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# STUDIES ON ANTIMICROBIAL ACTIVITY OF *ZIZIPHUS RUGOSA* LAM. PERICARP

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**Keywords:**

*Ziziphus rugosa*, Extracts, MIC, Antibacterial, Antifungal

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**ABSTRACT:** The desire of the present study was to investigate *in-vitro* antimicrobial activity on different crude extracts of *Ziziphus rugosa* pericarp. The antibacterial assay was carried out against *Bacillus subtilis* MTCC 1133, *Staphylococcus aureus* MTCC 7443, *Escherichia coli* MTCC 1692 and *Salmonella typhi* ATCC 19430. Meanwhile, *Candida albicans* ATCC 18804, *Aspergillus niger* ATCC 16404, *Trichoderma harzianum* MTCC 3832 and *Polyporus rubidus* MTCC 140 were used for the antifungal assay. Both activities were carried out by cup plate method using 100, 200, and 300mg/ml of hexane, ethanol, and aqueous extracts. The hexane extract showed MIC at 100 mg/ml for *B. subtilis*, 60 mg/ml for *S. aureus* and *S. typhi*, and 80 mg/ml for *E. coli*. The ethanolic extract showed 80 mg/ml for all tested organisms. The aqueous extract showed 80, 40, 60, and 100 mg/ml for *B. subtilis*, *S. aureus*, *E. coli*, and *S. typhi*, respectively. Among the extracts, aqueous extracts showed highest level activity against both gm +ve (*B. subtilis* 26mm) and gm -ve (*S. typhi* 24mm) at 300 mg/ml. Regarding antifungal activity, all the extracts at all the concentrations formed a similar zone of inhibition against *A. niger*. Therefore, the awesome constituents in the plant would solve some infectious as well as nutritional deficiency diseases.

**INTRODUCTION:** Plant constituents perform their role either at the molecular or cellular level. They show antibacterial, antiviral, neuro-degenerative and other activities [1]. The tribal and rural community of Sata Pokhran use medicinal plants to treat a wide spectrum of human ailments [2]. The residents of Keffi in north central Nigeria use about 40 plant species to treat skin diseases. They view herbal treatments as more effective and cheaper when compared to orthodox medicine [3].

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Because of antimicrobial resistance, 10 million people will stop living every year by 2050, if not a global response to the problem of antimicrobial resistance (AMR) is increased [4]. The plant *Ziziphus rugosa* belongs to the family Rhamnaceae. It occurs in all forest districts. It grows up to 6,000 ft in the Western Ghats and also in dry deciduous forests. It is a large straggling thorny shrub. Thorns usually solitary and recurved. Wood is moderately hard and reddish. Leaves large elliptic usually cordate. Flowers paniculate, petals absent, styles - 2, fruits small and single-celled drupe [5].

It is a famine edible fruit and medicinal plant of the Western Ghats. The deseeded pulp is the source for preparation of dosa and juice. It contains macro, micronutrients and low toxic elements [6].

The folk medicinal healers of Sylhet and Moulvibazar district use *Z. rugosa* fruit to treat tumors or cancers. Besides, they use it for sedative, hepatoprotective, blood purifier, and cardiotonic. They take one teaspoon of fruit powder orally with one cup of hot water twice a day [7]. The local people and traditional healers of Thalamalai hills, Vadavathur village in Namakkal district, use the bark of *Z. rugosa* in the form of powder to treat ulcer, skin disease and cough [8]. The Kanikkar of Agasthiarmalai Biosphere Reserve applies paste from one handful of leaves of *Z. rugosa* (before the morning bath) externally to treat scabies and ringworm infection [9]. The root of this plant possesses antibacterial and antifungal efficacy [10]. The pericarp exhibits antibacterial, insecticidal, and free radical scavenging assay [11]. Therefore, the present study aims to investigate antimicrobial activity of different solvent extracts of *Z. rugosa* Lam. pericarp.

