested was placed on the surface of agar. The compound diffuses from the filter paper to the agar. The concentration of the compound will be highest next to the disc and will decrease as distance from



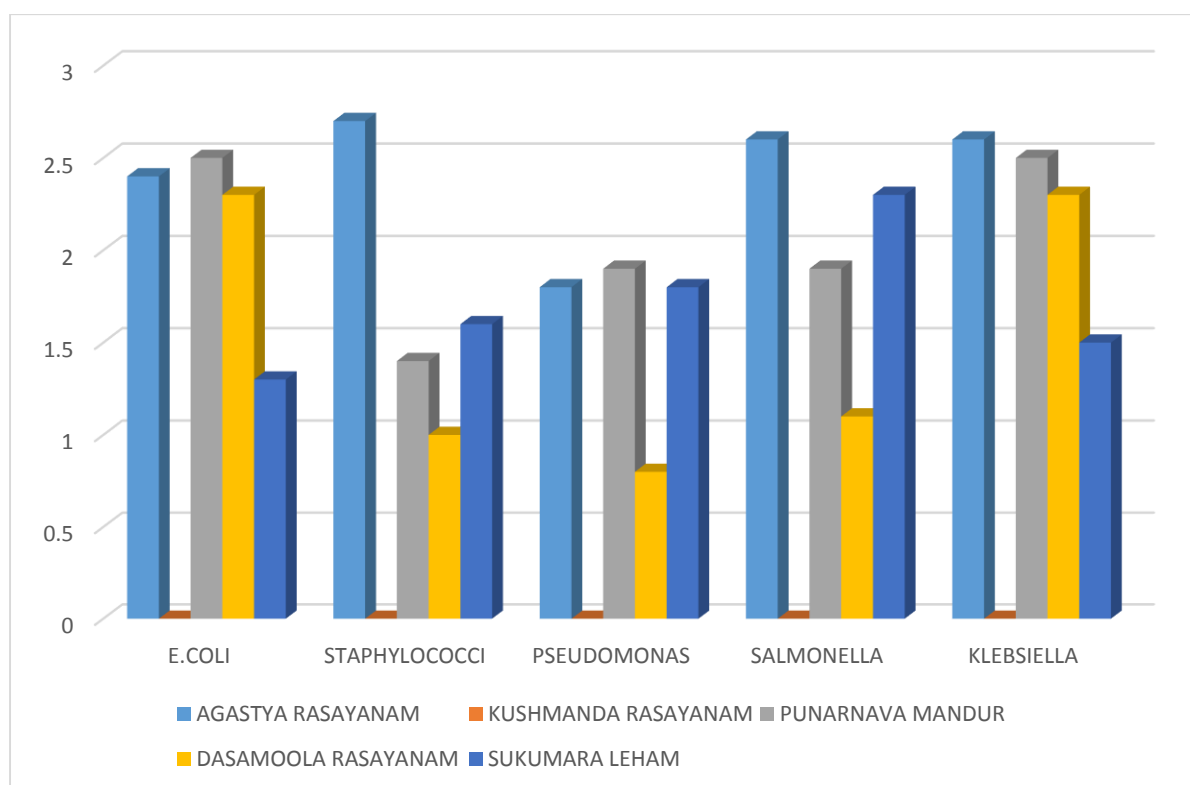
the disc increases. If the compound is effective against bacteria at certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the compounds effectiveness, the larger the clear area around the filter paper, the more effective the compound

#### **KILLING OR DISPOSING**

After the experiments were conducted the bacteria was killed by autoclaving for 20 minutes. All the glass wares used was also autoclaves to remove any bacteria if present

# OBSERVATION

Name of the Rasayanam	Escherichia coli	Staphylococcus aureus	Pseudomonas aeruginosa	Salmonella typhi	Klebsiella pneumoniae.
Agastya Rasayanam	2.4cm	2.7cm	1.8cm	2.6cm	2.6cm
Kushmanda Rasayanam	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition
Punarnava Manduram	2.5cm	1.4cm	1.9cm	1.9cm	2.5cm
Dasamoola Rasayanam	2.3cm	1cm	0.8cm	1.1cm	2.3cm
Sukumara Lehnam	1.3cm	1.6cm	1.8cm	2.3cm	1.5cm



