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Research Article

Investigation of Broad-Spectrum Antimicrobial Potential in Selected Ethnomedicinal Plant Extracts

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Abstract

In the present investigation the antimicrobial activity of *Emblica officinalis* Gaertn, *Syzgium aromaticum* (L.) Merr. & L.M.Perry, *Eucalyptus globulus* Labill., *Solanum tuberosum* L, *Pyrus communis* L, *Prunus armeniaca* L. were evaluated. The plant material was extracted through maceration method in different polar and non-polar solvents that are petroleum ether, chloroform, methanol, Distill water having different concentrations of 30%,50%,70% and 100% and the results were noted against *Candida albicans* (C.P.Robin) Berkhou and *Staphylococcus aureus* Rosenbach. Well diffusion method is used for this purpose. The zones of inhibitions were measured against these strains and result were compared with commercially available standard antimicrobial discs. The standard discs used in the experiment were Flucanozole and Clindomycin. Chloroform extraction (30 %) *Emblica officinalis* showed the maximum inhibitory zone of 16.5±0.5 in 30% concentration of chloroform and in the same way *Prunus armeniaca* showed minimum inhibitory zone of 10.5±0.7 in 30% concentration of methanol against *Candida albicans. Syzgium aromaticum* showed highest inhibitory zone of 25.16±0.76 in 30% concentration of chloroform while *Solanum tuberosum* showed lowest inhibitory zone of 10.1±0.7 in 100% concentration of chloroform against *Staphylococcus aureus*

Keywords: *Emblica, Syzgium, Euclayptus, Solanum, Pyrus, Candida albicans, Staphylococcus aureus*

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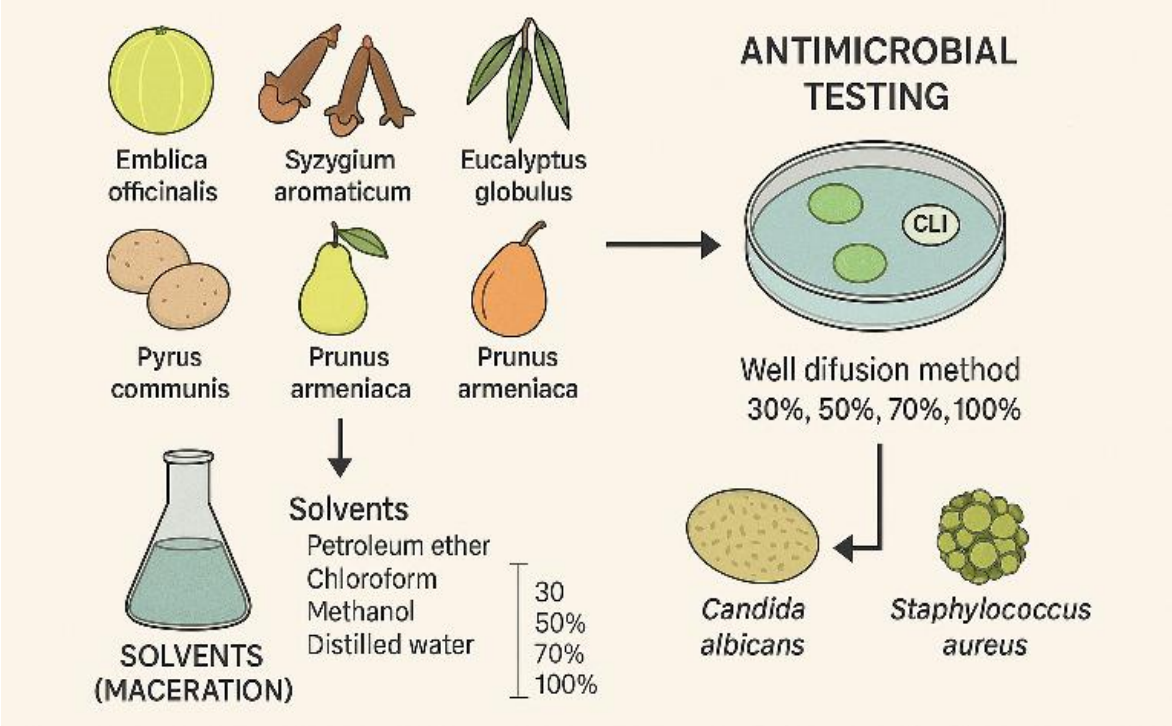
Graphical Abstract



1: Introduction

Herbs and spices have been used excessively in reduction of inflammation, prevent from infection, helps to reclaim the liver and cleanse the lungs and protect the cell from damage because it causes rheumatoid arthritis, osteoporosis, heart disease.(Dobre *et al.,* 2011). Apricot fruit is rich in vitamins and minerals. The apricot has been used in folk medicine as cure for various diseases. The bark of this plant is characterized as astringent to pacify irritated skin.it is used in treatment of hemorrhages, spasm, infertility and eye inflammation. Vaginal infections can be heal up by apricot kernel and its oil has been used in cosmetics and pharmaceutical agent.(Yigit *et al.,* 2009).Apples possess phenolic compounds that had many beneficial properties for the human health. It has high antioxidant, anti-inflammatory, anti-allergic, anti-thrombosis and antimicrobial activities. Microbial growth and metabolism is affected by phenolic compounds.(Alberto *et al.,* 2006).The buds of *Syzygium aromaticum* (clove) is being used in folk medicine as diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment properties and condiment with

