WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Comparative Study of *Alium Sativum* And *Zingiber Officinale* Against Respiratory Tract Infectional Organism *Staphylococcus Aureus*

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ABSTRACT

The present study investigation was aimed to identify as the phtyochemiacl analysis and antibacterial properities *of Allivum sativum* and *zngibier officinale*. Properties of ginger and garlic cure diseases like anti- tumor, in cardiovascular disorder, in liver damage, arthritis, cramp, sprain, constipation, vomiting, and hypertension. Phytochemical analysis of ginger and garlic extract in solvent extract of methanol and aqueous extract was performed. In those the presence of alkaloids, saponins, flavonoids, tannins, carbohydrate, Glycosides are identified. The biochemical test was conducted to identify the bacteria present in respiratory tract infection and identified as *Staphylococcus aureus*. Agar well diffusion method, agar disc diffusion method was conducted to see the antimicrobial activity of aqueous and methanol extract of *Allium sativum and Zingiber officinale* against S*taphylococcus aureus*. The agar well diffusion method showed that zone of inhibition of garlic extract was higher than the ginger extract in different concentration. The diameter of zone inhibition in ginger and garlic aqueous extract showed that 10.5 ± 0.2 mm to 16.0± 0.2mm and methanolic extract of ginger and garlic showed that 23.0 ± 0.2mm to 15.5 ± 0.2mm in various concentration. In addition, antibacterial activity of disk diffusion method of ginger and garlic extract shows that zone of inhibition was 14.0 ± 0.2mm to 11.0 ± 0.2mm for *Staphylococcus aurues* while zone of inhibition in disk diffusion method of aqueous extract of garlic less effective then methanolic extract of ginger.

Keyword: *Allium sativum and Zingiber officinale*, S*taphylococcus aureus*, Disk diffusion method, Agar well diffusion method

1. INTRODUCTION

Garlic (Alium sativum L.) is under family *Liliacea*. It is an erect annual herb with superficial adventitious root, bulbs, composed of a disk like stem. It has been used as long traditional medicinal plant, started with a direction of preparing medicinal remedy written in a cuneiform character in about 3000 BC. Garlic family Liliaceae, fall within the onion group. The most often used portions of garlic for therapeutic purpose are the bulb and cloves. Other members of the family include onion, leek, and shallot. There are many culinary and medical uses for garlic (**Karuppiah and Rajaram, 2012**). It has a strong flavor that mellows and becomes better when cooked. It has been used to treat infection such cold flu, diarrhea, asthma, sore throats, gastrointestinal discomfort, and respiratory tract infection (**Abubakar, 2009; Shobhana *et al.*, 2015**).

1.1 GARLIC

The perennial bulbous plant known as *Allium sativum*, also known as ('aayu' in youba, 'ayoishi' in Igbo, and 'tafarunua' in Hausa) originated in middle Asia and is now farmed all over the world. Allium sativum is a scientific name of garlic. It is commonly known as garlic. It is an odoriferous plant belonging to a lilacease family. Garlic is a common plant and easily provides the market. It is a small perennial herb with narrow flat leaves, and is confined all sides by membranous patches. Garlic was grown mostly in Northen Nigeria.

The scientific classification of garlic given below:

Kingdom : Plantae

Clade :*Tracheophytes*

Clade : *Monocots*

Order : *Asparagales*

Family : *Amaryllidaceae*

Subfamily : *Allioidae*

Genus : *Allium*

Species : *A. sativum*

The medicinal property of garlic due to its "Sulphur" content which was believed to be responsible for it is medicinal value. Raw garlic is used to treat colds and coughs. Garlic is an herbal ingredient for lowering high blood pressure, fighting heart alignments and cholesterol. It is used mainly as spice and also for its medicinal property

Naturally occurring plant have played an important role in the discovery of new therapeutic agent. The therapeutic uses include beneficial effect on the cardiovascular system, anticancer, antibiotics, anti-inflammatory, hypoglycemic and hormone like effect.

Garlic has a maximum height of 2 feet. The primary component of the plant that utilized as medication is the bulb. Each garlic bulb made up of 4 to 20 cloves. Each garlic clove could weigh as much as 1 gram. Garlic supplements may be made from fresh, aged, dried garlic. It is frequently employed as seasoning. It lowers blood pressure, lowers cholesterol, and prevents atherosclerosis, among other heart disorders. The medicinal potency of garlic is due to glycoside, vitamin B, C and D, allisatin . It is also contain volatile sulphur oil, which has a vermifugal action (**Arshad *et al*., 2016**)

Garlic is rich in compounds like Allicin, Sulphur, Zinc, and Calcium that have health benefits, beauty benefits as well as antibiotics and antifungal properties. It is also a rich source of selenium. Selenium is known to fight cancer and it works on vitamin E in body to boost antioxidant powder. Garlic as a medicinal plant widely used and found to be very effective on infections.

Garlic is a mostly useful plant for medicinal, it control infection. Garlic has been used from the ancient times in India and China for a valuable effect on the heart and circulation, cardiovascular disease and regular use of garlic may help to prevent cancer, to treat malaria, and to raise immunity. Garlic has also proposed to treat asthma, colds, diabetes, and antibacterial effect against food pathogens like *S.aureus.*

Numerous features of garlic, such as antibacterial, anti-neoplastic, anti-cardiovascular, immune-stimulatory, and hypoglycemic activity, have been discovered. Gram-positive and Gram-negative bacteria are both susceptible to garlic. According to studies, raw garlic juice is effective against a variety of pathogenic bacteria, as well as against strains that are resistant to antibiotics. It also stops pathogenic strains from manufacturing toxins. A study of antibacterial activities of garlic was reported by (**Harris *et.al*., 2001**) subsequent research revealed that garlic also had pharmacological effects.

