

ANTI-MICROBIAL SCREENING OF SOME MEDICINAL PLANT EXTRACTSMansoor Shazia^{1*}, Mohd. Ali Saadia¹, Fatima Jasmine², Jamal Mohammad Arif³ and Mustafa Huma⁴¹Department of Biotechnology, Integral University, Kursi Road, Lucknow, India²Department of Chemistry, Integral University, Kursi Road, Lucknow, India³Department of Biochemistry, College of Applied Medical Sciences, University of Hail, Hail, KSA⁴Council of Science and Technology, U.P. Lucknow, India

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ABSTRACT

Medicinal plants are the best source to obtain a variety of herbal drugs. The use of plant extracts and photochemical both with known anti-microbial properties can be of great importance in therapeutic treatments. The plants have provided a good source of anti-infective agents and many of them remain highly effective in the fight against microbial infections. Therefore in the present study seven medicinal plants that are *Embllica officinalis*, *Ficus bengalensis*, *Myristica fragrans*, *Acacia arabica*, *Aloe barbadensis*, *Ricinus communis* and *Zizyphus jujuba* were screened for potential anti-bacterial activity against medically important bacterial strains, such as *Pseudomonas aurogenosa*, *Proteus vulgaris*, *Staphylococcus aureus* and *Streptococcus cereviceae*. The anti-microbial activity was determined in methanolic extracts using agar well diffusion method. *Streptococcus cereviceae* showed resistance against the plant extracts. *Embllica officinalis* and *Aloe barbadensis* showed strong anti-bacterial activity against all the tested bacterial strains. Hence, this plant extract can be used to evaluate any bioactive natural products that may serve as leads in the development of new pharmaceuticals that can address the unmet therapeutic needs.

KEY WORDS: medicinal plants, anti-microbial activity, *Ficus bengalensis*, *Myristica fragrans*, *Ricinus communis*, *Zizyphus jujuba*, bacterial strains.

***Corresponding Author**E-mail: humamustafa1@yahoo.co.in**INTRODUCTION**

Plants are used as sources of medicinal compounds and important therapeutic aid for maintenance of human health since ancient times. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies in approximately 80% of the world's population¹. Some therapeutic benefits can be traced to specific plant compounds. Such as many herbs contain dozens of active constituents that, together, combine to give the plant its therapeutic value. Consequently, it is believed that the whole plant has more effective healing properties than its isolated constituents. Any part of the plant may contain active components. About 80% of individuals from developed countries are using traditional derived from medicinal plants. Therefore, these plants should be investigated to understand their properties, safety and efficacy in a better way. The use of plant extracts and photochemical both with known anti-

microbial properties can be of great importance in therapeutic treatments.

The natural products, either as pure compounds or as standardized plant extracts, provide indefinite opportunities for new drug because of the unmatched availability of chemical diversity. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. The anti-microbial activities of the plant extracts on microorganisms have been studied by a large number of researchers in various parts of the world.²⁻¹⁰ It has anti-viral, anti-bacterial, anti-cancer, anti-allergy, and anti-mutagenic properties. It is rich in phenols, flavonoids and tannins.

In the present study seven different plants were selected such as *Embllica officinalis* Gaertn, *Ficus bengalensis* L., *Myristica fragrans* Houtt, *Acacia arabica*, *Aloe barbadensis*, *Ricinus communis* L. and *Zizyphus jujuba*. The *E. officinalis* Gaertn belongs to the family

Euphorbiaceae. It is found throughout India, Pakistan, Bangladesh, China, Sri Lanka and the Malayan Peninsula. It is a deciduous tree 8-15 m tall. The leaves, bark, fruit, root bark, etc are used to cure many diseases. The plant is known for its digestion power and improving liver function and also act as liver-protective. It has anti-viral, anti-bacterial, anti-cancer, anti-allergy, and anti-mutagenic properties. It is rich in phenols, flavonoids and tannins. *E. officinalis* was included as a positive control in the study.

