



Research Article

INVITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF CLOVE BUDS(*EUGINEA AROMATICA*)A.Mounika¹, M.Sushma², Lahari Sidde³, Sankara Malathi⁴, Konapalli Rajani⁵.

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| Article History: | Abstract |
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| Received on: 01-06-2020 Revised on : 11-08-2020 Accepted on : 25-08-2020 | Bioactive compounds from Eugenia aromaticum were extracted by Soxhlet using DCM ,DCM;WATER,WATER and extracts were examined for its phytocomponents along with Clove oil. These bioactive plant compounds were screened for possible antimicrobial activities against two strains of MDR S. aureus. Antimicrobial activity of extracts using agar diffusion disc variant method. The objective of this study was to evaluate technical variants used in screening methods to determine antibacterial activity of natural products. Thus, a varied range of natural products of plant were tested against two bacterial species,m Staphylococcus aureus ATCC 25923 and klebesella pneumonia ATCC 25922, by two variants of the agar diffusion method (well and disc), two variants of the bio autographic method (direct and indirect) and by microdilution assay. We concluded that the well-variant of the diffusion method was more sensitive than the disc-variant, it was found extract 1 ie,DCM and extract 2 i.e DCM;WATER had shown best results on antimicrobial activity ,phytochemical screening test are done for three extracts invitro evaluation of antimicrobial activity was performed. |
| Keywords: Agar diffusion, antimicrobial activity, bioautographic methods, minimum inhibitory concentration, minimum bactericidal concentration, natural products. | |

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Doi: <https://doi.org/10.46956/ijhd.vi.95>**INTRODUCTION**

Cloves are an aromatic herb that has many useful purposes. The aroma of the clove is pleasant yet spicy and can be used to make drawers and closets smell nice. Cloves has some medicinal purposes as well and it tastes good in certain dishes like spice cake. Cloves like to grow in hot [1-2]. Tropical climates like the islands of Indonesia. The clove plant is an evergreen tree that can reach a height of thirty or forty feet high. The leaves of the clove are leathery textured and are covered with many tiny depressions. The part of the clove that is used is the flower buds of the clove. The aromatic oils of the

clove have a stimulant and irritant effect. Cloves can increase blood circulation and raise a person's temperature slightly. The oils of the cloves have been known to stimulate and disinfect a body as it travels through the body. Clove can be used to promote the flow of saliva and gastric juices [3]. If you have stomach pain or gas in the stomach then the use of cloves can help to relieve the stomach pain. You can prepare a cup of clove tea by taking a cup of boiling water and adding a teaspoon of clove powder to it. Then let the water and clove steep for a few minutes before you drink it. Clove tea has been known to relieve nausea and vomiting. When cloves are used externally on the body it can relieve the pain in chronic rheumatism, toothache and lumbago. Cloves can also help to relieve the pain of muscle cramps and some nerve conditions. You can apply clove oil along the nerve where the pain is. To deodorize a smelly drawer or closet you can put

some cloves in a small container that is open and let it sit in the drawer or closet. Leave the container open and put it in a place that it will not spill. Or you can tie up a handful of cloves in a handkerchief and put it in a closet or drawer. The scent of cloves is pleasant and nice. Cloves are a very useful herb that has many uses for the body and the aroma. Cloves can be used to spice up foods like a ham or cake. Cloves can be purchased in a powder form or it can be in a dried whole flower bud form. You can buy cloves in a grocery store in the spice section. Clove is an herb. People use the oils, dried flower buds, leaves, and stems to make medicine. Clove is used for upset stomach and as an expectorant. Expectorants make it easier to cough up phlegm. Clove oil is used for diarrhoea, hernia, and bad breath. Clove and clove oil are used for intestinal gas, nausea, and vomiting. Clove is applied directly to the gums (used topically) for toothache, for pain control during dental work, and for a complication of tooth extraction called "dry socket." It is also applied to the skin as a counterirritant for pain and for mouth and throat inflammation. In combination with other ingredients, clove is also applied to the skin as part of a multi-ingredient product used to keep men from reaching orgasm too early (premature ejaculation). In foods and beverages, clove used as a flavoring. In manufacturing, clove is used in toothpaste, soaps, cosmetics, perfumes, and cigarettes. Clove cigarettes, also called kreteks, generally contain 60% to 80% tobacco and 20% to 40% ground clove. Eugenol, one of the chemicals in clove, acts like menthol to reduce the harshness of tobacco smoke [4].