## MATERIALS AND METHODS:

**Collection of Material:** For the research purpose, the plant *Ziziphus rugosa* Lam. showed in **Fig. 1** was collected with f1owers and fruits at Sokkalapuram near Pallapatti, Karur district during June 2017. It was identified and authenticated at Botanical Survey of India, Coimbatore. The voucher specimen of the plant refers the No. BSI/SRC/5/23/2017/Tech.570.

**FIG. 1: EXPERIMENTAL PLANT *ZIZIPHUS RUGOSA***

**Preparation of Extract:** The hexane and ethanolic extracts were prepared with the aid of the previous literature method [12] with some changes. The aqueous extract was prepared by maceration method. To make these successive solvent extracts, 70 gm of processed pericarp was packed and kept in the Soxhlet apparatus. It was subjected to continuous hot extraction first with 700 ml of 95% hexane at a suitable temperature until the solvent in the siphon became colorless. Then the extract was collected in China dish. The collected and air dried marc was extracted with 700 ml of 99.9% ethanol at a suitable boiling point until the solvent became clear. Finally, extracted the same dried marc with 400 ml of distilled water at 20-100 ºC by maceration method for an hour. To get the pure extract, filtered the macerated extract primarily by cotton cloth and followed by muslin cloth. To remove the solvent from the extracts, they were concentrated and dried in air.

**Microorganisms used for this Study:**

**G+:** *Bacillus subtilis* MTCC 1133, *Staphylococcus aureus* MTCC 7443.

**G⁻:** *Escherichia coli* MTCC 1692, *Salmonella typhi* ATCC 19430.

*Candida albicans* ATCC 18804, *Aspergillus niger* ATCC 16404, *Trichoderma harzianum* MTCC 3832 and *Polyporus rubidus* MTCC 140.

**Preparation of Test Inocula for Bacteria:** One loopful of each bacterial strain was transferred into sterile nutrient broth in Laminar air flow chamber using sterilized Pasteur loop (3 mm diameter). These slants were incubated at 37 ºC for 24 h which showed sufficient growth organism in the broth.

**Preparation of Test Inocula for Fungi:** One loopful of each selected fungal strain was transferred into sterilized Sabouraud's agar medium in the Petri plate individually by streaking technology. These plates were incubated at 25 ºC for seven days.

**Minimum Inhibitory Concentration (MIC):** The MIC of different crude extracts of *Z. rugosa* was determined by liquid dilution method [13]. A series of test tubes containing 5ml of sterile nutrient broth medium (which labeled as 20, 40, 60, 80, 100 mg/ml, blank and negative control) were placed in the test tube stands. Then 50 µl of test organism was transferred, followed that the respective concentration of the extract was added under Laminar airflow chamber. They were mixed well and incubated at 37 ºC for 24 h. These test tubes were examined carefully, and the result was recorded based on the turbidity.

**Assay of Antibacterial Activity:** The antibacterial activity of different extracts (hexane, ethanol and aqueous) of *Z. rugosa* was evaluated by cup plate method [13] (diffusion plate technique) for 100, 200 and 300 mg/ml dilutions. To the test tube containing 30 ml of molten agar medium at 45 ºC, 0.5 ml of standard bacterial inoculum (24 h broth culture) was added. This inoculated agar medium was transferred to Petri plates by pour plate technique. The plates were rotated gently and allowed to solidification under Laminar airflow chamber. Four wells were bored with the help of (5 mm diameter) sterile borer in all the plates aseptically. Out of 4 wells, three wells were filled with the respective concentrations of drug extract. The rest well was filled with norfloxacin (positive control). The plates were kept in the refrigerator for 30 min. This facilitates the even diffusion of extract into the agar medium. The plates were incubated at 37 ºC in an upright position for 24 h. The clear zone around each cup was measured using scale.