carminative and stimulant activity. *Syzygium* species have been reported to possess antibacterial and anti-inflammatory activity (Pandey and Singh 2011).The *Eucalyptus* are used in cure of sore throat and infections caused by bacteria. The gram positive bacterium such as *Staphylococcus aureus* is mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning the gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia. Essential oils of the leaves are used in the treatment of lung diseases.(Ghalem and Mohamed 2008). Effective antimicrobial agent, was detected in *Pyrus communis* and *P. pyraster* leaves and bark, as well in the flowers and fruits of *P. communis* and other *Pyrus* species.(Kundaković *et al.,* 2014).*Allium sativum* is native to India, China and has a valuable effect on the heart and circulation of blood, cardiovascular disease and regular use of *A. sativum* may help to prevent cancer, to treat

malaria, and to raise immunity. *A. sativum* has been used to treat asthma, candidiasis, colds, diabetes.(O and Adeniyi 2008). *A. sativum* has been recognized as a potential medicinal value for thousands of years to different micro-organisms.(Kumar *et al.,* 2014). *Terminalia chebula* Retz has traditionally and locally used in the treatment of the asthma, vomiting, hiccup, and heart, bleeding piles, gout, sore throat and bladder diseases.(Manzoor *et al.,* 2013).

Essential oils may be progressive against antimicrobial activity as these are mixtures of natural volatile compounds deriving from the plant secondary metabolism, mainly monoterpenes, sesquiterpenes, and their oxygenated derivatives. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant proprieties(Dobre *et al.*, 2011).The fruit of *E. officinalis* contain flavonoids (quercetin), phyllemblin, ascorbic acid, gallic acid alkaloids (phyllantine, phyllantidine) and tannins that undergo antibacterial activity(K *et al.*, 2012). The fresh apricot fruit contains carbohydrates, vitamins C and K, β-carotene, niacin, and thiamine. Organic acids, phenols, volatile compounds, esters, and terpenoids have also been isolated that are part of our dietery proteins.(D. Yigit1 2009).The apple skin contains catechin, procyanidin, caffeic acid and chlorogenic acid, polyphenols flavonoids that leads to antimicrobial activity.(Alberto *et al.,* 2006). *Syzygium* species have been reported to possess antibacterial and anti-inflammatory activity and ethanolic and methanolic extracts of clove and increase the antibacterial activity of clove by introducing metal ions.(Pandey and Singh 2011).The presence of arbutin which is considered as phenolic compound in leaves and also present in bark of *P. pyraster* while the presence of phenolics in *P. spinosa* was linked with disease resistance of pyrus species.(Kundaković *et al.*, 2014). Garlic has therapeutic effect, this effect of garlic is possible because of its oil- and water-soluble organosulfur compounds, which are responsible for the typical odor and flavor of garlic. Thiosulfinates play an important role in the antibiotic activity of garlic.(Kumar *et al.,* 2014).Clove has Essential oil are phenylpropanoides such as carvacrol, thymol, eugenol and cinnamaldehyde that must be used to evaluate the antibacterial potential against 100 number of gram negative bacilli. (Saeed and Tariq 2008).

The purpose of this research was to determine the antimicrobial activity of medicinal plants including *Emblica officinalis, Syzgium aromaticum, Eucalyptus globulus, Solanum tuberosum, Pyrus communis, Prunus armeniaca* effectively used in face wash.

2: Methodology

The present study deals with the evaluation of antimicrobial activity of some medicinally important plants.

2.1: plant material and test organism

Plant material was collected from different locations. The Cloves of *Syzygium aromaticum* were collected from local market. Amla (*Emblica officinalis)* was collected from local herbalists. *Prunus armeniaca* was collected from the local markets during the month of June. *Eucalyptus globulus* was collected from LCWU during February. *Prunus armeniaca* was collected from local herbalist during April. *Solanum tuberosum* was collected from local markets. The present investigation deals with the study of antimicrobial activity of some medicinally important plants, e.g. *Syzygium aromaticum, Emblica officinalis, Prunus armeniaca, Eucalyptus globulus,Pyrus communis,Solanum tuberosum* by well diffusion method against bacterial strain *Staphylococcus aureus* and fungal strain *Candida albicans.* For the determination of antimicrobial activity of plant extracts the utilized organisms was Fungi (*Candida albicans*) and Bacteria (*Staphylococcus aureus*).

2.2: Solvent Extraction by Maceration Method

250g of ground plant material was extracted in sequence with 500ml of polar and non-polar solvents .The extraction was carried out by soaking the powder in each of the solvent for the period of 8 days. e.g. P. ether, Chloroform, Methanol and Water. The residue

was filtered and the filtrate was preserved in the labeled amber colored glass jars, whereas the residue was further soaked in the next solvent in series.

Plate 1: Extraction of different plant materials (A: *Syzygium aromaticum* with petroleum ether **(B)**Extraction of different plants with petroleum ether**(C)**Extraction of *Pyrus communis* with methanol**(D)** Extraction of *Emblica officinalis* with chloroform **(E).***Syzygium aromaticum* with methanol **(F)***Syzygium aromaticum* with choloroform

All the crude extracts for the antimicrobial activity were tested by following the methodology of Aftab et al. (2020) according to well diffusion method.

2.3.1: Medium Preparation:

The bacteria were cultured on the nutrient agar medium (Cruickshank *et al.,* 1975).