The *Ficus bengalensis* L belong to the family Moraceae. They are large deciduous tree, distributed throughout India mostly wild but few are cultivated as well. Various parts of the plant like bark, leaves, tender shoots, fruits, seeds, and latex are of medicinal importance. The bark contains tannin, rubber and wax. The plant parts are used in diseases of blood, vagina, uterus and leucorrhea, burning sensation, gonorrhoea, diarrhea, dysentery, hemorrhoids and gastrohelcosis. *Ricinus communis* L. (Euphorbiaceae) is a soft wooden small tree, wide spread throughout tropics and warm temperature regions of the world.

Aloe barbadensis Miller is a perennial succulent plant belonging to the Liliaceae family (sub-family of the Asphodelaceae¹¹). The plant is made of turgid green leaves joined at the stem in a rosette pattern. *Zizyphus jujuba* (Rhamnaceae) is a thorny rhamnaceous plant that is widely distributed in Europe and Southeastern Asia. Fruits of this plant are edible and different parts of *Z. jujuba* have been found to have multiple medicinal properties.

Acacia arabica belongs to family Fabaceae, and English name is Indian Gum, Arabica tree. In Ayurvedic medicine, babul is considered a remedy that is helpful for treating premature ejaculation. *Acacia bar* is a powerful astringent. The barks from the branches yield 7-12 percent tannins and used for asthma, bronchitis, diabetes, dysentery, diarrhea and skin diseases. *Myristica fragrans* Houtt (nutmeg) is one of the plants commonly found in Asian countries. It contains many bioactive compounds including camphene, elemicin, eugenol, isoelemicin, isoeugenol, methoxyeugenol, pinene, sabinene, safrol, myristic acid, myristicin, elimicin and lignin compounds etc. The present study was undertaken to evaluate the anti-microbial activities of these plants. All the above plants taken were of medicinal values and are easily available.

MATERIALS AND METHODS

Plant Collection

The plants and plant materials were collected from different places in and around the campus of Integral University, Lucknow, Uttar Pradesh, India. The

taxonomic identification of these plants was verified in Taxonomy, National Botanical Research Institute, Lucknow in the Department of Herbarium. The fresh plant materials were washed under running tap water. Then they were shade dried and was powdered with the help of mortar and pestle. The fine particles were separated using muslin cloth and stored in clean and dry container until used

Crude Extract Preparation

The crude extracts of plant parts were prepared in soxhlet apparatus. The plant parts (leaves, fruit and flowers) were dried, powdered and weighed. Powdered plant parts (20 g) were soaked in 200 ml organic solvent using soxhlet apparatus. Briefly, the extraction was carried out at room temperature until exhaustion. The extracts were filtered and air dried at 40° C. The weight of each dried residue was recorded. The extracts were reconstituted in dimethyl sulphoxide (DMSO). All the extracts were prepared and kept at 4° C until used. **Determination of total phenolic content**

The total phenolic content of the extracts were determined by the Folin-Ciocalteu method with few modifications¹² using gallic acid as standard. Four hundred microliter DMSO solution of gallic acid and 1.6 ml Na₂CO₃ (7.5%) in distilled water were added to 2 ml of Folin-Cocalteu reagent (diluted 10 folds). Then 400 µl of aqueous solution of plant extracts were mixed with the same reagents as described above and incubated at 37° C in the dark and for 1 hour and absorbance was read at 765 nm in a UV-VIS spectrophotometer. All determinations were carried out in triplicate and the results are expressed as mg gallic acid equivalent (GAE)/g of extract.

Bacterial and Fungal Strains

The microbial strains were obtained from the National Chemical Laboratory (NCL), Pune, India. The studied bacterial strains were *Streptococcus aureus* ATCC2079, *Escherichia coli* ATCC 2065, *Proteus vulgaris* ATCC 2027 and one anti-fungal strain *Sacchromyces cereviseae* ATCC3090.