The name “Allium sativum” is derived from the celtic word “all” meaning burning a stinging, and the Lathin “sativum” meaning ‘planted or cultivated’. This medicinal plant is mainly used as condiments and for different cooking. The use of higher plants and their extracts to treat infections is an ancient practice in traditional medicine. Many plants have been used because of their antimicrobial treats, which are chiefly synthesis during secondary metabolism of the plants. The herbal medicine may be in the form of powders, liquid or mixtures, ointments and incision are may be raw or boiled.

Traditional medicine is the sum of total of knowledge skills and practices based on the theories, beliefs and experiences indigenous to different cultural that are used to maintain health, as well as to prevent, diagnose , improve or treat physical and mental illness, (Thomas *et.al.*,). In many developing countries, a large proportion of the population relies on traditional practitioners of medicinal plants in order to meet health care needs. Garlic (*Allium sativum*) is one of those plants that were seriously investigated over several years and used for century to light infection disease.

Allicin is an organosulfur compound found in garlic (the activity ingredient); it shows inhibitory effect on some pathogenic bacteria. The best known and well studied effect of Allicin was illustrated by controlling and killing activity to *Staphylococcus aureus* (MRSA). *Allium sativum* could manage and regulate the oxidative stress status by trapping (binding and subsequent deactivating) the harmful oxidant agents (free radicals).

1.2 GINGER

Ginger, *Zingiber officinale* is an erect slim, herbaceous perennial plant that possess a fleshy and thick underground rhizome and having one or more aerial leafy stems, that grows up to 1.25m tall. Ginger is cultivated in countries with tropical climates such as China, Jamaica, Brazil, West Africa, Australia, and some regions of the United States (**Suruchi *et.al*., 2016**). A green, straight stalk-like stem around 60cm high emerges from the rhizome during the first year of growth. Every year, the leaves on it grow to a length of 12 to 30cm.

The scientific classification of ginger is given below:

Kingdom : Plantae

clade : Tracheophytes

clade : Angiosperm

clade : Monocot

clade : Commelinids

order : Zingiberaceae

Genus : Zingiber

Species : *z.officinales*

The crop benefits from warm, sunny conditions for growth and it might benefits from shad on hot days, especially when it is young. However shading is frequently viewed as unnecessary. Both the aroma and flavor of ginger are distinctive fragrant, and strong.

Fresh ginger, powdered ginger is widely used as a spice. Fresh ginger has wide application in cooking. Ground ginger is used mostly for cooking purposes and may also as flavor in processed foods. Preserved ginger on the other hand is used in the production of processed foods such as jams, marmalades, cake and confectioneries (**Sharifi-Rad *et.al.*, 2017**)

Ginger plants have been used as spice and medicine in China and India for ages. Ginger plants were grown in pots and carried abroad on long sea voyages to prevent scurvy. Oil extracted from this plants exhibit antimicrobial activity due to the presence of components such as eugenol, thymol, 1, 8-cineole, linalool and terpinoeol (**Suruchi *et.al.*, 2016**). Fresh ginger has application in the treatment of cold-induced diseases, nausea, asthma, cough, colic, heart palpitation, swelling, dyspepsia, loss of appetite and rheumatism. It serves as remedy for asthma and cough when fresh ginger juice is mixed with small quantity of fresh lemon juice and honey (**Ponmurugan and Rajaram, 2012**). Ginger play important roles due to the presence of certain constituents such as gingerol paradol,shogoal, zingerone, terpenoids and ginger flavonoids (**Arshad *et al.*, 2014**).

Ginger (rhizome) is the root of the *Zingiber officiale* plant, which can be utilized as a medication or as pleasant condiments. In addition to ginger, there are other important followers to this plant family including turmeric, cardamom.

Zingiber officinale creates sets of flower sprouts (pink and white) that developed into yellow flowers. As a result of the hot weather the plant exhibits beautiful appearance and the habituation. *Zingiber officinale* is usually as scenery across subequatorial homes. Fully developed *Zingiber officinale* roots are fibrous. The syrup from ancient ginger rhizomes is highly strong and usually utilized like condiments. It is as typical component of cooking in many Asian countries also this return to its pleasant relish which makes the taste of many foods dishes an extremely delicious.

Zingiber officinale is a medicinal plant that has been widely used all over the world, as antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases. Ginger has direct antimicrobial activity and thus can be used in the treatment of bacterial infections.

The Zingiberaceae plants have strong and medicinal properties and characterized by their tuberous or non tuberous rhizomes. Ginger is relative inexpensive due to their easy availability. Universally accepted and well tolerated by the most people. In many countries including Bangladesh, ginger is used in boiled food preparation.

Stomach infections and other health issues can be resolved because to the antibacterial properties of ginger's gingerols and shogaols. It has potent antibacterial effects, and ginger's active ingredients stop intestinal bacteria from replicating. Additionally, it prevents the growth of *E.coil* and other bacteria.

Antibacterial properties of ginger could be attributed to its phenolic components. In the past, ginger was used to cure intestinal infections, particularly those that had an impact on digestive health. In light of this, study will evaluate the antibacterial activity of ginger against the bacteria that are most frequently present in the digestive tract.

The size of seed ginger, called rhizome, is essential to the production of ginger. The large the rhizome, the faster ginger will be produced and therefore the faster it will be sold onto the market. Prior to planting the seed rhizomes, farmers are required to treat the seed and rhizome root from seed-borne diseases. There are various ways that farmers do seed treatment in India. These include dipping the seeds in cow dung emulsion, smoking the seeds before storage, or hot water treatment (**Kodoth Prabhakaran *et.al.*, 2019**).

The patients with Diabetes mellitus, cataract might have developed due to glycation. Ginger is chosen because of its anti-glycating property. The traditional medicinal uses of ginger are to treat rheumatoid arthritis and gastric ulcer. Androgenic, antioxidant effect of ginger has been known worldwide to treat diseases.