2.3.2: Potato dextrose agar (PDA) medium Preparation

It is basically used for fungal culture preparations by following the methodology of Johansen (1940). The recipe was prepared by taking 3.9 grams of PDA (potato dextrose agar) in 100 ml of distilled water, provided it gentle heat on hot plate and then the preparation was made microbe free through sterilization by autoclaving. The pH was adjusted at 5.5.

2.3.3: Fungal Slants

For the preparation of fungal slants 5ml of potato dextrose agar was added in test tube and cotton plugged and were then sterilized in autoclave. The test tubes were then placed in diagonal position and the medium was allowed to solidify. For the preparation of slants, the fungi from the culture plates was transferred to these slants with the help of inoculating needle under aseptic conditions and incubated for 5 days at 30°C. These slants were further used for the inoculum preparation.

2.3.4: Bacterial Slants

For slant preparation 5 ml of agar was poured in each test tube. The tubes were cotton plugged and then sterilized by autoclaving. The test tubes were then placed in the diagonal position and allowed the medium to solidify at room temperature. An inoculating needle was used to transfer the bacterial colonies to the test tube in laminar air flow in aseptic conditions. The slants were labeled and incubated for 24 hours and were further used for the inoculum preparation.

2.3.5: Minimum Inhibitory Concentration (MIC)

MIC of the plant specimens against the fungal and bacterial strain was done by following the methodology of Doughari and Nuya (2008) with slight modifications.

2.3.6: Statistical analysis for antimicrobial activity

As the experiment was done in triplicate manner so mean and standard deviation values of the inhibitory zone were calculated in each case. The data presentation was done as Mean ± S.E. (M± standard error). The treatment effects were compared after Snedecor and Cochran (1980) and a significant difference among replicates was presented as Duncan's multiple range tests, in the form of probability <p> values by using Costat, cs6204 W.exe. Computer software. Standard error was calculated by formulaeby (Alfonso *et al*.1985). The data was further analyzed by Analysis of Variance (ANOVA) with two ways Complete Randomized Designs to have an idea about the least significant difference among means, treatments and the types of Microorganisms used. For result analysis

Duncan's Multiple Range test was applied and thus the results were tabulated.

3. Results

In the present investigation, efforts have been made to determine the antimicrobial activity of medicinal plants commonly known as *Syzygium aromaticum, Emblica officinalis, Prunus armeniaca, Eucalyptus globulus* and *Solanum tuberosum* (Figure 1 to Figure 10). For this purpose, different polar and non-polar solvents were used for extraction, and their antimicrobial properties were evaluated by measuring the inhibitory zones against various microbes in millimeters. The results showed that *Emblica officinalis* exhibited the maximum inhibitory zone of 16.5±0.5 in the chloroform extract of the inflorescence (Figure 1).It is experimented that *Syzygium aromaticum* showed maximum inhibitory zone of 12.8±0.7[a] in 30% concentration of petroleum ether while *Syzygium aromaticum* showed minimum inhibitory zone of 0.5±0.5b in 70% concentration of methanol against *Candida albicans* (Figure 2)

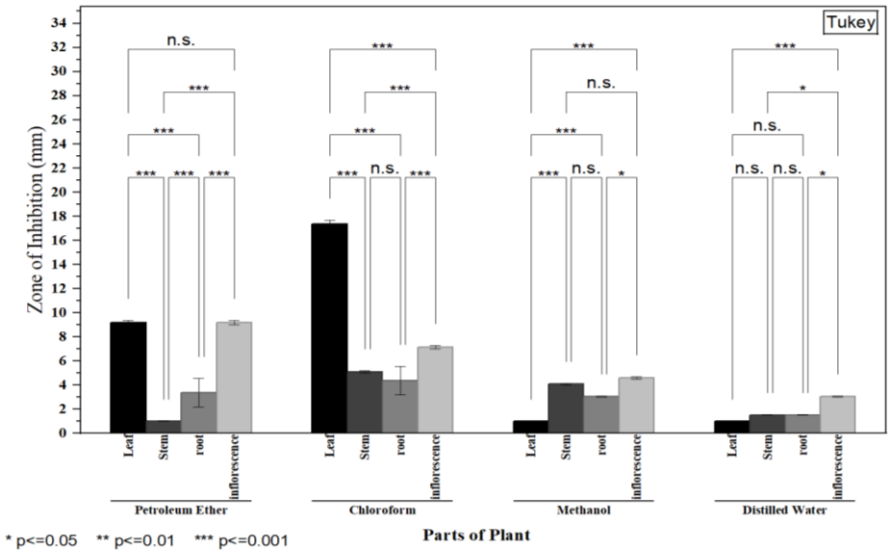


Figure 1: Inhibitory zone (mm) produced by *Emblica officinalis* plant extract against *Candida albicans*