Anti-bacterial Activity

The anti-bacterial activity of different plant species were evaluated by agar well diffusion method for solvent extract using Nutrient Agar medium for the assay. The microorganisms were activated by inoculating a loopful of the strain in the nutrient broth (20ml) and incubated overnight on a rotary shaker at 37° C. Then 0.2 ml of inoculum was inoculated into the molten Nutrient Agar media and after proper homogenization it was poured into the Petri plate. For agar well diffusion method, a well was made in the seeded plates 1.5x10⁴ bacteria with the help of a well cutter (0.85 cm). The test compound

was introduced into the well and the plates were incubated at 37° C for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract. The experiment was done three times and the mean values are presented.

RESULTS

The ethno-botanical information of the screened plants is given in Table 1. The crude plant extract was prepared by using leaf from *E. officinalis*, *F. bengalensis*, *A. arabica*, *A. barbadensis*, *R. communis* and *Z. jujuba* and fruits were used in *M. fragrans* and *E. officinalis*. The total phenolic content in each plant extract was calculated spectrophotometrically at 765 nm by using gallic acid as standard. The mg gallic acid equivalent (GAE/g) of the plant extracts was calculated. The results are given in table 2.

The crude methanolic plant extract of *E. officinalis*, *F. bengalensis*, *M. fragrans*, *R. communis*, *Z. jujuba*, and *A. arabica* showed considerable anti-bacterial activity against *Staphylococcus aureus*. The maximum anti-bacterial activity was shown by *E. officinalis*, followed by *M. fragrans*, *R. communis*, *Z. jujuba* and *A. arabica*. The minimum anti-bacterial activity was shown by *F. bengalensis*. The diameter of zone of inhibition in *E. officinalis* methanolic fruit extract was found to be 32 mm whereas in the methanolic *E. officinalis* leaf extract gave the zone of inhibition to 20mm. Similarly, the diameter of zone of inhibition of crude extract of *M. fragrans* and *R. communis* was found to be 30 mm as shown in figure 1.

The crude methanolic plant extract of *E. officinalis*, *F. bengalensis*, *M. fragrans*, *R. communis*, *Z. jujuba*, *A. barbadensis* and *A. arabica* showed considerable anti-bacterial activity against *Pseudomonas vulgaris*. The maximum anti-bacterial activity was shown by *A. barbadensis*, *A. arabica*, *E. officinalis*, *R. communis* followed by *M. fragrans*, and *Z. jujuba*. The minimum anti-bacterial activity was found in the crude extract of *F. bengalensis*. The diameter of zone of inhibition in *A. barbadensis* methanolic extract was found to be 30 mm whereas in *A. arabica* extract gave the zone of inhibition to 24 mm. Similarly, the diameter of zone of inhibition of crude extract of *E. officinalis*, *M. fragrans* and *R. communis* was found to be 20 mm, 16 and 19 mm respectively. (Figure 2).

The 6 crude methanolic plant extract of *E. officinalis*, *F. bengalensis*, *M. fragrans*, *R. communis*, *Z. jujuba*, *A. barbadensis* and *A. arabica* showed considerable anti-bacterial activity against *Escherichia coli*. The maximum activity was shown by *A. barbadensis*, *E. officinalis*, *A.*

arabica, *R. communis* followed by *M. fragrans*, and *Z. jujuba*. The minimum anti-bacterial activity was shown by *F. bengalensis*. The diameter of zone of inhibition in *A. barbadensis* methanolic extract was found to be 33 mm whereas in *E. officinalis* fruit and leaf extract gave 26mm and 24 mm zone of inhibition respectively. The methanolic *A. arabica* leaf extract gave the zone of inhibition to 24 mm as given in figure 3.

The crude methanolic plant extract of 3 out of 7 plants, *E. officinalis*, *A. barbadensis* and *A. arabica* showed considerable anti-fungal activity against *Streptococcus cereviceae* and *F. bengalensis*, *M. fragrans*, *Z. jujuba* and *R. communis* did not showed any activity. The maximum activity was shown by *A. barbadensis*, *E. officinalis*, followed by *A. arabica*. The diameter of zone of inhibition in *E. officinalis* methanolic fruit extract was found to be 24 mm whereas in *E. officinalis* leaf extract gave the zone of inhibition to 20 mm. Similarly, the diameter of zone of inhibition of crude extract of *A. barbadensis* and *A. arabica* was found to be 25 and 18 mm respectively as shown in figure 4.