**Assay of Antifungal Activity:** The antifungal activity of different extracts (hexane, ethanol and aqueous) of *Z. rugosa* was tested against the fungal strains (*Candida albicans* ATCC 18804, *Aspergillus niger* ATCC 16404, *Trichoderma harzianum* MTCC 3832 and *Polyporus rubidus* MTCC 140) by diffusion plate method [13]. To prepare the seeded Sabouraud's Dextrose Agar (SDA) plates, 30 ml of sterilized SDA medium was poured into each sterile plate. After solidification, each plate was swabbed with respective inoculum by using cotton buds. Four wells (5 mm diameter) were bored using sterile borer in all the plates aseptically. Out of four wells, three wells were injected with 15 µl of respective concentration of extracts. The rest well was injected with 15 µl of standard drug fluconazole (positive control). After charging them, the plates were kept in the refrigerator for 30 min. After that, the plates were kept at 25 ºC in BOD incubator for three days. As usual, the clear zone around each cup was measured using scale.

**RESULT:** The results for the MIC of different crude extracts are showed in **Table 1** and **Fig. 2**. The hexane extract showed 100 mg/ml for *Bacillus subtilis*, 60 mg/ml for *Staphylococcus aureus* and *Salmonella typhi*, and 80 mg/ml for *Escherichia coli*. The MIC of the ethanolic extract revealed 80 mg/ml against all the tested microorganisms. The aqueous extract showed 80, 40, 60, and 100 mg/ml for *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* respectively.

**TABLE 1: THE MIC TEST OF *Z. RUGOSA* PERICARP**

| S. no. | Organisms | Hexane mg/ml | | | | | | | Ethanol mg/ml | | | | | | | Aqueous mg/ml | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 20 | 40 | 60 | 80 | 100 | Std | -ve | 20 | 40 | 60 | 80 | 100 | Std | -ve | 20 | 40 | 60 | 80 | 100 | Std | -ve |
| 1 | *B. subtilis* MTCC 1133 | + | + | + | + | - | - | + | + | + | + | - | - | - | + | + | + | + | - | - | - | + |
| 2 | *S. aureus* MTCC 7443 | + | + | - | - | - | - | + | + | + | + | - | - | - | + | + | - | - | - | - | - | + |
| 3 | *E. coli* MTCC 1692 | + | + | + | - | - | - | + | + | + | + | - | - | - | + | + | + | - | - | - | - | + |
| 4 | *S. typhi* ATCC 19430 | + | + | - | - | - | - | + | + | + | + | - | - | - | + | + | + | + | + | - | - | + |

Std: standard, -ve: negative, +: growth, - : No growth

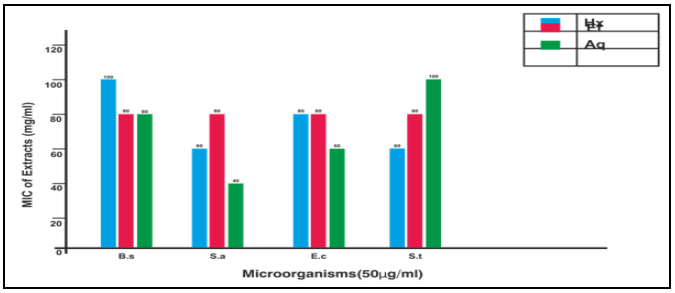


**FIG. 2: MIC VALUES OF DIFFERENT CRUDE EXTRACTS OF *Z. RUGOSA* LAM. PERICARP**

The results for antibacterial activity of hexane, ethanol, and aqueous extracts are showed in **Plate 1, 2** and **3, Table 2** and **Fig. 3**.

The hexane extract at 100 mg/ml did not form a zone against *Bacillus subtilis* showed in **Plate 1a**. But this extract formed greatest zone (14 mm) at 300 mg/ml. On the other hand, this extract formed a similar zone of inhibition against *Salmonella typhi* at 100mg/ml showed in **Plate 1d**. At the same time, increased concentrations (200 and 300 mg/ml) did not reveal activity. The zone of inhibition (14 mm) formed at lower concentration was almost equal to standard (15 mm). This extract formed lowest zone against *Staphylococcus aureus*, and *Escherichia coli* showed in **Plate 1b** and **c**. The ethanolic extract at all the concentrations possessed

higher level activity 22, 23 mm against both gm +ve organisms and 20 and 21 mm for gm -ve organisms showed in **Plate 2a, b,** and **c, d** respectively. Though, this extract formed greatest zone (23 mm) against *Staphylococcus aureus*, the zone formed by standard was more (42mm). The aqueous extract formed the highest level activity against both gm +ve (*Bacillus subtilis*) and gm -ve (*Salmonella typhi*) organisms showed in **Plate 3a** and **3d**. The zone 22 mm formed at 300 mg/ml of aqueous extract was equal to (25 µg/ml) of standard norfloxacin against *Escherichia coli* showed in **Plate 3c**. All the extracts possessed lesser activity against *Staphylococcus aureus* showed in **Plates 1b, 2b,** and **3b**.