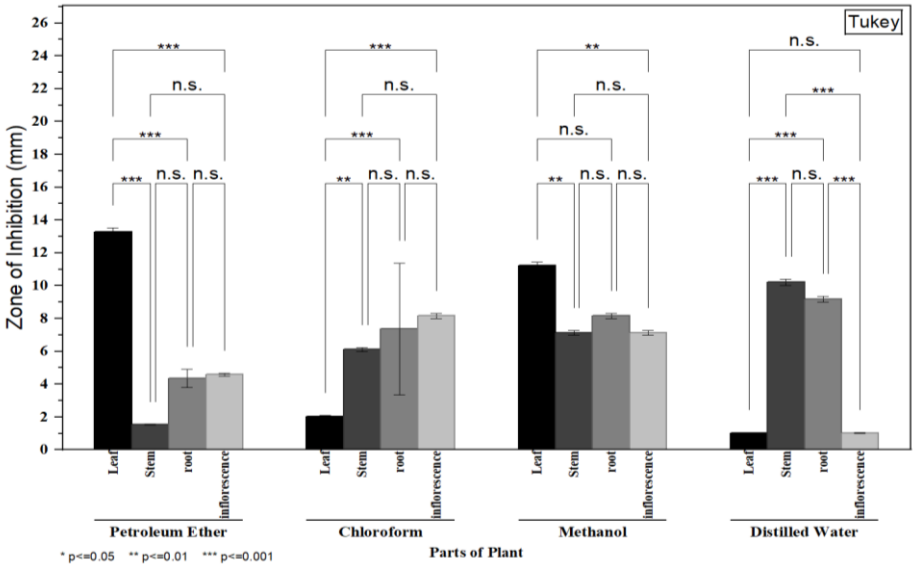


Figure 2: Inhibitory zone (mm) produced by *Syzygium aromaticum* plant extract against *Candida albicans*

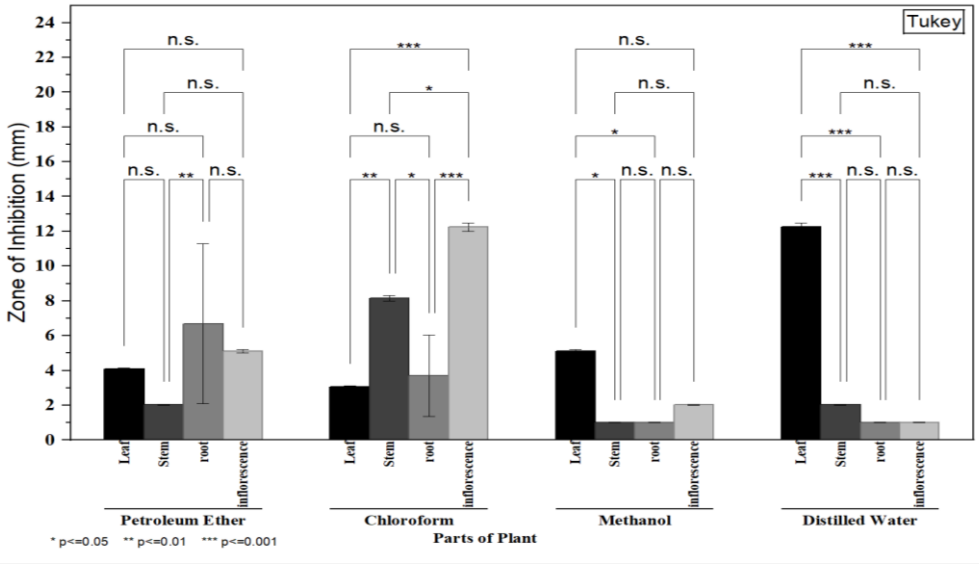


Figure 3: Inhibitory zone (mm) produced by *Eucalyptus globulus* plant extract against *Candida albicans*

Figure 3 proves that *Eucalyptus globulus* showed maximum inhibitory zone of 12.1 ± 1.04^{c} in 30% concentration of distill water while *Eucalyptus globulus* showed minimum inhibitory zone of 0.2 ± 0.2^{e} in 70% concentration of chloroform against *Candida albicans*

It is examined that that *Solanum tuberosum* showed maximum inhibitory zone of 11.1 ± 0.7^{c} in 100% concentration of methanol while *Solanum tuberosum* showed minimum inhibitory zone of 0.33 ± 0.5^{b} in 50% concentration of petroleum ether against *Candida albicans* (Figure 4)

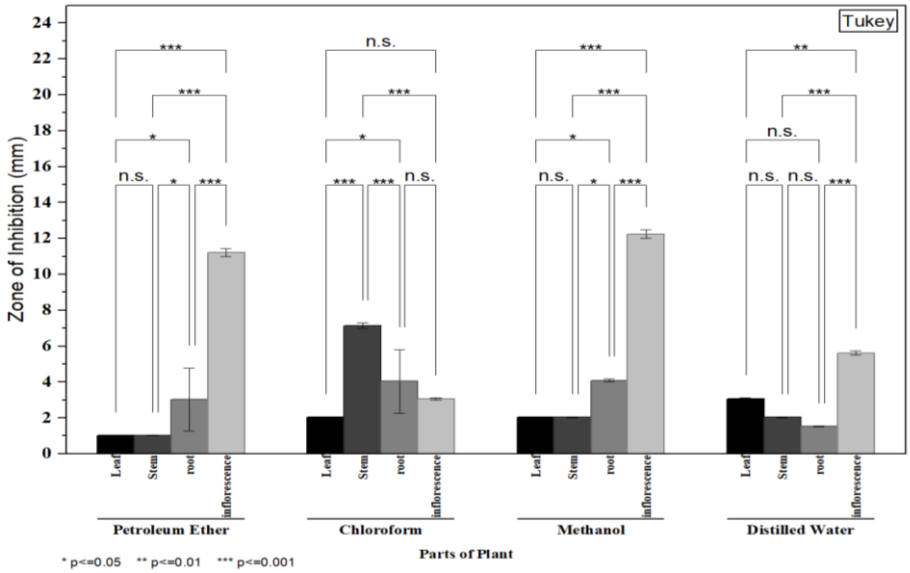


Figure 4: Inhibitory zone (mm) produced by *Solanum tuberosum* plant extract against *Candida albicans*