Zingiber officinale roscoe is a rhizomatous perennial herb, reaching up to 90cm long. Rhizomes are aromatic, thick lobed, pale yellowish, bearing simple alternate distiches narrow oblong-lancelet leaves. The herb develops several lateral shoots in clumps, which begin to dry when the plant matures. Leaves are long and 2-3cm broad with sheathing bases, the blade gradually tapering to point.

In florescence solitary, lateral radical peduculate oblong cylindrical spikes. Flowers are rare, rather small, calyx superior, gamosepalous, three toothed, open by splitting on one side, corolla of three sub-equal oblong tolanceolate connate greenish segments (**Yousaf *et.al.*, 2015**).Ginger rhizome is extensively consumed as a spice in foods and beverages because of its characteristic pungency and piquant flavor. It is used in a variety of foods and also in carbonated drinks, in liquors and as a preserve in sugar syrup.

1.3 STAPHYOCOCCUS AUREUS

Staphyococcus aureus is a Gram-positive, spherically shaped bacterium, a member of the Bacillota, and is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobic that can grow without the need for oxygen. Although, *S.aureus* is usually acts as a commensal of the human microbial organism. It can become an opportunistic pathogen, being 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 protein toxins, and the expression of a cell-surface protein that binds to inactivates antibodies. *S.aureus* is one of the leading pathogens for deaths associated, with antibacterial resistance and the emergence of antibiotic-resistant strains, such as methicillin-resistant**.**

Staphylococcus aureus is the most medically important member in terms of pathogenicity of the group. Two other less important members are *Staphylococcus epidermis* and *Staphylococcus saprophyticus*. It is commonly present in the upper respiratory tract and intestinal tract. They grow on a variety of media and ferment many carbohydrates without gas. Colonies may be white, gray, or golden yellow, the species name *aureus* is derived from the Latin word, 'aureus' meaning golden. They are halophilic (salt 'loving').

The increased usage of antibiotics has induced micro organisms to acquire resistance factors which have become a public health challenge. As a result there is an urgent need to find the alternative of chemotherapeutic drugs in treatment particularly those of plants origin which are easily available and have considerably less side effects.

Respiratory tract infections (RTIs) are caused by the invasion of the respiratory tract by infectious micro-organisms such as bacteria and virus. Lower respiratory infections, such as pneumonia, tend to be far more serious conditions than upper respiratory infections, such as the common cold. Typical infections of the upper respiratory tract include tonsillitis, pharyngitis, laryngitis, sinusitis, certain types of influenza, and the common cold. Symptoms of URIs can includes cough, sore throat, runny nose, nasal congestion, headache, low grade fever, facial pressure and sneezing (**Eccles, *et.al.*, 2007**).

Lower respiratory tract infections are generally more serious than the upper respiratory infections. LRIs are the leading cause of death among all infectious diseases (**Beaglehole,*et.al*,. 2004**). The two most common LRIs are bronchitis and pneumonia (**Antibiotic Exert Group, 2006**). Lower respiratory tract infection, is often used as a synonyms for pneumonia, can also be applied to other types of infections including lungs abscess and acute bronchitis.

The use of higher plants and their extracts for treating infectious diseases has long been practiced in many different forms including powder, liquid or mixtures. The research works in is geared to words creating more awareness on the use of herbal medicine such as ginger and garlic in overcoming antibiotic drug resistance.

The antimicrobial activity of spices is due to its specific phytochemical or essential oil. The main factors that determine the antimicrobial activity are the type of composition of the spice, amount used, type micro-organism, composition of the food, pH value and temperature of the environment. Several reports had been published that describe the antibacterial and anti-fungal properties of different herbs and spices

The goal of this study was to assess comparative study of ginger and garlic aqueous extract and methanol extract effective against *Staphylococcus aureus* in respiratory tract infection respectively.

2. AIM AND OBJECTIVE

2.1 AIM

To examine the antimicrobial activity of *Zingiber officinale* and *Allium sativum extract* against *Staphylococcus aureus* in respiratory tract infection.

2.2 OBJECTIVES

- To prepare *Zingiber officinale* and *Allium sativum* extract
- To isolate bacteria from respiratory tract samples (sputum by swab)
- To perform phytochemical screening of *Zingiber officinale* and *Allium sativum*
- To prepare culture for Staphylococcus aureus

- To maintain suitable method for biochemical screening
- To evaluate antimicrobial activity of *Zingiber officinale* and *Allium sativum* extract by well diffusion method and disc diffusion method.

3. MATERIALS AND METHODOLOGY

3.1 CLEANING AND GLASSWARES

Glassware's such as petri-plates, beakers, pipettes, conical flask and test tube used for various experiment were cleaned with detergent washed with tap water, finally with distilled water and dried in Hot air over 80^0 C. The glassware' was sterilized 121^0C for 15 minutes.

3.2 STERILIZATION OF MATERIALS

Sterilization of culture media containers and the instruments are done at 121 lbs for 15 minutes, which is essential for complete sterilization.

3.3 COLLECTION OF PLANTS MATERIALS

The ginger and garlic was collected from local market at Tiruvannamalai. The health and infection-free condition of the plant was guaranteed.

3.4 PREPARATION OF AQUEOUS EXTRACT OF GINGER AND GARLIC

The ginger rhizome was washed with distilled water to get rid of sand particle and air dried at ambient temperature for six week. The garlic bulb were separated into cloves, the cloves skin were peeled off and the cloves were sliced and also air dried at ambient temperature for about seven week. The dried materials were pounded using a sterile laboratory mortal and grinded using a sterile electric blender to obtain a homogenous sample. Ten grams each of ginger powder was soaked in 100ml of distilled water and 100ml of methanol separately. The flasks were incubated at room temperature for 72 hours. The crude extracts were centrifuged at 3000rpm for 10 minutes at 28 ± 2^OC, and supernatant filtered with filter paper. The extract were concentrated using a rotary evaporator. It was then stored in sterile sample bottles and preserved in the refrigerator at 4^oC until further uses. Portion of the plant extract were then subjected to phytochemical screening.