ORIGIN OF CLOVE

Cloves are the pink flowering bud of a form evergreen tree (*Eugenia aromatica*) (fig 01), which are dried until brown and used for medicinal and spicing purposes. Indigenous to the Moluccas spice islands of Indonesia, cloves also grow naturally in India, the West Indies, Tanzania, Sri Lanka, Brazil and Madagascar. With their sultry sweet aromatic flavor and powerful essential oil compounds, cloves have been used for hundreds of years as a nutritional spice for food and a remedy for a variety of health concerns. For over 2,000 years, both Indian and Chinese traditional medicine made extensive use of clove flowers and clove oil. Arabic traders brought the buds to Europe in 4th century A.D., and in the seventh and eighth century A.D. Europe, cloves became very popular as a medicinal flower, due to

their ability to preserve foods, and mask the smell of poorly-kept foods [5].

ACTIVE CONSTITUENTS OF CLOVE OIL

Approximately, 72-90% of the essential oil extracted from cloves has Eugenol⁶⁻⁷. Other essential oil ingredients of clove oil are,

1. Acetyl eugenol.
2. Beta-caryophyllene and vanillin
3. Crategolic acid, tannins, gallotannic acid, methyl salicylate (painkiller)
4. Flavonoids eugenin, kaempferol, rhamnetin, and eugenitin
5. Triterpenoids like oleanolic acid.
6. The dried buds of cloves contain about 15 - 20 percent of essential oils, and the bulk of this is eugenol. A kilogram of dried buds provides about 150 ml (1/4 of pint) of eugenol.

Health Benefits of Organically Certified Clove [8-9]

Clove is a natural antiviral, antimicrobial, antiseptic, and anti-fungal agent. It also holds aphrodisiac and circulation-stimulating capacities. The oil of cloves has been used in a variety of health conditions including indigestion, generalized stress, parasitic infestations, cough, toothaches, headache, and blood impurities. In fact, the expert panel German Commission recently approved the use of its essential oil as a topical antiseptic and anesthetic.

Clove may play a therapeutic role in the following conditions

- Powerful germicidal properties
- Anti-Bacterial
- Antiseptic
- General Stress Reliever
- Anti-Inflammatory.
- Blood Purifier
- General Immune System Booster
- Premature Ejaculation
- Indigestion
- Cancer Prevention
- Cardiovascular Health
- Prevention from toxic exposure

MEDICINAL USES CLOVE OIL

Clove oil is produced by a steam distillation process. So you're probably better off buying the oil rather than trying to make it yourself. Clove oil is available almost anywhere. Clove oil is an unusually powerful antioxidant. Antioxidant capacity is measure by

ORAC (Oxygen Radical Absorption Capacity). Although the dried buds or powders rank highly among anti-oxidants, clove oil is the monster antioxidant.

BIOLOGICAL SOURCES [7]

SCIENTIFIC CLASSIFICATION

Table 01: Scientific classification

| | |
|----------------------|--|
| Kingdom | Plantae |
| Phylum | Angiosperms |
| Unranked | Eudicots |
| Unranked | Rosids |
| Order | Myrtaceae |
| Family | Syzygium |
| Genus | Aromaticum |
| Species | <i>S. aromaticum</i> |
| Binomial name | <i>Syzygium aromaticum</i> (L.) Merrill & Perry |



Fig 01: *Eugenia aromatica*

PROPERTIES OF CLOVES [10-13]