EFFECT OF AYURVEDIC RASAYANAM ON  
ESCHERICHIA COLI AND STAPHYLOCOCCUS  
AUREUS BACTRIA

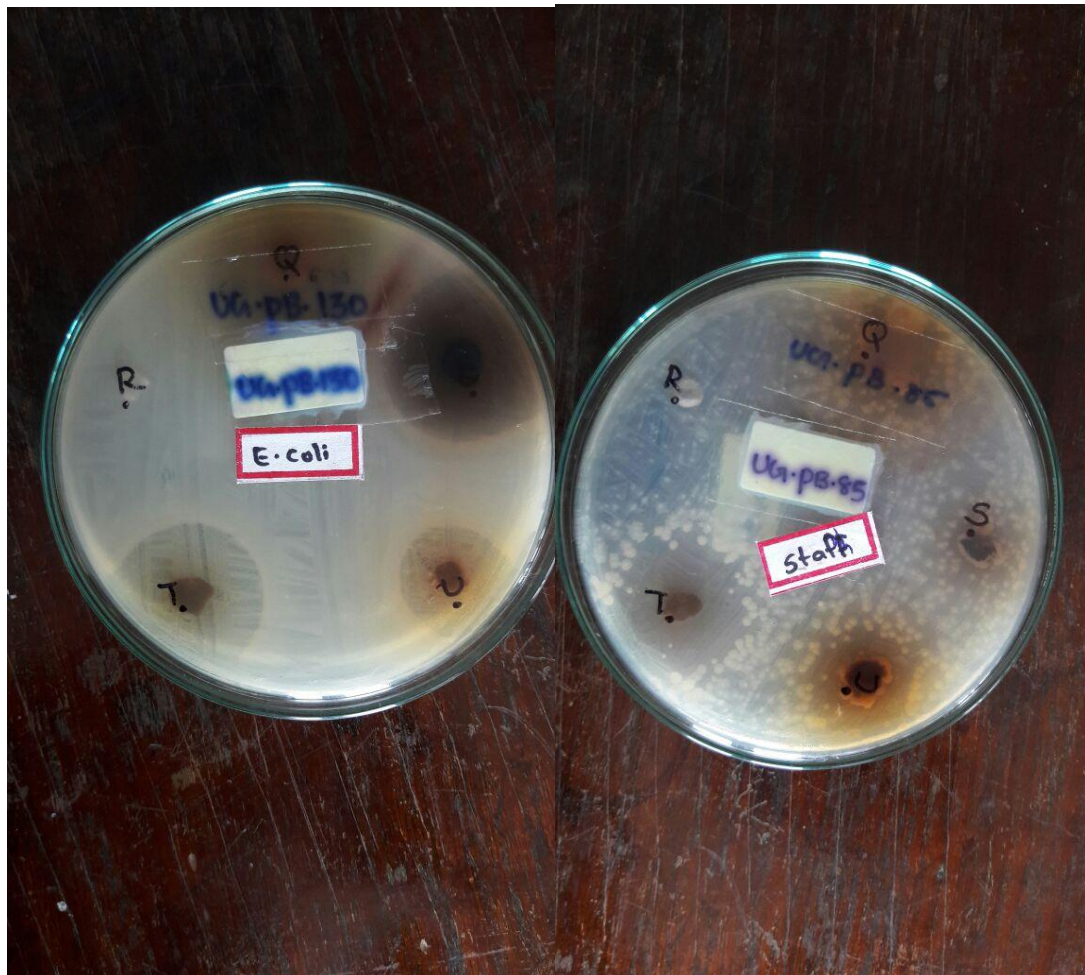


FIGURE 1: PLATES SHOWING ANTIBACTERIAL ACTION ON AGASTYA RASAYANAM, KUSHMANDA RASAYANAM, PUNARNAVA MANDURAM, DASAMoola RASAYANAM, SUKUMARA LEHAM