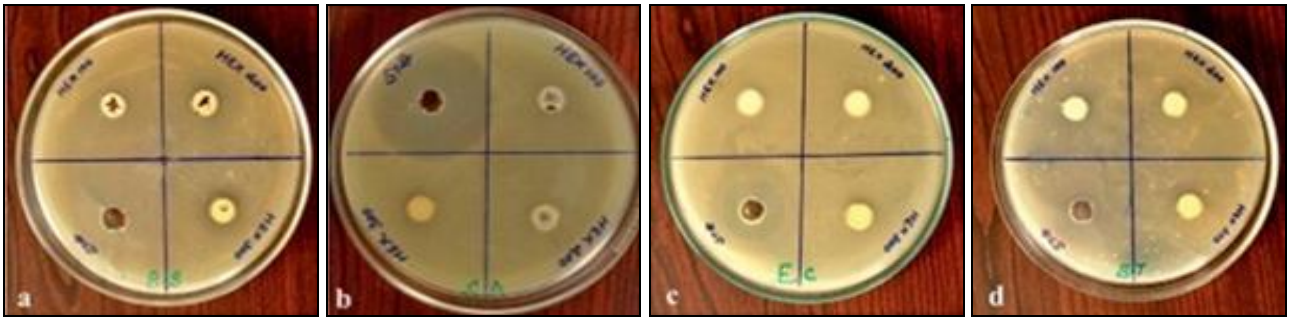

**PLATE 1: ANTIBACTERIAL ACTIVITY OF HEXANE EXTRACT**

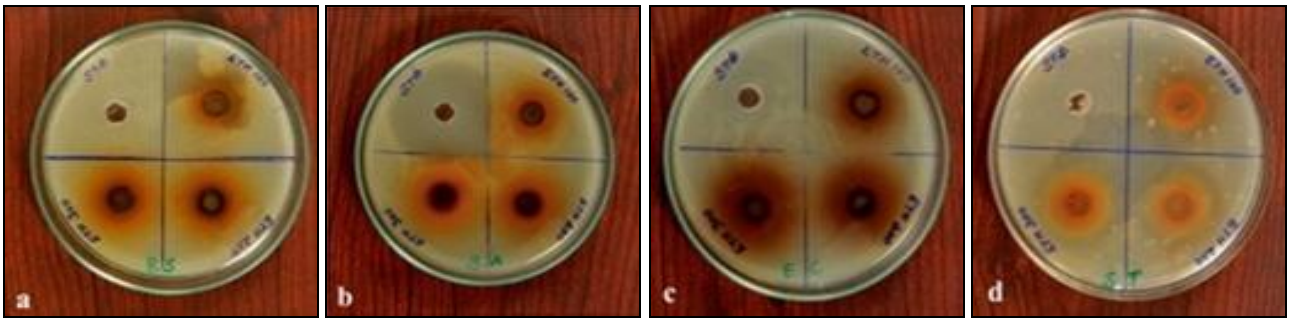

**PLATE 2: ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT**

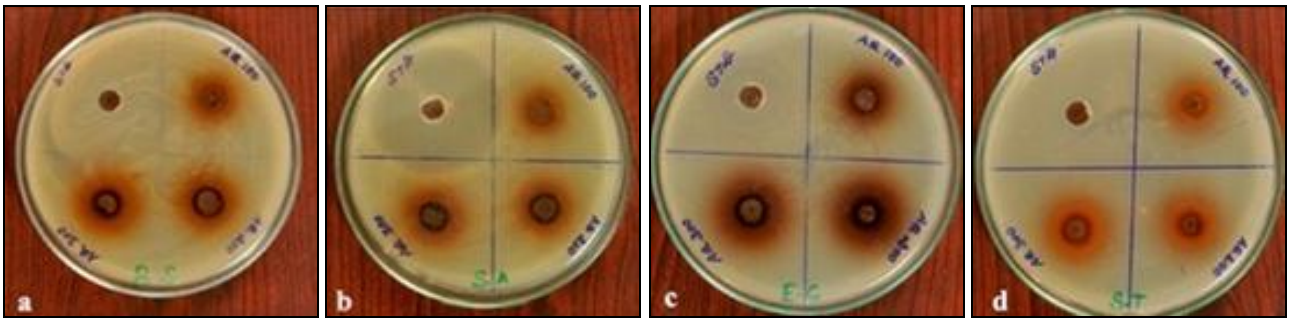

**PLATE 3: ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACT**

**TABLE 2: ANTIBACTERIAL ACTIVITY OF *Z. RUGOSA* PERICARP.**

| S. no. | Microorganisms | Zone of growth inhibition in mm | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hexane extract mg/ml | | | | Ethanol extract mg/ml | | | | Aqueous extract mg/ml | | | |
| | | 100 | 200 | 300 | Std Norfloxacin | 100 | 200 | 300 | Std | 100 | 200 | 300 | Std |
| 1 | *Bacillus subtilis* MTCC 1133 | - | 12 | 14 | 10 | 16 | 20 | 22 | 12 | 18 | 22 | 26 | 14 |
| 2 | *Staphylococcus aureus* MTCC 7443 | 12 | 12 | 10 | 40 | 17 | 21 | 23 | 42 | 14 | 17 | 21 | 44 |
| 3 | *Escherichia coli* MTCC 1692 | 11 | 12 | 13 | 20 | 17 | 19 | 20 | 22 | 14 | 18 | 22 | 22 |
| 4 | *Salmonella typhi* ATCC 19430 | 14 | - | - | 15 | 14 | 18 | 21 | 12 | 16 | 22 | 24 | 12 |