Cloves are the dried flower buds of the clove tree, an evergreen tree that grows in tropical climates. While cloves are mostly used for culinary purposes today, the health benefits of cloves have been known for centuries. The Chinese used cloves to get rid of bad breath over 2000 years ago, and it is even said that anyone who had an audience with the Emperor was required to chew on cloves so that their breath was sweet! It was also considered an aphrodisiac in China as well as Persia. Cloves have powerful medicinal properties. They are stimulating and have antibacterial, antiviral, antifungal and antiseptic properties. They are also a natural anesthetic (due to the eugenol oil) which is why they were often used

for dental procedures in centuries past and are still used in some cultures to remedy toothache. It is the oil that is derived from the cloves that is so powerful, and this is often used for medicines both topically and internally. This oil contains compound that helps with blood circulation and can stimulate the skin when applied directly to it. Cloves are a great spice to heal ailments of the digestive system. They are well known for relieving flatulence and can actually help promote good digestion as well as metabolism. They may also help relieve vomiting and diarrhea as well as a host of other digestive disorders. Cloves have been well known as an all around healing herb and it's not just digestive problems that cloves are reputed to help with. In fact, they are used in tropical Asia to treat conditions such as scabies, cholera, malaria and tuberculosis. As an antispasmodic it can be applied topically to relieve muscle spasms or in a tea to ease coughing. It can also treat skin problems like styes and sores when applied as an ointment. It is said a paste of milk, salt crystals, and cloves can be a great headache remedy. Cloves are believed to have other health benefits that aren't necessarily connected with an immediate illness. For instance, they can make a great mosquito repellent, as well as a moth repellent. Clove studded oranges are often used to repel many kinds of insects in tropical climates. Sucking on cloves may even reduce the craving for alcohol. Today, the health benefits of cloves are not mentioned much in the Western world, but this ancient spice is still a popular herb with Ayurvedic healers who use it in teas and powders both topically and internally. It is even found in the arsenal of aromatherapy practitioners. Although used from centuries in all over the world, clove is a resident of Molucca islands. It has been widely used in the Ayurveda – Indian traditional medicine, and Chinese traditional medicines. In the modern world, it is largely cultivated in Zanzibar, Indonesia, Sri Lanka, India, and Pakistan.

USES OF CLOVE [14-17]

Clove

is very well known as spice as well as herb all over the world. An English name clove, has been derived from the Latin word 'nail' as the shape resembles to small sized nails. It is widely used for medicinal as well as culinary purposes. Cloves are actually the dried flower buds of tree that is member of Myrtaceae family. Clove is an evergreen tree that

bears sanguine flowers in clusters. The medicinal uses of this dried bud are as follows:

Cholera

The intake of cloves is very much effective in the treatment of cholera.

Add some four grams of cloves to boiling water. Boil it, till the half water gets evaporated. Drink this water to prevent from severity.

Digestive disorder

Cloves boost the digestive system of the body as, it regulates the enzyme flows. Intake of this herb reduces the irritation level in intestine and cures indigestion problem. Mix the powdered cloves with teaspoonful of honey, and consume this mixture before going to bed.

Coughs: Take a clove with rock salt and chew it thoroughly to ease down soreness of throat. It also helps in relieving the inflammation of pharynx. You may even burn a clove and chew it, to get relieved from severe cough. Clove oil when mixed with honey, gives amazing effect of recurring cough.

Teeth troubles

From ancient era, clove is used in India and china to cure tooth aches. They are highly beneficial in reducing the pain of tooth. Due to its antiseptic properties, it is also guards the tooth from infections. Apply the clove oil which reduces the pain in gums and decayed tooth.

Headaches

Ground the cloves into powder form and mix it with salt crystals. Add milk to this mixture, it is very effective remedy for headaches.