3.5 PHYTOCHEMICAL SCREENING OF ZINGIBER OFFICINALE

In order to identify different classes of active chemical element in the collected plant extract, a qualitative photochemical analysis was performed using accepted techniques.

3.6 ALKALOIDS TEST (MAYER'S TEST)

- 2ml of ginger extract was taken in a test tube.
- Add 1 ml of hydrochloric acid to each tube.
- A few drop of saturated picric acid solution were added.
- They are observed for the result.
    - POSITIVE RESULT **:** A creamish precipitate obtained.
    - NEGATIVE RESULT **:** No precipitate formation appears.

3.7 TANNINS TEST (BRAYMER'S TEST):

- Take 2ml of the ginger extract was dissolved in 10ml of distilled water and filtered
- Take 2ml of the extract in the test tube.
- Then add 2ml $FeCl_3$ to them.
- They are observed for the result
    - POSITIVE RESULT **:** Appearance of black precipitate.
    - NEGATIVE RESULT **:** No appearance of black precipitate.

3.8 STEROIDS TEST (SALKOWSIS TEST)

- Take 10 ml of the ginger extract in the test tube.
- Mixed 10 ml of chloroform and filtered.
- Then 2ml filtrate was added to 2ml acetic anhydride.
- Then added by concentrated H_2SO_4 to the extract.
- They are observed for the result.
    - POSITIVE RESULT : Formation of blue green ring.
    - NEGATIVE RESULT : No formation.

3.9 FLAVONOIDS TEST

- 2ml of the ginger extract was taken in a test tube.
- Then diluted NaOH and diluted HCL were added.
- They are observed for the result.
    - POSITIVE RESULT : Solution turn to colorless
    - NEGATIVE RESULT : The presence of yellow solution

3.10 CARDIAC GLYCOSIDES

- 2ml of ginger extract was taken in the test tube.
- Then added 1ml of glacial acetic acid, 1ml of $FeCl_3$.
- They are concentrated with 1ml of H_2SO_4.
- They are observed for the result.
    - POSITIVE RESULT : Absence of green blue colour
    - NEGATIVE RESULT : Formation of green color.

3.11 SAPONINS TEST (FROTHING TEST):

- Take 0.5 ml of the ginger extract was taken in a tube.
- Add 5ml distilled water into the tubes.
- They are observed for the result.
    - POSITIVE RESULT : Formation of frothing.
    - NEGATIVE RESULT : No formation of frothing.

3.12 PHYTOCHEMIACL SREENING OF ALLIUM SATIVUM

3.13 ALKALOIDS TEST(MAYER'S TEST)

- 1 ml of garlic extract was taken in a test tube.
- Add 0.2 ml of dilute hydrochloric acid.
- Then add observed for the result.
    - POSITIVE RESULT : Appearance of yellowish precipitate.
    - NEGATIVE RESULT : No precipitate formation.

3.14 SAPONINS TEST (FOAM TEST):

- 1ml of garlic extract was taken in a test tube.
- Diluted with 5 ml of distilled water.
- The contents are heated in a boiling water bath for few minutes.
- They are observed for the result.
    - POSITIVE RESULT : Formation of foam.
    - NEGATIVE RESULT : No foam formation.

3.15 TANNINS TEST (BRAYMER'S TEST):

- Take 1 ml of garlic extract in a test tube.
- Boiled gently for 2 minutes.
- Then allowed to cool the tube.
- Add 2 drops of a 5% ferric chloride solution to the extract.
- They are observed for the result.
    - POSITIVE RESULT : Appearance of dirty green precipitate.
    - NEGATIVE RESULT : No appearance of dirty green precipitate.

3.16 STEROIDS TEST (SALKOWSIS TEST):

- Take 2 ml of the garlic extract in a test tube.
- Mixed 2 ml of chloroform in that tube.
- Then added by concentrated sulfuric acid to the extract.
- They are observed for the result.
    - POSITIVE RESULT : Formation of reddish brown ring.
    - NEGATIVE RESULT : No ring formation.

3.17 PHENOL TEST

- 1ml of the garlic extract was taken in a test tube
- The treated with 3%ferric chloride in to tube.
- They are observed for the result.
    - POSITIVE RESULT : Appearance of deep blue color.
    - NEGATIVE RESULT : No appearance of deep blue color .

3.18 FLAVONOIDS TEST:

- 1ml of the garlic extract was taken in a test tube.
- Mixed with 1ml of sulphuric acid into a tube.
- They are observed for the result.
    - POSITIVE RESULT : Formulation of orange color.
    - NEGATIVE RESULT : No color formation.

3.19 CARBOHYDRATES TEST :

- Take 2ml of garlic extract in the test tube
- They treated with 1ml Molisch reagent in to tube
- They were concentrated with few drop sulfuric acid in tube and shaken well
- They are observed for the result.
    - POSITIVE RESULT : Violet color ring formation.
    - NEGATIVE RESULT: No violet ring formation.

3.20 COLLECTION OF SAMPLE:

Sputum sample was collected from clinical isolates of respiratory tract infection patient at Arunai medical hospital in Tiruvannamalai. The samples were carefully labeled. The sample were inoculated in suitable agar medium and maintained in 4 ^{0}C for the further screening.

3.21 ISOLATION OF THE TEST ORGANISM:

- The samples were inoculated in the nutrient broth and nutrient agar plates.
- The nutrient broth was prepared and sterilized in test tubes.
- The collected sample were inoculated in nutrient broth and incubated for 24 hours at 37^{0}C for the isolation of bacteria.
- The nutrient agar plates were inoculated and incubated for 48 hours at 28 ^{0}C.