Based on our results, it is concluded that plant extracts have great potential as anti-microbial compounds against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms. The *E. officinalis* and *A. barbadensis* showed stronger activity than the other plants against all the tested bacterial strains. Therefore, *E. officinalis* and *A. barbadensis* can be selected for further analysis. It can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals that address fulfill therapeutic needs.

DISCUSSION

Saeed and Tariq¹³ focused on anti-bacterial potential of aqueous infusions and aqueous decoctions of *Embllica officinalis* (amla) and *Coriandrum sativum* (coriander) against 345 bacterial isolates belonging to 6 different genera of Gram -ve bacterial population isolated from urine specimens by employing well diffusion technique. Aqueous infusion and decoction of *Embllica officinalis* exhibited potent anti-bacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *K. ozaenae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. paratyphi A*, *S. paratyphi B* and *Serratia marcescens* but did not show any anti-bacterial activity against Gram negative urinary pathogens.

Saeed and Tariq¹³ showed potent anti-microbial activity in aqueous infusion and decoction of *Embllica officinalis* against *Staphylococcus aureus*, *S. haemolyticus*, *S. saprophyticus*, *Microcococcus varians*, *M. lylae*, *M. roseus*, *M. halobius*, *M. sedenterius*, *Bacillus subtilis*, *B. megaterium* and *Candida albicans*. In the present study

the organic solvent extract of *Emblica officinalis* was used as a positive control and it showed potent anti-bacterial activity with *Proteus vulgaris*, *Staphylococcus aureus* and *E. coli* in all the extracts.

The bark of *Ficus bengalensis* is used in inflammations, swelling of neck, gonorrhoea, scabies, mouth wash for toothache and for strengthening gums, the steeped freshly burnt bark has been found to cure cases of obstinate hiccup. The latex is used in inflammations and hemorrhages. In the Indian system of medicine, the leaf, root and seed oil of *Ricinus communis* L have been used for the treatment of the inflammation and liver disorders¹⁴, hypoglycemic¹⁵, laxative¹⁶ etc. Various studies have revealed that *Aloe vera* leaf skin (AVLS) possesses many pharmaceutical activities, including antibacterial activity¹⁷⁻¹⁸. Similarly *Z. jujuba* have been found to have multiple medicinal properties such as anti-fertility, analgesic, and anti-diabetes.

The gum of *Acacia arabica* was also applied to open wounds as an antiseptic balm. A decoction of the bark, mixed with rock salt should be used as a gargle in treating tonsillitis. *M. fragrans* extract has been shown to contain anti-bacterial activity against different genera of bacteria¹⁹. In the present study, all the methanolic extract of the above plants when tested against a panel of bacterial strains showed considerable anti-bacterial strains against the different bacterial strains used as shown in the results. These plant extracts will be evaluated further against more pathogenic bacteria.

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Table1:Ethnobotanical information of some medicinal plant species selected for anti-microbial activity

| S.No. | Scientific name of Plant | Family | Common Name | Part used |
|-------|-----------------------------------|---------------|-------------|----------------|
| 1. | <i>Acacia arabica</i> | Fabaceae | Kikar | Leaf |
| 2. | <i>Aloe barbadensis</i> | Liliaceae | Ghikuvar | Leaf |
| 3. | <i>Emblica officinalis</i> Geartn | Euphorbiaceae | Amla | Leaf and Fruit |
| 4. | <i>Ficus bengalensis</i> L. | Moraceae | Bar | Leaf |
| 5. | <i>Myristica fragrans</i> | Myristicaceae | Jaiphal | Fruit |
| 6. | <i>Ricinus communis</i> | Euphorbiaceae | Arand | Leaf |
| 7. | <i>Zizyphus jujuba</i> | Rhamnaceae | Ber | Leaf |