Results proved that *Pyrus communis* showed maximum inhibitory zone of 12±1[c] in 100% concentration of petroleum ether water while *Pyrus communis* showed minimum inhibitory zone of 0.33±0.5[a] in 30% concentration of methanol against *Candida albicans* (Figure 5)

Results proved that *Emblica officinalis* showed maximum inhibitory zone of 12.5±1.5[a] in 100% concentration of methanol while *Emblica officinalis* showed minimum inhibitory zone of 2.5±0.5[b] in 30% concentration of chloroform against *Staphylococcus aureus* (Figure 6)

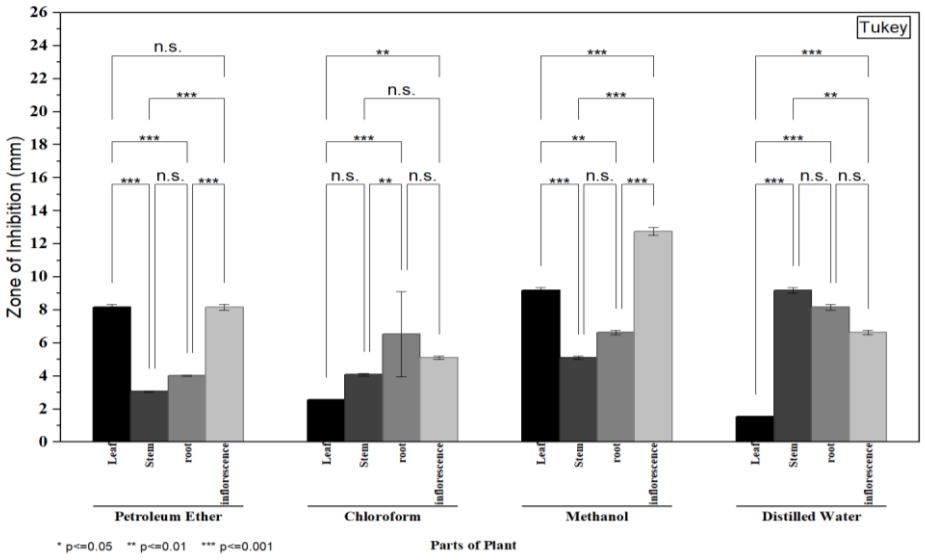


Figure 5: Inhibitory zone (mm) produced by *Prunus armeniaca* plant extract against *Candida albicans*

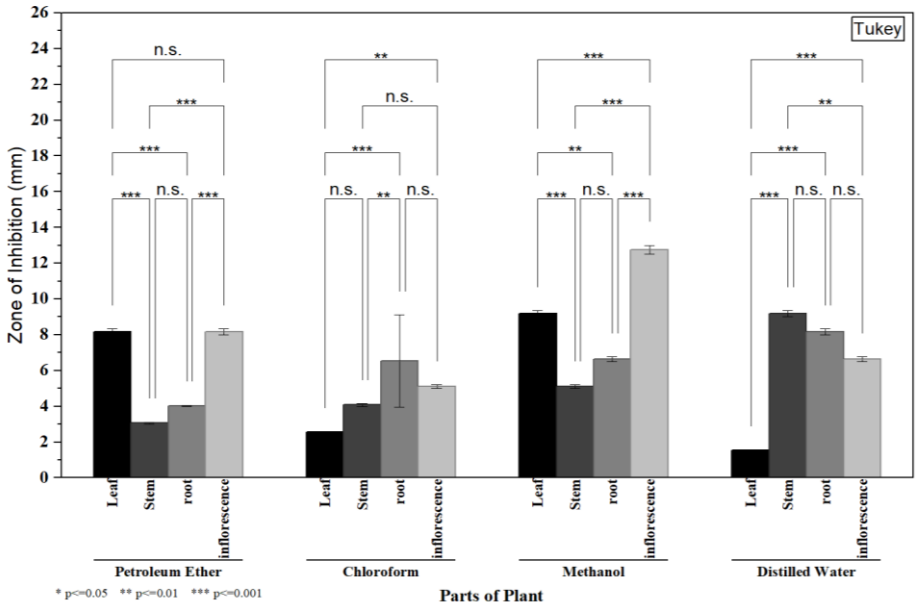


Figure 6: Inhibitory zone (mm) produced by *Emblica officinalis* plant extract against *Staphylococcus aureus*

Results proved that *Syzgium aromaticum* showed maximum inhibitory zone of 25.16±0.76[d] in 30% concentration of chloroform while *Syzgium aromaticum* showed minimum inhibitory zone of 0.5±0.5[b] in 50% concentration of petroleum ether against *Staphylococcus aureus* as shown in Fig 7. Results proved that *Eucalyptus globulus* showed

maximum inhibitory zone of 16.1±0.76^{cd} in 30% concentration of chloroform while *Eucalyptus globulus* showed minimum inhibitory zone of 1±0.5^{bc} in 30% concentration of petroleum ether against *Staphylococcus aureus* mentioned in Figure 8.