3.22 IDENTIFICATION OF BACTERIA'S MORPHOLOGICAL CHARACTERISTICS

3.22.1 GRAM STAINING

- A thin smear was made from the colonies of agar plate and heat fixed.
- The smear was covered with 2-3 drops of crystal violet for a minute.
- The slide was washed with water and then covered with gram iodine for one minute.
- Again the smear was washed to decolorize the slide gently by adding acetone/alcohol till it de-stain the Gram's iodine.
- Then the slide was counter stained with saffranin for 30 seconds.
- Once again the slide was washed with water blot dried with tissue paper and viewed under the oil immersion microscope, to identify the type of bacteria present in it.

3.22.2 COAGULASE TEST:

3.22.2 a. SLIDE TEST:

- Place two separate drops of saline on a slide.
- Using a sterile inoculating loop, emulsify one or two colonies of organism in one drop to make thick suspension of bacteria.
- Add a loop full of plants to both the suspension and mix gently.
- Look for immediate coagulase clumping of the mixture within 10-15 seconds.

3.22.2 b. TUBE TEST

- Dilute the plasma 1:10 with saline.
- Take 2 test tubes and add 0.5 ml of diluted plasma to each.
- Inoculate a tube with bacterial colonies to make a cloudy suspension.
- Alternatively, add about 5 drops of thick 18-24 hours broth cultures.
- Incubate both tubes at 35 ^{0} C for 1 to 4 hours in water bath.

- After that examine both tubes for the presence or absence of clots.

3.23 CATALASE TEST

- Use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean ,dry glass slide
- Place a drop of 3% hydrogen peroxide in the glass slide
- Observe for the evolution of oxygen bubble

3.24 COLONY CHARACTERISTICS

Based on the morphological characteristics the organism was inoculated on the selective Media's.

3.24.1 SELECTIVE MEDIA FOR STAPHYLOCOCCUS AUREUS

- The Mannitol salt agar was prepared and poured in petriplates and allowed to get solidifies
- The samples were streaked on the plates and incubated at 37 ^{0}C for 24hrs
- After incubation, the plates were observed for bacterial growth and formation of golden yellow colony.

3.25 BIOCHEMICAL TEST

The biochemical test was conducted by the following methods as described by **Cappuccino and Sherman** (**1999**) to identify the bacteria.

3.25.1 INDOLE TEST

- Tryptophan broth was prepared, sterilized and dispensed into sterile test tubes.
- Inoculate the tubes of tryptophan broth with the test organism at 37 ^{0}Cfor 24 hours
- After incubation add 0.5ml of Kovac's reagent and shake
- Allow to stand for few minutes and observe the ring formation.

3.25.2 METHYL RED TEST

- MR-VP broth were prepared, sterilized and dispersed into sterile test tubes.
- Inoculate the tubes with the test organism and incubates at 37^0 C for 24 hours.
- After incubation add 5 to 6 drop of methyl red solution and shake.
- Allow to stand for few minutes and read result.
    - POSITIVE RESULT : Red color
    - NEGATIVE RESULT: Yellow color

3.25.3 VOGES –PROSKAUER'S TEST

- MR-VP broth was prepared, sterilized dispersed into sterile test tubes.
- Inoculate the test tubes with organism and incubates at 37^O C for 24 hours.
- After incubation add 3 ml of Barritt's reagent A and 3ml of Baaritt's reagent B and shake.
- Allow to stand for few minutes and read results.
    - POSITIVE RESULT : Red color
    - NEGATIVE RESULT: Yellow color

3.25.4. CITRATE UTILIZATION TEST

- Simmon citrate agar was prepared, sterilized dispensed into sterile test tubes.

- Slants were made and inoculate with the organisms.
- The test tubes were incubated at 37^{O} C for 24 hours.
- After incubation read for results.
    - POSITIVE RESULT : Prussian Blue Color
    - NEGATIVE RESULT: Green color

3.25.5. CATALASE TEST

- This test was performed to detect the enzyme catalase.
- This enzyme was responsible for protecting bacteria from hydrogen peroxide accumulation, which can occur during aerobic metabolism.
- The hydrogen peroxide accumulates and becomes toxic to the organisms. Catalase breaking hydrogen peroxide into water and oxygen.
- To perform this test a small amount of test organism was plased in the lid of the petriplates or glass slide.
- Then a drop of hydrogen peroxide was added to the petri plate or slide. Observe the result for the presence or absence of catalase.
    - POSITIVE RESULT : Bubble formation
    - NEGATIVE RESULT: No bubble formation

3.25.6. OXIDASE TEST

- This test is performed to identify the bacteria which possess the enzyme oxidase.
- Place the oxidase disc on a clean glass slide.
- Add a drop of test organism on the disc and observe the color changes.
    - POSITIVE RESULT : Dark Purple Color
    - NEGATIVE RESULT: No color change

3.25.7. TRIPLE SUGAR TEST

- The Triple sugar iron agar medium was prepared, Sterilized and dispensed into sterile test tubes.
- Slants were made and incubated with the test organism.
- The test tubes were incubated at 37^{O} C for 24 hours.
- Then observe for the gas, alkaline and acid production in it.

3.25.8. UREASE TEST

- The Christensen's urea agar medium was prepared, sterilized and dispensed into sterile test tubes.
- Slants were made and incubated with the test organism.
- The test tubes were incubated at 37^{O}C for 24 hours.
- Then observe the color changes.

3.26 ANTIBACTERIAL ACTIVITY

3.26.1 CHARACTERISTICS OF THE MEDIUM

The main characteristics of the medium (Mullen Hinton agar medium) was support the growth of the organism, normally tested.

3.26 2. PREPARATION OF INOCULUM

24 hours old culture of selected bacteria was mixed in nutrient broth, turbidity was observed after 24hours of incubation.

3.26.3 DISC PREPARATION

Wattman NO:1 filter paper disc (5mm) was prepared. The discs were sterilized by autoclave at 121^{O}C. After sterilization the moisture disc were rinsed with plant extract and allowed to dry at room temperature.