Table 2: The mg Gallic acid equivalent (GAE/g) of the plant extracts.

| S.No. | Scientific name of Plant | Gallic Acid Equivalent $\mu\text{g}/\mu\text{l}$ |
|-------|-----------------------------------|--|
| 1. | <i>Acacia arabica</i> | 1.76 $\mu\text{g}/\mu\text{l}$ |
| 2. | <i>Aloe barbadensis</i> | 1.33 $\mu\text{g}/\mu\text{l}$ |
| 3. | <i>Emblica officinalis</i> leaves | 3.91 $\mu\text{g}/\mu\text{l}$ |
| 4. | <i>Emblica officinalis</i> fruit | 2.4 $\mu\text{g}/\mu\text{l}$ |
| 5. | <i>Ficus bengalensis</i> | 2.56 $\mu\text{g}/\mu\text{l}$ |
| 6. | <i>Myristica fragrans</i> | 3.6 $\mu\text{g}/\mu\text{l}$ |
| 7. | <i>Ricinus communis</i> | 0.73 $\mu\text{g}/\mu\text{l}$ |
| 8. | <i>Zizyphus jujuba</i> | 0.35 $\mu\text{g}/\mu\text{l}$ |

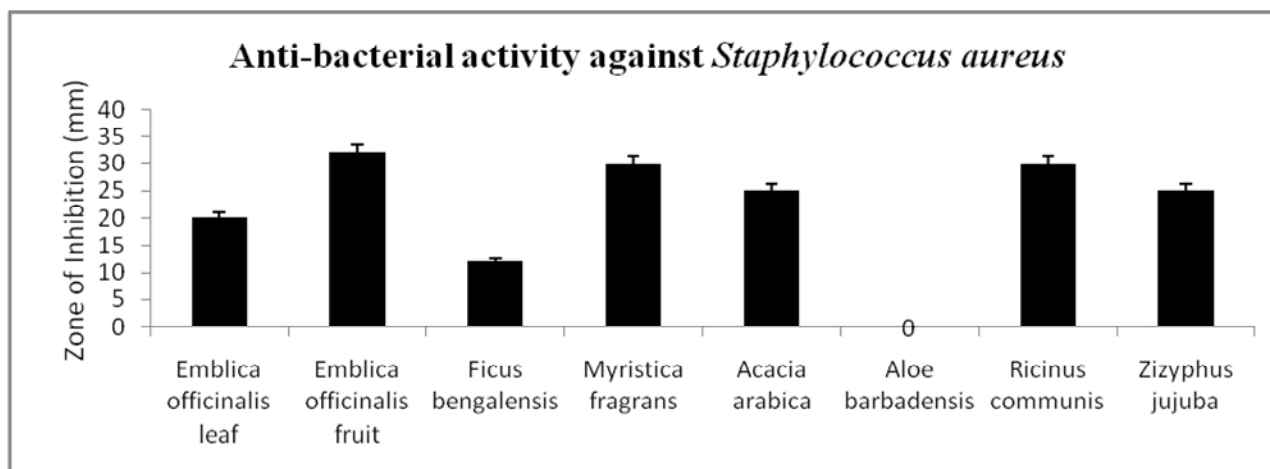


Figure 1: The anti-bacterial activity of methanolic extracts of the different plants against *Staphylococcus aureus*.