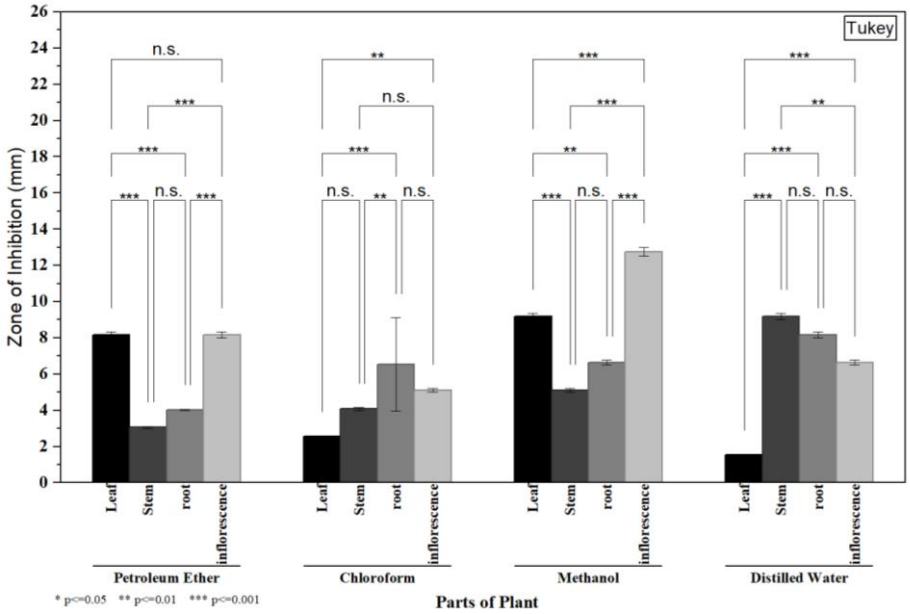


Figure 7: Inhibitory zone (mm) produced by *Syzygium aromaticum* plant extract against *Staphylococcus aureus*.

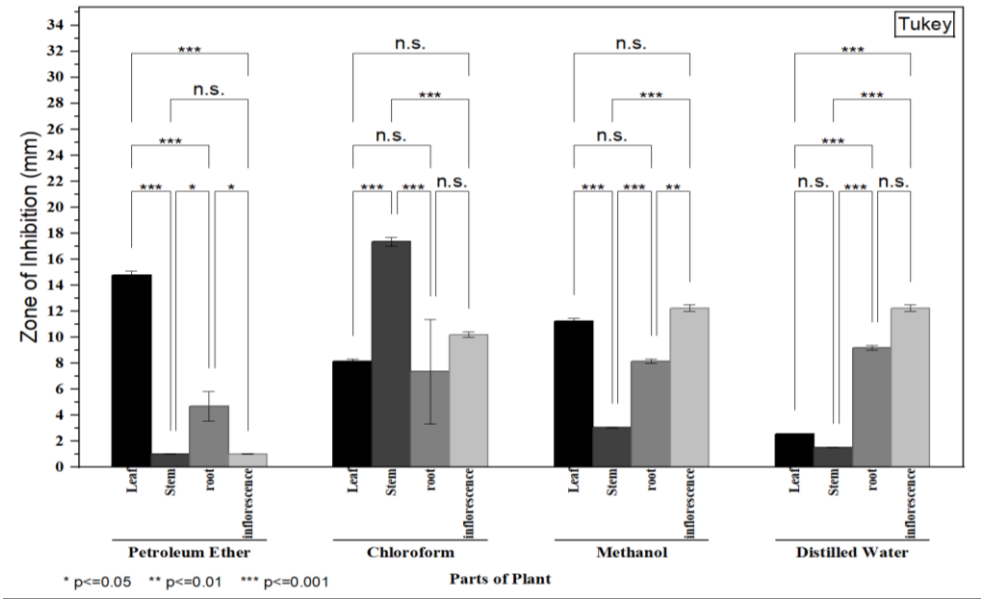


Figure 8: Inhibitory zone (mm) produced by *Eucalyptus globulus* plant extract against *Staphylococcus aureus*.

Results proved that *Solanum tuberosum* showed maximum inhibitory zone of 10.1±0.7^{e} in 100% concentration of chloroform while *Solanum tuberosum* showed minimum inhibitory zone of 0.5±0.5^{b} in 50% concentration of petroleum ether against *Staphylococcus aureus* (Figure 9). Results proved that *Pyrus communis* showed maximum inhibitory zone of 12.1±0.7^{b} in 50% concentration of methanol while *Pyrus communis* showed minimum inhibitory zone of 0.1±0.2^{e} in 100% concentration of distill water against *Staphylococcus aureus* (Figure 10).