## EFFECT OF AYURVEDIC RASAYANAM ON PSEUDOMONAS AND SALMONELLA TYPHI

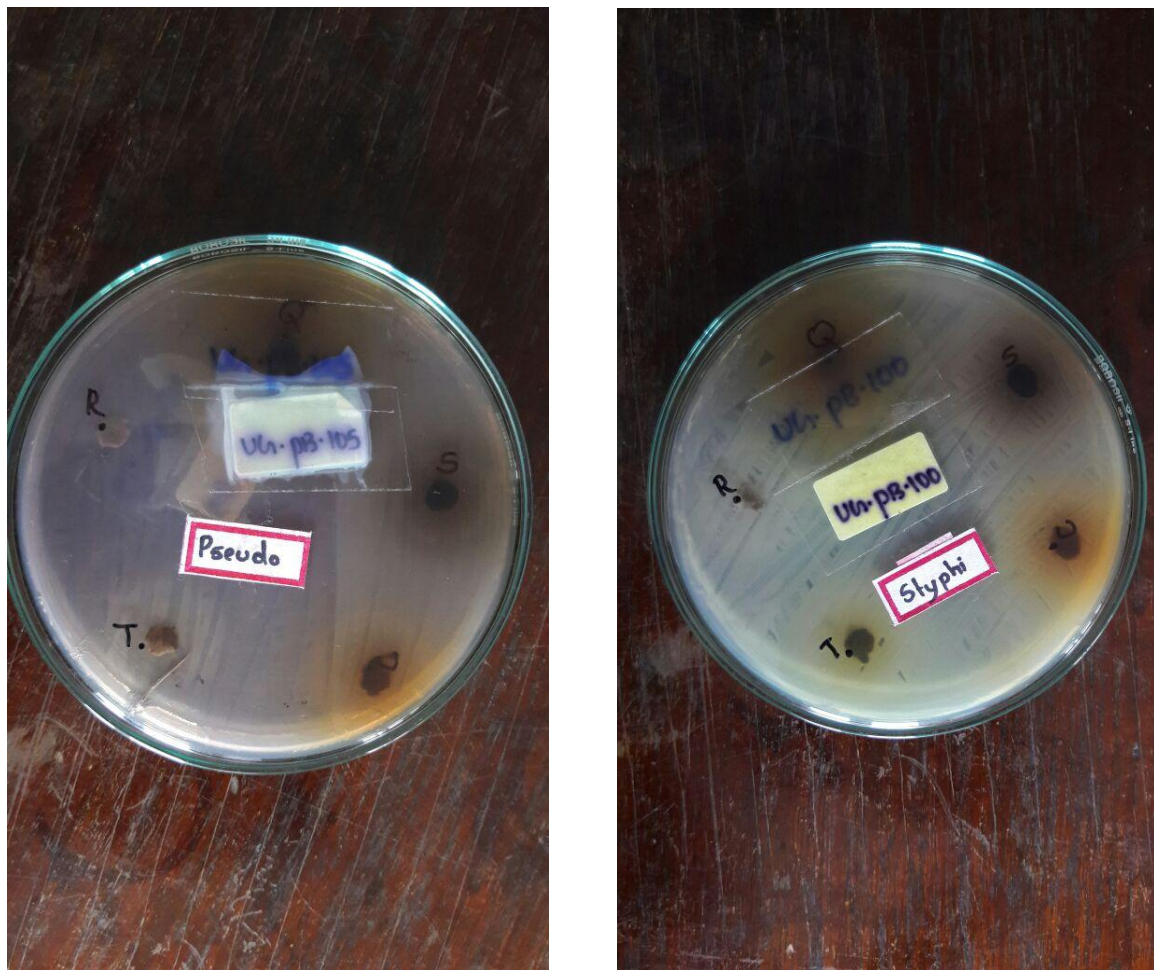


FIGURE 2: PLATES SHOWING ANTIBACTERIAL ACTION ON AGASTYA RASAYANAM, KUSHMANDA RASAYANAM, PUNARNAVA MANDURAM, DASAMoola RASAYANAM, SUKUMARA LEHAM

## EFFECT OF AYURVEDIC RASAYANAM ON KLEBSIELLA



FIGURE 3: PLATES SHOWING ANTIBACTERIAL ACTION ON AGASTYA RASAYANAM, KUSHMANDA RASAYANAM, PUNARNAVA MANDURAM, DASAMoola RASAYANAM, SUKUMARA LEHAM

# RESULT

The objective of this experiment was to determine the anti bacterial effect on Ayurveda Rasayanams against five strains of bacteria which included four gram negative and one gram positive strain bacteria. Gram negative strain include Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Klebsiella pneumoniae. Gram positive strain include Staphylococcus. The five Ayurvedic Rasayanam used for the study were Agastya Rasayanam, Kushmanda Rasayanam, Punarnava Manduram, Dasamoola Rasayanam and Sukumara Lethem. E. coli showed the highest inhibition in Punarnava Mandur of 2.5cm and the least inhibition was shown by Sukumara Leham 1.3cm. Dasamoola Rasayanam and Agastya Rasayanam showed moderate inhibition of 2.3cm and 2.4cm

In case of Salmonella typhi Agastya Rasayanam showed the highest activity with a zone of inhibition of 2.6cm. Dasamoola Rasayanam showed the least activity with a zone of inhibition 1.1cm. Punarnava Mandur and Sukumara Leham exhibited moderate activity against the bacterial strain with a zone of inhibition 1.9 cm and 2.3cm respectively.

Punarnava Mandur has highest inhibition against Pseudomonas aeruginosa with a zone of inhibition of 1.9 cm. Dasamoola Rasayanam has the least activity with a zone of inhibition 0.8cm. Sukumara Leham and Agastya Rasayanam showed moderate activity against bacterial strain with a zone of inhibition 1.8cm.

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the *Klebsiella pneumoniae* the highest activity was shown by Agastya Rasayanam with a zone of inhibition 2.6cm .SukumaraLeham

Showed the least bacterial activity of 1.5cm whereas DasamoolaRasayanam and PunarnavaMandur showed moderate activity of 2.3cm and 2.5cm respectively. Agastya Rasayanam showed the highest zone of inhibition of 2.7cm against the bacteria *Staphylococcus aureus* whereas DasamoolaRasayanam showed the least bacterial activity of 1cm and moderate activity was shown by PunarnavaMandur with 1.4 cm and SukumaraLeham with 1.6cm respectively KushmandaRasayanam failed to give any inhibition against all five strains of bacteria.