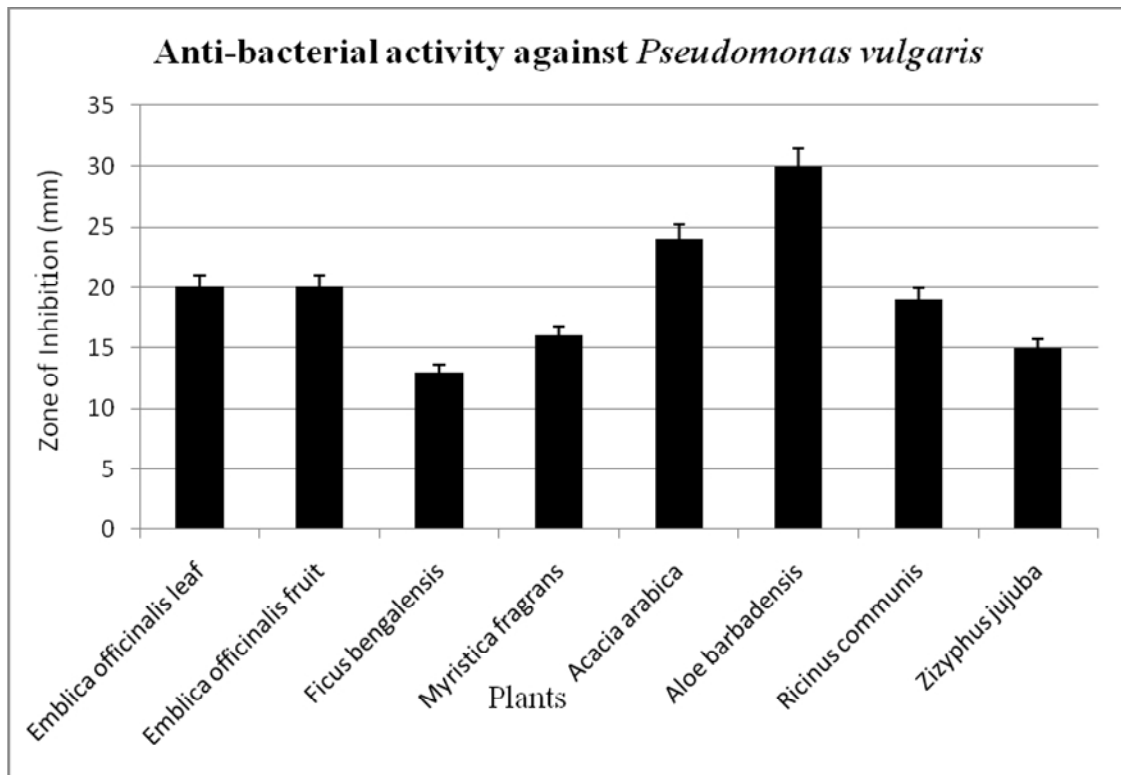


Figure 2: The anti-bacterial activity of methanolic extracts of the different plants against *Pseudomonas vulgaris*