- Paper disc soaked in plant extract solution were allowed to stand for a period of one hour to ensure full saturation of the extract preparation. The discs were then aseptically removed from extract solution and allowed to dry in an oven at 25^{O}C.

3.26.4. ASSAY OF ANTIBACTERIAL ACTIVITY

Overnight cultures were kept ready to antimicrobial activity. Assay of the antibacterial activity of plant were done by disc diffusion assay, agar well diffusion assay.

3.26.5. ANTIBACTERIAL ACTIVITY USING DISC DIFFUSION METHOD

- The bacterial strains were swabbed on Muller Hinton agar plates.
- The disc was placed on the agar plates on different dilution.
- 20ml of dilution were added on each disc
- The plates were incubating at 37 ^{0}C for 24 hours.
- The zone of inhibition was calculated

3.26.6 ACTIVITY ON AGAR WELL DIFFUSION

- Muller Hinton agar was prepared and the test organism were swabbed on the Muller Hinton agar plates
- The MHA plates were punched with cork borer to 4mm.
- The 20ml of sample was poured in the bore with micropipette
- The plates were allowed to standby for 30 minutes.
- The plates were incubated at 37 ^{0}C for 48 hours.
- The antibacterial activity is measured by measuring the zone of inhibition area.

4. RESULT AND DISCUSSION

The current study reveals that garlic extract shows golden – yellow, gummy residue with a pungent offensive smell. The ginger extract was obtained a brown with spicy – sweet smell **FIGURE I**. Phytochemical analysis of the ginger and garlic reveals constituents such as phenol, alkaloids, flavonoids, carbohydrates, glycosides, tannins, steroids, in methanolic and aqueous extraction, as shown in **TABLE I** and **II**.

To 2ml of ginger and garlic extract, few drop of Mayer reagent was added. The formation of creamy white precipitate obtained indicated the presence of alkaloids. Alkaloids are chemical compounds that occur naturally and contain basic nitrogen atoms. They are frequently used as medications and recreational drugs due to their pharmacological effects.

0.5 ml of ginger and garlic extracts, were mixed with 5ml of distilled water. The contents were heated in a boiling water bath. Frothing indicates that presence of saponins. Saponins cause the red blood cell hemolysis. Because of their potential antibacterial properties against pathogenic organism, antibacterial activity was investigated.

2 ml of ginger and garlic extract were dissolved in 10 ml of distilled and filtered. To these take 2ml of extract and 2ml of ferric chloride solution was added. Appearance of black precipitate indicated presence of tannins.

2 ml of ginger and garlic extract were diluted with sodium hydroxide and 1ml hydrogen chloride was added. The yellow colour turn to colorless indicates the presence of flavonoids. It acts as anti-inflammatory and antioxidants properties.

2 ml ginger and garlic extract was added to 1ml glacial acetic acid and 1ml of ferric chloride, and 1 ml of concentrated sulfuric acid. The observed green blue colour indicated the presence of cardiac glycosides.

2ml of ginger and garlic extract was treated with 3% ferric chloride solution. The appearance of deep blue colour indicated the presence of phenol. These compound for mainly possess the antioxidant, anti inflammatory properties.

2ml of garlic and ginger extract was treated with 1ml of Molisch reagent, to these concentrated sulfuric acid was added. The observed violet colour ring indicated the presence of carbohydrates.

The morphological properties of the suspected isolated colonies were used to identify them using Gram stain (size, shape, arrangement). Under oil immersion lens (100x), the bacteria were identified as Gram–positive, pink colour colonies of spherical shaped as shown in **FIGURE II.**

Further study of *Staphylococcus aureus* identified presence of coagulase by clot formation of colonies in both slide and tube test. The catalase test was performed as to use loop full of colonies placed in sterile or dry glass slide. Place a drop of 3% hydrogen peroxide in the glass slide and the observed oxygen bubble indicates the presence of catalase as shown in **FIGURE III.**

The colony morphology of the bacterial isolates was studied by Streak Plate Method on Mannital salt agar. The organism shows golden yellow colonies shown in **FIGURE IV.**

The growth of the bacterial colonies in plates and their Gram stain, biochemical test result shows *Staphylococcus aureus* isolates tested are positive for catalase and Voges – Proskauerand. The TSI test revealed that no gas and no acid production in it. Negative for indole, methyl red, oxidase, simmon citrate, urease test and which were shown in **TABLE III** and **FIGURE V.**

4.1 ANTIBACTERIAL ACTIVITY USING WELL DIFFUSION METHOD

The antibacterial activity of ginger and garlic using agar well diffusion method are given in **TABLE IV.** It indicates that the zone of inhibition of the garlic extract was higher than that of the garlic extract for the different concentration. The diameter for zone of inhibition for garlic aqueous extract ranged from 5.5 ±2.0 to 16.0 ± 2.0 mm at various concentration used. The ginger aqueous extract shows lower zone of inhibition that ranged from 6.0± 2.0 to 10.5 ± 2.0 mm at various concentration shown in **FIGURE VI.**

The zone of inhibition of methanolic extract of garlic was ranged from 15.5 ±2.0 mm to 23.0 ± 2.0 mm at various concentrations. The zone of inhibition of methanolic extract of ginger was ranged from 8.5 ± 2.0 mm to 15.5 ± 2.0 mm at various concentrations shown in **FIGURES VII**.