Earache

Boil cloves in sesame oil and pour three to four drops of oil in ear. It will immediately cure earaches. Apart from the medicinal uses, it is used in culinary worldwide. It is main ingredient of the spice in Indian curries and also included in the preparation of curry powder. Clove oil is also used in the production of bath salt, soaps, and perfumes

NATURAL HERBAL REMEDIES USING CLOVES [1-7]

Natural herbal remedies using cloves includes: Relieving toothaches, earaches, nausea, hypertension and pain from burns and wounds. Also

helps respiratory problems, great air freshener, mosquito repellent, fly deterrent, and ant killer.

CLOVES COME IN 3 DIFFERENT FORMS

Cloves (myrtaceae) come in 3 different forms, whole, ground and oil. All three forms have the same properties with differing degrees of potency. Oil has the highest potency and is best used diluted with carrier oil like almond oil. Whole cloves are medium potency the oils are still in them and they can be ground in a mortar and pestle for use. Ground cloves are the least potent, most of the oil has already been released.

MEDICINAL USES AND PHARMACEUTICAL PREPARATIONS

Western studies have supported the use of cloves and clove oil for dental pain. However, studies to determine its effectiveness for fever reduction, as a mosquito repellent and to prevent premature ejaculation have been inconclusive. Clove may reduce blood sugar levels. Tellimagrandin II is an ellagitannin found in *S. aromaticum* with antiherpes virus properties. The buds have antioxidant properties. Clove oil can be used to anesthetize fish, and prolonged exposure to higher doses (the recommended dose is 400mg/l) is considered a humane means of euthanasia. In addition, Clove oil is used in preparation of some toothpastes, laxative pills and Clovacaine solution which is a local anesthetic and used in oral ulceration and anti-inflammations. Eugenol (or clove oil generally) is mixed with Zinc oxide to be a temporary filling.

EXPERIMENTAL PROCEDURE

Extraction of clove buds with dichloromethane:

- 1) Weigh 5 g of dry cloves. Grind them to a coarse powder using a mortar and pestle. Reweigh the powder and record the weight.
- 2) Transfer the ground cloves to a 100 mL round bottom flask. Add 50 mL of dichloromethane and a few boiling chips in the round bottomed flask.
- 3) Assemble the soxhelt extraction apparatus. Extraction was continued until 150 to 200 cycles are completed from the thimble of the soxhlet apparatus water through the condenser.
- 4) Ask the instructor to inspect your equipment set up. Turn on the heating mantle.

5) After completion of the cycles, a thick viscous liquid is obtained in the round bottomed flask. Now Stop the extraction when approximately 30-40 mL of extract has been collected.

6) Allow the extract to cool to room temperature.

EXTRACTION OF CLOVE BUDS WITH DICHLOROMETHANE : WATER (1:1)

1) Weigh 5 g of dry cloves. Grind them to a coarse powder using a mortar and pestle. Reweigh the powder and record the weight.

2) Transfer the ground cloves to a 100 mL round-bottom flask. Add 50 mL of dichloromethane and water (1:1) and a few boiling chips in the round bottomed flask.

3) Assemble the soxhlet extraction apparatus. Extraction was continued until 150 to 200 cycles are completed from the thimble of the soxhlet apparatus. water through the condenser

4) Ask the instructor to inspect your equipment set up. Turn on the heating mantle.

5) after completion of the cycles, a thick viscous liquid is obtained in the round bottomed flask. Now Stop the extraction when approximately 30-40 ml of extract has been collected.

6) Allow the extract to cool to room temperature.

EXTRACTION OF CLOVE BUDS WITH WATER

1) Weigh 5 g of dry cloves. Grind them to a coarse powder using a mortar and pestle. Reweigh the powder and record the weight.

2) Transfer the ground cloves to a 100 mL round-bottom flask. Add 50 mL of water and a few boiling chips in the round bottomed flask.

3) Assemble the soxhlet extraction apparatus. Extraction was continued until 150 to 200 cycles are completed from the thimble of the soxhlet apparatus. water through the condenser

4) Ask the instructor to inspect your equipment set up. Turn on the heating mantle.