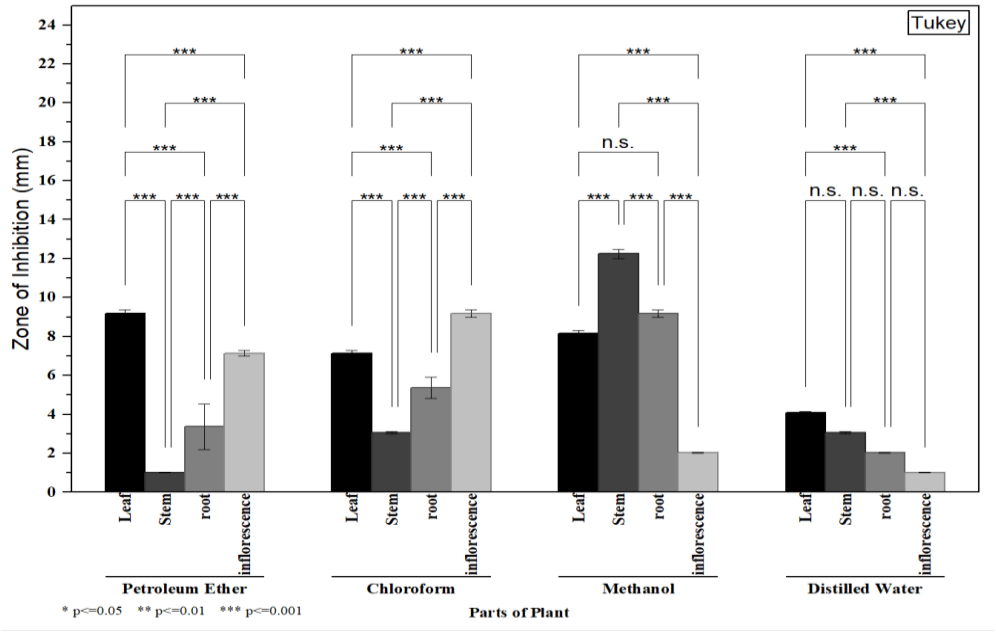


Figure 9: Inhibitory zone (mm) produced by *Solanum tuberosum* plant extract against *Staphylococcus aureus*.

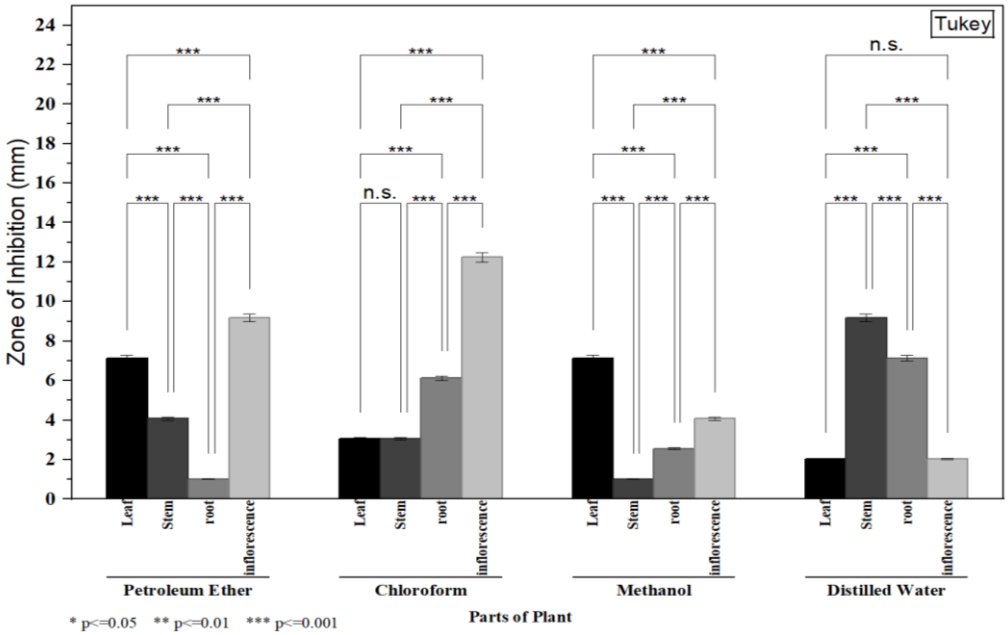


Figure 10: Inhibitory zone (mm) produced by *Prunus armeniaca* plant extract against *Staphylococcus aureus*.

Results proved that *Prunus armeniaca* showed maximum inhibitory zone of 11.8±0.7^{e} in 100% concentration of chloroform while *Prunus armeniaca* showed minimum inhibitory zone of

1±0.5[a] in 50% concentration of methanol against *Staphylococcus aureus*.

Comparison among all six plants revealed that *Emblica officinalis* showed maximum inhibitory zone of 16.5±0.5[d] in 30% concentration of chloroform and in the same way *Prunus armeniaca* showed lowest maximum inhibitory zone of 10.5±0.7[a] in 30% concentration of methanol against *Candida albicans*. *Syzgium aromaticum* showed highest maximum inhibitory zone of 25.16±0.76[d] in 30% concentration of chloroform while *Solanum tuberosum* showed lowest maximum inhibitory zone of 10.1±0.7[e] in 100% concentration of chloroform against *Staphylococcus aureus.*

4. Discussion

The present investigation is done for checking the antimicrobial activity of medicinally important plants against different microbial strains.The use of herbal and medicinal plants as the first medicine is a universal phenomenon.in ancient times different cultures are using the herbal remedies.Plants used as herbal remedies are not only important as drugs but also as food supplements with vitamins and minerals. Medicines derived from plants are widely available safer and cheaper than the synthetic medicines that are easily available but expensive and sometimes can cause harmful impacts.

The results indicate that the plant is antimicrobial in nature as it has produced some value for the zone of inhibition against bacterial and fungal strains while some of them are antimicrobial in nature. Antimicrobial discs were run to compare the zones of inhibition against fungus *Candida albicans* and bacteria like *Staphylococcus aureus.* It has been experimented that *Emblica officinalis* showed maximum inhibitory zone of 16.5±0.5[d] in 30% concentration of chloroform and in the same way *Prunus armeniaca* showed minimmum inhibitory zone of 10.5±0.7[a] in 30% concentration of methanol against *Candida albicans.*

Syzgium aromaticum showed highest maximum inhibitory zone of 25.16±0.76[d] in 30% concentration of chloroform while *Solanum tuberosum* showed minimum inhibitory zone of 10.1±0.7[e] in 100% concentration of chloroform against *Staphylococcus aureus.* Firstly, *Emblica officinalis* showed maximum inhibitory zone of 16.5±0.5[d] in 30% concentration of chloroform while *Emblica officinalis* showed minimum inhibitory zone of 0.8±1.04[b] in 50% concentration of petroleum ether against *Candida albicans*. (Dobre *et al.,* 2011).D.yigit also work with Candida and significant anti-candida activity was also observed with the methanol extract of bitter apricot kernels against *Candida albicans,* consisting of a 14 mm in diameter of inhibition zone and a 0.625 mg/mL MIC value.