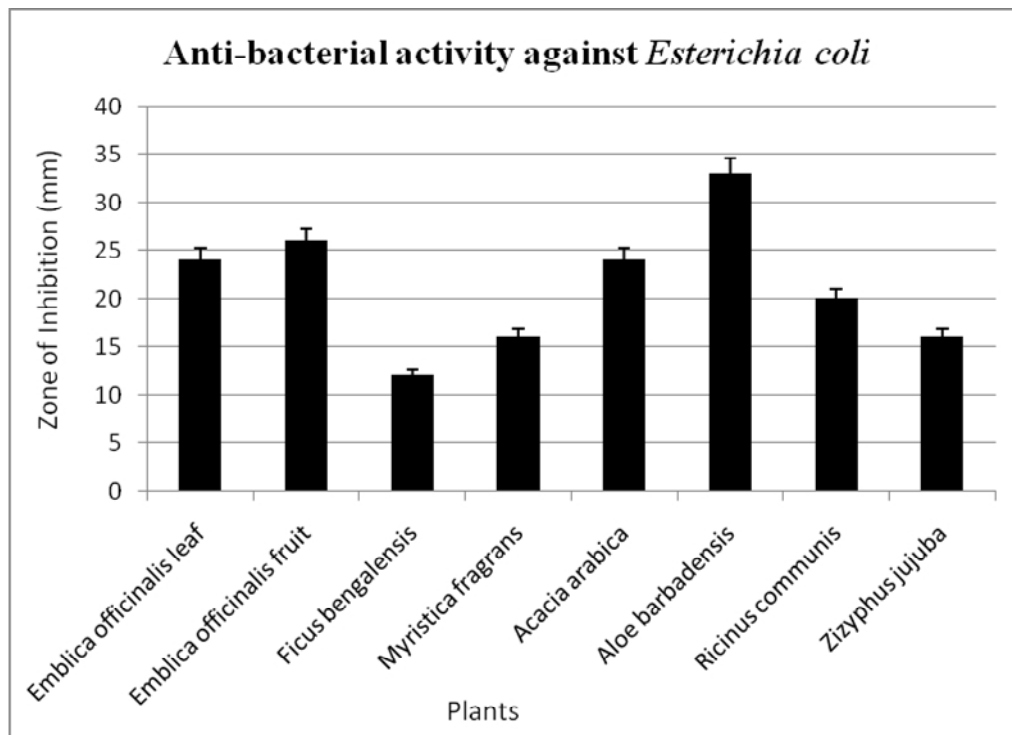


Figure 3: The anti-bacterial activity of methanolic extracts of the different plants against *Escherichia coli*

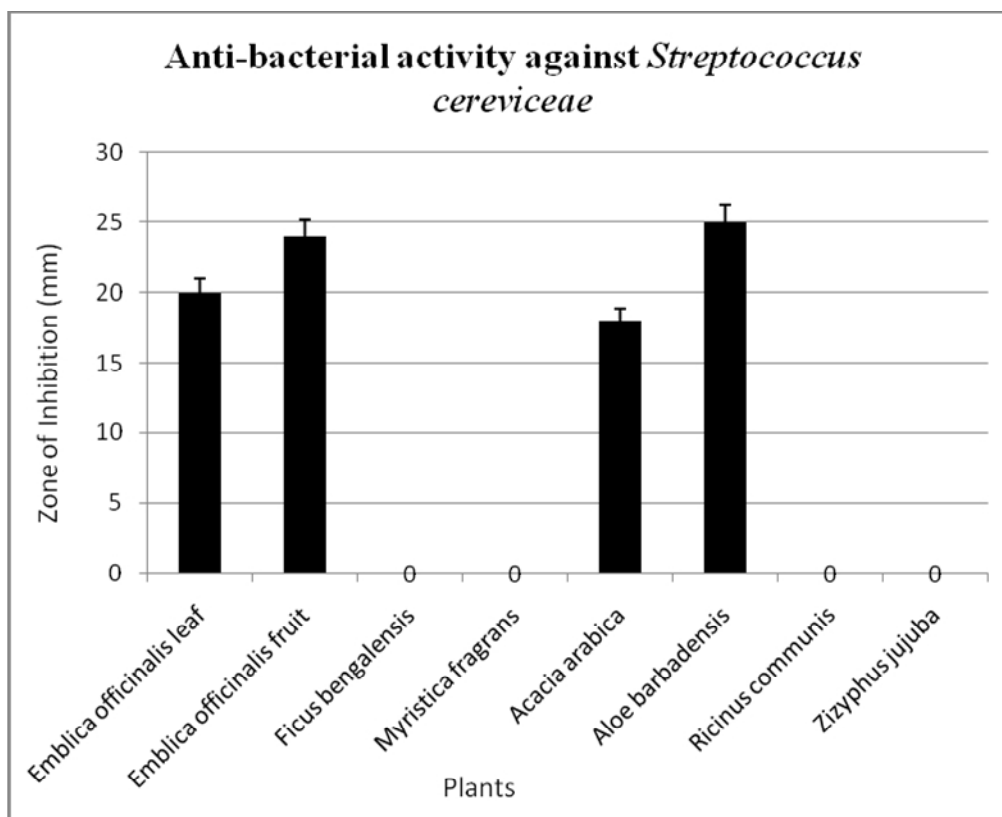


Figure 4: The anti- fungal activity of methanolic extracts of the different plants against *Streptococcus cereviceae*

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