5) after completion of the cycles, a thick viscous liquid is obtained in the round bottomed flask. Now Stop the extraction when approximately 30-40 ml of extract has been collected.

6) Allow the extract to cool to room temperature.

PHYTOCHEMICAL SCREENING

After completion of extraction procedure with three solvents i.e, with dichloromethane, dichloro methane:water and onlywater. The obtained three extracts are subjected to phytochemical screening tests to identify the chemical constituents present in the obtained extracts. The following tests are performed for the three extracts. (Table no:3)

TEST FOR REDUCING SUGARS

10 ml of deionized water was added to 1ml or 1.0g of sample in a test tube and followed by addition of few drops of Fehling solution and heat at 40°C in water bath. Brick red precipitate indicates positive result for reducing sugar.

Test of Tannins

2.0 gm of aqueous extract was taken in test tube, after addition of few (2-3) drops of 5% ferric chloride green colour is observed, and is confirmation of presence of tannins.

Test of Phlobatannins

10ml of aqueous extract was boiled with few drops of 1% conc. hydrochloric acid for 10 min., deposition of red precipitate at the base of the test tube indicates presence of phlobatannins.

Test of Saponins

1.0 gm of aqueous extract was added into a test tube followed by addition of 5.0 ml of deionized water, tubes was shaken vigorously, allowed it for few minutes. If froth remains for 15min, it means saponins are present.

Test of Terepenoids: To the 5.0 ml of aqueous extract, 2.0 ml of chloroform was added, followed by addition of 3.0 ml concentration H₂SO₄. The reddish brown interface indicates the presence of terpenoids.

Test of Steroids

The development of greenish colour when 2.0 ml of extract dissolved in 2.0 ml of chloroform and treated with H₂SO₄ and acetic acid.

Test of Glucosides

2.0 ml of extract was dissolved in 2.0 ml of CHCl₃, 2.0 ml H₂SO₄ was added carefully and shaken gently. A reddish brown colour interface is the indication for the presence of glucoside.

Determination of antimicrobial activity

To determine the antimicrobial activity, disc plate technique was performed. Based on the zone of inhibitions obtained by the three extracts, comparative study of their qualitative antimicrobial activity was done.

Test microorganisms taken for the study

Bacteria of gram positive and gram negative were employed as test organisms. These include gram positive staphylococcus aureus and gram negative k.pneumonia, which were obtained as fresh pure cultures from RDT hospital, Bathalapalli, Ananpatur dist. The bacterial strains were maintained on Nutrient agar media in the refrigerator at 4°C prior to use.

STERILIZATION

All the glassware used in the experiment was sterilized in hot air oven at 160°C for four hours. The media used in the experiments were sterilized in an autoclave at 15 lbs per square inch for 15 minutes.

COMPOSITION OF NUTRIENT AGAR MEDIA

Nutrient agar medium was preferred as the media for bacteria. The following composition

Peptone :5g
Beef extract :3g
NaCl :5g
Agar :20g
Distilled water :1lit

After preparation it was mixed well until the contents are dissolved and then autoclaved at 15 lbs per square inch for 15 minutes

Nutrient broth does not contain agar. Nutrient broth is used for enrichment of specified bacteria. Nutrient agar medium was prepared and poured into sterilized petridishes and placed it in laminar air flow.

DETERMINATION OF ANTIMICROBIAL ACTIVITY

For the determination of the antimicrobial activity, standard disc diffusion method was adopted and three types of discs were used, i.e., discs containing standard antibiotics were used as positive control, discs containing plant crude extract or latex were used as sample discs, and discs containing the DMSO were used as negative control. Punch machine was used to prepare the discs having the diameter of 6 mm from the Whatman-1 filter paper. All