Syzgium aromaticum indicated maximum inhibitory zone of 12.8±0.7[a] in 30% concentration of petroleum ether while *Syzygium aromaticum* showed minimum inhibitory zone of 0.5±0.5[b] in 70% concentration of methanol against *Candida albicans.* Pandey and Singh (2011) investigated maximum antimicrobial activity of methanolic extract of *Syzygium aromaticum* was obtained against *Staphylococcus aureus.* In present research,it was concluded that *Eucalyptus globulus* showed maximum inhibitory zone of 12.1±1.04[c] in 30% concentration of distill water while *Eucalyptus globulus* showed minimum inhibitory zone of 0.2±0.2[e] in 70% concentration of chloroform against *Candida albicans. Eucalyptus globulus* have great potential as antimicrobial agents in the treatment of infectious organisms.(O and Adeniyi 2008). Justify in his work that the active components of the Eucalyptus globulus showed antimicrobial activity and capable for the exact mechanism of action that will contribute greatly to the development new pharmaceuticals.

It is examined that that *Solanum tuberosum* exhibited maximum inhibitory zone of 11.1±0.7[c] in 100% concentration of methanol while *Solanum tuberosum* showed minimum inhibitory zone of 0.33±0.5[b] in 50% concentration of petroleum ether against *Candida albicans* mentioned in Same work was done by(Mbaeyi-Nwaoha and Emejulu 2013).on Solanum tuberosun and showed that *S. tuberosum* exhibit less inhibition with distill water. Results proved that *Pyrus communis* showed maximum inhibitory zone of 12±1[c] in 100% concentration of petroleum ether water while *Pyrus communis* showed minimum inhibitory zone of 0.33±0.5[a] in 30% concentration of methanol against *Candida albicans.* These minimum zone of inhibi-

tion is supported by (A *et al.,* 2011).That different types of extracts from neem leaves were found to have inhibitory effect on Candida albicans but C.albicans is a weakest fungus and show less zone of inhibition.

Emblica officinalis showed maximum inhibitory zone of 12.5±1.5^{a} in 100% concentration of methanol while *Emblica officinalis* showed minimum inhibitory zone of 2.5±0.5^{b} in 30% concentration of chloroform against *Staphylococcus aureus*. It was also determined by(Nahor and Ahmed 2011). Extracts of *E. officinalis* showed that they are effective against all the tested pathogens *S. aureus, Salmonella sp., S. typhi, S. paratyphi, B. cereus, B. subtilis, E. coli,* and S. aureus is best in controlling their growth in vitro in culture condition. They all have a bacteristatic and bactericidal activity when tested in vitro using crude preparation.

Syzgium aromaticum showed maximum inhibitory zone of 25.16±0.76^{d} in 30% concentration of chloroform while *Syzgium aromaticum* showed minimum inhibitory zone of 0.5±0.5^{b} in 50% concentration of petroleum ether against *Staphylococcus aureus*, while for determination of antimicrobial activity, research work was done and it is evaluated that *Syzgium aromaticum* showed zone of inhibition of 19.5mm at 2000 ppm conc.and minimum zone is 8mm at 1000ppm concentration.(Kumar *et al.,* 2014).

Eucalyptus globulus have maximum inhibitory zone of 16.1±0.76^{cd} in 30% concentration of chloroform while *Eucalyptus globulus* indicated minimum inhibitory zone of 1±0.5^{bc} in 30% concentration of petroleum ether against *Staphylococcus aureus.* Well diffusion method was used for this purpose but this method was contrary to the method of (Ghalem and Mohamed 2008). Broth dilution method was used to count the inhibition activity.but effective results were made with well diffusion method. *Solanum tuberosum* showed maximum inhibitory zone of 10.1±0.7^{e} in 100% concentration of chloroform and (Mbaeyi-Nwaoha and Emejulu 2013). Evaluated that *Solanum tuberosum* have maximum zone of inhibition of15.7±0.6 ^{a} with distill water and it is justified that *S. tuberosum* contain antibacterial content.

According to the methodology followed it was concluded that in whole experiment, if comparing all six plants, *Emblica officinalis* determined the maximum inhibitory zone of 16.5±0.5d in 30% concentration of chloroform and in the same way *Prunus armeniaca* showed minimum inhibitory zone of 10.5±0.7^{a} in 30% concentration of methanol against *Candida albicans. Syzgium aromaticum* showed highest inhibitory zone of 25.16±0.76d in 30% concentration of chloroform while *Solanum tuberosum* showed lowest inhibitory zone of 10.1±0.7^{e} in 100% concentration of chloroform against *Staphylococcus aureus.*(Nahor and Ahmed 2011). Supported this result that *Emblica officinalis* have maximum inhibitory zone against *Staphylococcus aureus.*

Author Contributions:

Conceptualization, HY..; methodology, AA. Software, HY and NR. Validation, AA and NR. .; formal analysis, HY; investigation, AA.; resources, HY.; data curation, AA.; writing—original draft preparation, AA.; writing—review and editing, AA and NR.; visualization, HY.; supervision, HY.; project administration, HY.. All authors have read and agreed to the published version of the manuscript

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Conflicts of Interest: The authors declare no conflicts of interest

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