4.2 ANTIBACTERIAL ACTIVITY USING DISK DIFFUSION METHOD

The results given in **TABLE V** show that ginger methanol extract are more effective against *Staphylococcus aureus* than aqueous ginger extract. The antibacterial activities are due to the presents of flavonoids and volatile oils which were dissolved in organic solvents. The zone of inhibition shows 14.0 ± 2.0 mm. whereas the garlic shows 11.0 ± 2.0 mm **FIGURE VII**

5. DISCUSSION

The result of the present study identify the phytochemical test, biochemical test and evaluates the antibacterial activity of the *Alliuvum sativum and zingiber officinale* against the respiratory tract infection, which conclude ginger and garlic can be considered as a potential antibacterial properties that can be act as drug medicine against the respiratory tract infection. Antibacterial evaluation of aqueous and methanol extract of ginger and garlic revealed a antibacterial disc diffusion and well diffusion. On the similar manner the organism were all susceptible of varying concentration of plant extract. The antibacterial activity of methanol and aqueous extract of ginger and garlic is attributed to the bioactive components (P**on murugan and Rajara, 2012 ; Dixon and Jeena, 2017)**

On similar that the presence of flavonoids, Carbonhydrates, sapanins ,triderpenes and alkaloids present in both *Allium sativum and Zingiber officinale* extract were consistent with those of previous studies (**Cheeks 1989, Abdullahi *et.al*., 2014; Aliyu *et.al*.,2017)**. According to **(Roy *et.al*., 2006),** Antibacterial evaluation of aqueous and methanol extract of constituent of garlic have a long been know and its antibacterial properties have been widely reported.

Garlic is also rich in anionic components such as nitrates, chlorides and sulfates and other water soluble components found in plants and these components may have antimicrobial properties (**Shobana *et.al*., 2009**) reported. Garlic was shown to have antimicrobial activity against *Staphylococcus aureus* (**Silva and Fernandes, 2010; Daka, 2011**).

There are several reports of the inhibitory effect of ginger in the form of extract against several bacteria Moderate to good antimicrobial properties of ginger were shown in previous studies of **(Ibrahim *et al*., 2003; Singh *et al*., 2008; Venugopal *et al*., 2009).** **Masaldha M.*et al*., 2001,** was observed *Staphylococcus aureus* is the most medically important pathogenic organism. They are produces golden yellow colonies. Gram positive rod shaped bacteria. The bacteria were grown on a variety of media and ferment many carbohydrates without gas production.

Saphylocoocus aureus is isolated on mannitol salt agar which medium that contain a high salt content (75% NaCl) and contain mannitol a sources of carbon and energy. *S.aureus* ferment mannitol and cause media turn yellow is reported by **Ochei, J. *et al*., 2000**. **Aliyu *et al*.,(2017)** reported that ginger extract greater antibacterial activity against a variety of bacteria, although mixed result is attributed to different ginger preparations and varying strengths. The low activity shown by ginger extract may also be due to the fact that the active components in the extract may not have had activity in vitro against the clinical isolates or theat the concentration used may not have been high enough to cause antimicrobial effect against organism as reported by **Cheek *et al*.,(1989**).

Antibacterial of activity of garlic and ginger's inhibition done by diffusion method. Garlic is greater than the zone of inhibition of the ginger extract**. (Abullani *et al.*,(2014)** reported the garlic extract shown that zone gave the widest zone of inhibition compared of the ginger extract against all the bacterial isolate.The activity of these plant may be attributed to the presence of secondary metabolies with them said **(Patra and saxena 2009).**

Malu SP.*, et al.*,(2008), as reported that the antibacterial activity of the extracts are expected perhaps due to the compounds like flavonoids and volatile oil which were dissolved in organic solvents. It is reported that sesquiterpenoids are the main component of ginger which attributes its antibacterial activity. **Roy *et al.*,2000**, the results obtained in study corroborate which explains that bioactive compounds of ginger rendering antimicrobial activity are volatile in nature and antimicrobial activity of ginger extract decreases upon storage.

The concentration of (100mg/ml) of ginger and garlic extract had the highest inhibitory effect about (14mm)inhibition zone of methanolic extract of the ginger against the *staphylococcus aureus* . on the similar reported **(Mushi, D.A et al., 2014)**,Ginger extract exerted more effect than garlic extract, having the highest mean zone of inhibition of 16.0 ± 0.21 at 100µg/ml concentration, while garlic extract had a mean zone of inhibition of 11.5 ± 0.69 at 100µg/ml concentration. This is in line with the work of who reported a zone of inhibition of 16mm for ginger extract as against 10mm for garlic extract at 100µg/ml concentration.

6. TABLE AND FIGURES

TABLE – I : PYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACT OF GINGER AND GARLIC EXTRACT

PHYTOCHEMICAL	METANOLIC GARLIC	METANOLIC GINGER
PHENOL	NEGATIVE	NEGATIVE
FLAVONOIDS	POSITIVE	POSITIVE
TANNINS	NEGATIVE	NEGATIVE
SAPONINS	POSITIVE	POSITIVE
STEROIDS	NEGATIVE	NEGATIVE
CARBOHYDRATES	POSITIVE	POSITIVE
ALKALOIDS	POSITIVE	POSITIVE

TABLE –II : PYTOCHEMICAL ANALYSIS OF AQUEOUS EXTRACT OF GINGER AND GARLIC EXTRACT

PHYTOCHEMICAL	AQUEOUS GARLIC	AQUEOUS GINGER
PHENOL	NEGATIVE	NEGATIVE
FLAVONOID	POSITIVE	NEGATIVE
TANNINS	POSITIVE	NEGATIVE
SAPONINS	POSITIVE	NEGATIVE
GLYCOSIDES	POSITIVE	NEGATIVE
ALKALOIDS	NEGATIVE	NEGATIVE
STEROIDS	NEGATIVE	NEGATICE

TABLE-III : MORPHOLOGICAL AND BIOCHEMICAL TEST FOR IDENTIFICATION OF THE ISOLATES

BIOCHEMICAL TEST	*STAPHYLOCOCCUS AUREUS*
INDOLE	NEGATIVE
METHYL RED	NEGATIVE
VOGES –PROSKAUER	POSITIVE
CITRATE UTILIZATION	NEGATIVE
TSI	A/A
UREASE TEST	NEGATIVE
CATALASE PRODUCTION	POSITIVE
COAGULASE TEST	POSITIVE
COLONY MORPHOLOGY ON SELECTIVE MEDIUM	MSA ON YELLOW COLOUR PIGMENT COLONIES
GRAM STAINING	GRAM POSITIVE
CELLULAR MORPHOLOGY	RODS