glassware were sterilized by the dry heat of sterilization. Nutrient agar media prepared in distilled water and sterilized in autoclave at 121 °C for 30 minutes. Pour the media into separate petri dishes and allowed to set as a firm gel on cooling. The thickness of gels layer should range between 2-3 mm. The test petri-dishes were incubated overnight at 37 °C and those showing no growth were selected for further work. The bacterial culture were transferred from inoculums to petri-dishes by using the sterilized aluminum wire loops, which were subsequently spread by streaking method. Discs that are soaked in different extracts, standard solution, and DMSO, dried and placed in different petridishes filled with sterile media and bacterial cultures. All the procedure was carried out in the strict aseptic condition using horizontal laminar flow cabinet. Bacterial cultures were incubated at 37 °C in incubator for 24 hours. At the end of the incubation period, zone of inhibition (mm) of the each extract was measured in comparison with the positive and negative control.

ZONE OF INHIBITION

After overnight incubation, examine your plates (keep them covered at all times). Measuring Antimicrobial Effectiveness with Zones of Inhibition (Table 04)

- a. The control plates should show uniform colonies over the entire surface of the plate. If the distribution is highly uneven, you will need to improve your inoculation technique and repeat the experiment.
 - b. If your disinfectants are effective at the concentrations you tested, you should see zones of inhibition around the disinfectant disks. The clear zones around each disk should have a uniform diameter, since diffusion of the compounds through the agar should be uniform in every direction. If this is not the case, suspect either your impregnation technique, or poor contact of the filter paper with the agar.
2. Measure the diameter of the zone of inhibition around each disk. Keeping the lid of the plate in place, use a ruler to measure the diameter of the clear area in millimeters. You will get three separate measurements for each disinfectant, one from each of the three test plates.

3. Are the diameters consistent across all three plates? Calculate the average and the standard deviation of the diameter of the zone of inhibition for each disinfectant.
4. Use the values from Table 02 (below) to evaluate the bacterial response to each compound.

Table 02: Diameter of zone of inhibition (mm)

| | |
|--------------|------------|
| Resistant | 10 or less |
| Intermediate | 11-15 |
| Susceptible | 16 or more |

RELATIVE PERCENTAGE INHIBITION

The relative percentage inhibition of the crude extract with respect to positive control was calculated by using the following formula (Table no:5)

Relative percentage inhibition of crude extract = $100 \times \frac{(a - b)}{(c - b)}$

Where, a: total area of inhibition of the test extract,

b: total area of inhibition of the solvent

c: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using Area of inhibitory zone = πr^2

Where r is radius of zone of inhibition

RESULTS AND DISCUSSION

Phytochemical screening results

Table 03 : Present phytochemical screening test for DCM,DCM:WATER ,WATER

| Tests | DCM | DCM:WATER | WATER |
|--------------------------|----------|-----------|----------|
| test for reducing sugars | Positive | Positive | Negative |
| Test for tannins | Positive | Positive | Positive |
| Test for phlobatannins | Positive | Positive | Positive |
| Test for saponins | Positive | Positive | Positive |
| Test for terpenoids | Positive | Positive | Negative |
| Test for steroids | Positive | Positive | Negative |
| Test for alkaloids | Positive | Negative | Negative |
| Test for glucosides | Positive | Negative | Negative |