In the whole experiment Agastya Rasayanam shows the highest inhibition and lowest inhibition of 2.7cm and 1cm against *Staphylococcus aureus*

## DISCUSSION

The present project compared the antibacterial activity of ayurvedic rasayanams (Agastya Rasayanam, Kushmanda Rasayanams, Punarnamandooram, and Sukumara Leham). From the study it is found that ayurvedic preparations have certain significant antibacterial activity.

Results show that staphylococci showed the highest inhibition against Kushmandarasayanam. *Staphylococcus aureus* is a gram-positive cocci, and they have the capacity to catalase and coagulase positive bacterium.

The lowest inhibition was shown by *Staphylococcus aureus* by Dasasmoolarasayanam where as Kushmandarasayanam did not show any inhibition in all five strains of bacteria.

The highest inhibition is 2.7cm and the least is 1cm which shows that staphylococcus has highest bacterial inhibition on Agastya rasayanam with staphylococcus bacteria and the same bacteria has least inhibition in Dasasmoolarasayanam which shows that the bacterial activity is less.

In Agastya rasayanam showed highest inhibition 2.7cm by the bacteria *Staphylococcus aureus*. Agastya rasayanam is used to cumulatively work on kapha, pitta and vata Dosha associated with old age. In

Agasthya Rasayanams *Aegle Marmelos*, *Oroxylum Indicum*, *Premna Serratifolia* are the main plants used and *Piper longum* – Pippali (Long pepper) is used in common in all the rasayanams. Jagdevsingh (2016), Agastya rasayanam is a good tonic for lungs its main action appears in respiratory tract. It is helpful in common cold, allergic rhinitis (hay fever), abdominal disease, irritable bowel syndrome. It contains herbs and other ingredients which cumulatively work on vata dosha.



Sukumaraleham shows highest inhibition in salmonella typhi 2.3 cm. the components used are Payasya, Ashwagandha Ricinus communis. The common component used in Punarnava and Dasamula is long pepper. This is used for treating abdominal pain, constipation, menstrual pain

Kushmandarasayanam does not show any inhibition. The component used in this rasayanam are Kushmanda (Kushmand or Petha) –

Benincasahispida, Ghee, Sugar, Piper longum-pippali, both Kushmanada and Dasamoolarasayanam use cardamom and cinnamon in common. It is used for Hemostatic (styptic), Nervine tonic & brain tonic, Pitta Dosha and Vata Dosha

J.V Hebbar,;(2017) used in the treatment of bleeding disorders, emaciation, chronic respiratory conditions requiring nutrition and strength and weight. It helps to minimize the side effects of anti-tuberculosis medications by supporting liver and improving digestion.

Punarnavamanduram shows highest inhibition in both estercia coli and klebsiella 2.5 cm. the composition of this rasayanam includes Punarnava, Nishoth roots, Pippali (Long Pepper), Zingiber Officinale. both in Dasamoola as well as Punarnava Elletariacardamomum is used and also Punarnava is used in Sukumaralehm as well as Purnavamandoor, This rasayana is used to treat Anemia, Ascites, liver disease, Jaundice. JLN Shastri (2012) medicinal uses include treatment of Anemia, early stages of liver disorders. Punarvana is an excellent diuretic reaction punarnava is used.

Dasamoolarasayanam shows highest inhibition in Estericia coli and klebsiella 2.3cm. It is made out of Aegle Marmelos, Premna Serratifolia, Oroxylum Indicum, Pippali (Long

Pepper).cinammon is present in both dasamoola and kushmandarasayanam.thisrasayanam is used in treating Common Cold, Acute Rhinitis, Asthma,abdominallump.RajshNayar (2017)it is an effective Ayurvedic Medicine for acute and chronic upper respiratory tract infections and for many acute and chronic diseases

# CONCLUSION

The field of Ayurvedic Rasayanams uses various herbal remedies to heal all soughts of diseases conditions and bring back the body into balance. These Ayurvedic Rasayana drug enhances physical strength and improves complexion. They are immune stimulators and rejuvenatives . In research five Ayurvedic Rasayanams were selected and their antibacterial effect was studied against five strains of bacteria of which one is gram positive (*Staphylococcus aureus*) and the other four is gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*) the antibacterial effect was studied using Kirby-Bauer Disc diffusion method. The highest inhibition zone was shown by Agastya Rasayanam. Kushmanda Rasayanam failed to give any inhibition zone against all five strains of bacteria. The maximum inhibition was shown against *Klebsiella pneumoniae*. Lowest inhibition zone was obtained by Dasamoola Rasayanam against *Pseudomonas aeruginosa*

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