TABLE-IV: ANTIBACTERIAL ACTIVITY OF GARLIC AND GINGER EXTRACT BY WELL DIFFUSION METHOD

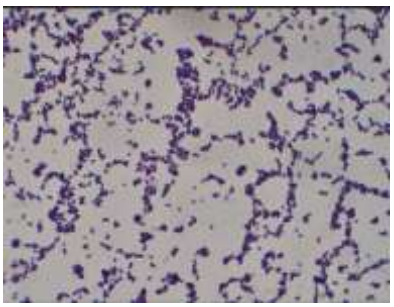
SAMPLE	CONCENTRATION	ZONE OF INHIBITION (AQUEOUS EXTRACT)	ZONE OF INHIBITION (METHANOLIC EXTRACT)
GARLIC	100	16.0± 2.0MM	23.0± 0.2MM
	50	13.0± 2.0MM	20.0± 0.2MM
	25	5.5 ± 2.0MM	15.5± 0.2MM
GINGER	100	10.5± 0.2MM	15.5± 2.0MM
	50	7.0 ±O.2MM	13.0± 2.0MM
	25	6.0±0.2MM	8.5± 2.0MM

TABLE –V : ANTIBACTERIAL ACTIVITY OF GINGER AND GARLC EXTRACT IN DISK DIFFUSION METHOD

SAMPLE	CONCENTRATION	ZONE OF INHIBITION AQUEOUS EXTRACT	ZONE OF INHIBITION METHANOLIC EXTRACT
GINGER	100	13.0 ±2.0MM	14.0±2.0MM
GARLIC	100	16.0±2.0MM	11.0±2.0MM

FIGURE I : **GINGER ANG GARLIC EXTRACT**

FIGURE II: GRAM STAINING FOR *STAPHYLOCOCCUS AUREUS*



F*IGURE III : CATALSE TEST FOR *STAPHYLOCOCCUS AUREUS

FIGURES IV: *STAPHYLOCOCCUS AUREUS* ON MSA PLATE

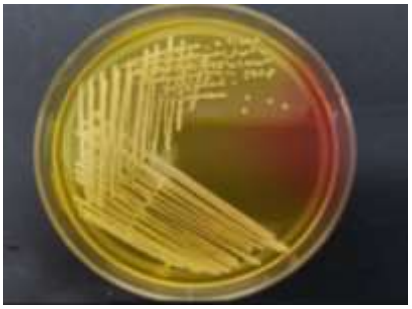


FIGURE VI SHOWING THE ANTIBACTERIAL ACTIVITY BY DISK DIFFUSION METHODS

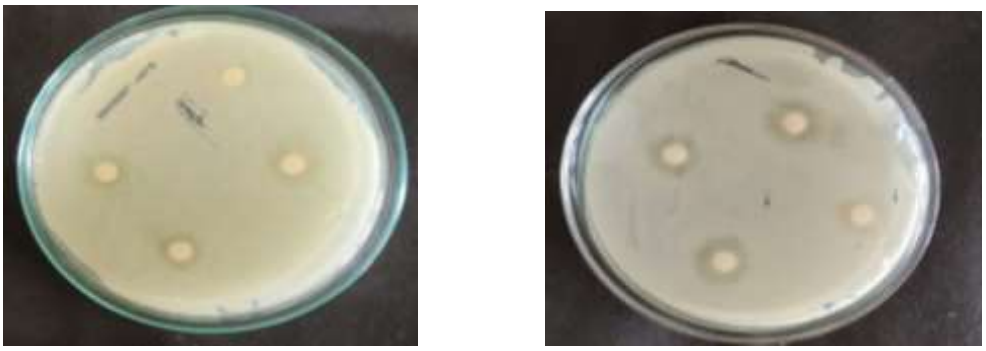


FIGURE VII : SHOWING ANTIBACTERIAL ACTIVITY BY WELL DIFUSION METHODS

7. CONCLUSION

The present study proved that the ginger and garlic extract are good sources for various photochemical like alkaloids, saponins, flavonoids, glycoside, and carbohydrates. Ginger and garlic extract are effective against the infection causing organism of *Staphylococcus aureus*. Garlic and ginger are good source of drug against the respiratory tract infection, gastrointestinal infection and other bacterial infections. However, it is important to assert that the garlic and ginger extract is easily prepared and of low cost. Based on the result of this study, methanolic extract of garlic make clear zones. This showed that the plant has antibacterial activity. The both spices are alternative and supplementary medicine to treat various infections. Further studies carry about pharmaceutical studies to support the uses of ginger and garlic as medicinal plant.

8. SUMMARY

Many people are suffered and affected from respiratory tract infection which are common cold, influenza, sinusitis, lung abscess. Respiratory tract infections are caused by the wide variety of microorganism, where *staphylococcus* caused upper respiratory tract infection. In current study is to analysis the therapeutic valuable of the ginger and garlic extract against the isolates of resident RTIs. Swab and sputum are taken. Staining, morphology, and biochemical tests were then used to determine it's identify. The current study to provides an introduction to the plant. Two solvent, methanol and aqueous were succession to extract the ginger and garlic plant. The inclusion of several phytochemical component, including flavonoids, saponins, alkaloids, tannins, glycosides contributes to the antibacterial action of the ginger and garlic extract. The goal to study is to determine the highest zone of inhibition against pathogenic organism from solvent extract of ginger and garlic. The ginger and garlic may have the therapeutic and preventative benefits, according to phytochemical analysis. This demonstrates how the ginger and garlic act against pathogenic organism and reduces the risk of microbial organism causing infection.

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