INVITRO ANTIMICROBIAL ACTIVITY

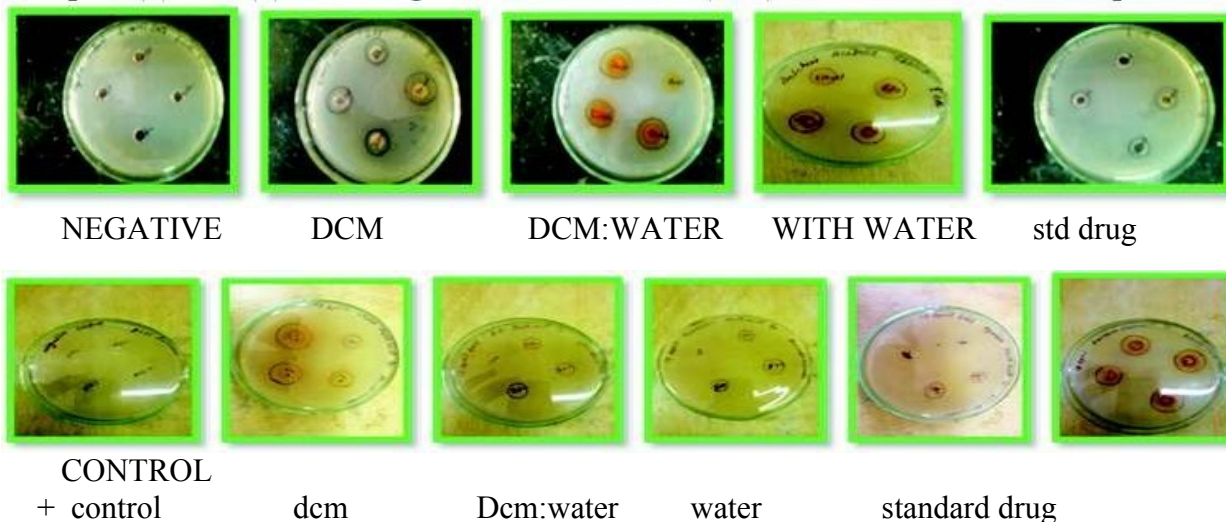
Diameter of the zone of inhibition and relative percentage of inhibition of DCM, DCM:WATER AND WATER extract of Clove buds against different pathogenic bacteria , DCM extract of clove buds showed the zone of inhibition (including diameter of disc 6 mm) of 23.00 mm against DCM,20.0 AGAINST DCM:WATER, 15 AGAINST water on the klebesella pneumonia culture and the standard drug used was levofloxacin and zone of inhibition was found to be 23mm. (fig 02) zone of inhibition was measured on another bacterial species of Streptococcus species. The zone of inhibition of DCM extract was found to be 21, on DCM:Water was found to be 18,with water zone of inhibition was found to be 14mm/ sensitive stain. The relative percentages of inhibition DCM, DCMandwater and water extracts was found to be 100 8.9,65 on klebisella pneumonia species.the relative percentage inhibition of streptococcus species of DCM,DCM:water and water extracts was found to be 87.5,75,58.33. It can be inferred that among all studied extracts, DCM extract of CLOVE BUDS ON KLEBISSELLA SPECIES showed the maximum zone of inhibition against all studied microbes as compared to other extracts.

Table 04 Zone of inhibition

| NAME OF THE MICROORGANISMS USED | CLOVE BUDS | | | Stand ar d drugs used | Zone of inhibition (mm/sensitive strain) |
|---------------------------------|---|--------------------|----------------|-----------------------|--|
| | ZONE OF INHIBITION(mm/sensitive strain) | | | | |
| | DCM EXT RACT | DCM:WATER EXTR ACT | WATER EXT RACT | | |
| KLEBESSELLA PNEUMONIAE | 23.00 | 20.00 | 15.00 | Levofloxacin | 23.00 |
| STREPTOCOCCUS | 21.00 | 18.00 | 14.00 | Flucloxacillin | 24.00 |

Table 05: Relative percentage inhibition of DCM, DCM:WATER

| Microbes used | Relative percentage inhibition | | |
|----------------------|--------------------------------|-----------|---------------|
| | DCM EXTRACT | DCM:WATER | WATER EXTRACT |
| KLEBESELLA PNEMONIAE | 100 | 86.9 | 65 |
| STREPTO COCCUS | 87.5 | 75 | 58.33 |



CONCLUSION

Finally we conclude that clove buds are extracted using soxhlet apparatus using different solvents like DCM, DCM; WATER (1:1), WATER, phytochemical screening test are done for three extracts and invitro evaluation and antimicrobial activity was performed. It was found that extract 1 i.e. with DCM, extract 2 i.e. dcm;water had shown best results on antimicrobial activity. Further studies are done for three extracts for the therapeutic activities.

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