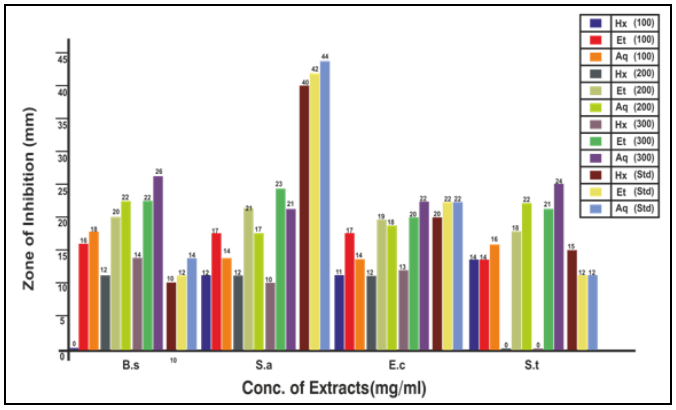


**FIG. 3: ANTIBACTERIAL ACTIVITY OF DIFFERENT CRUDE EXTRACTS OF *Z. RUGOSA* LAM. PERICARP**

**Antifungal Activity:** The results for antifungal activity of hexane, ethanol, and aqueous extracts are shown in **Plate 4, 5,** and **6, Table 3,** and **Fig. 4**. All the extracts at all the concentrations formed a similar zone of inhibition against *Aspergillus niger* showed in **Plate 4b, 5b,** and **6b**.

The ethanolic extract at all the concentration also formed similar and almost similar zone against *Candida albicans*, and *Polyporus rubidus* respectively showed in **Plate 5a** and **5d.**

All the extracts at 300 mg/ml formed the highest zone only against *Trichoderma harzianum* showed in **Plate 4c, 5c,** and **6c**. Both hexane and aqueous extract formed almost similar zone against *Candida albicans*, and *Trichoderma harzianum* showed in **Plate 4a, 6a,** and **4c, 6c** respectively.

In the hexane extract, the standard drug formed the least zone against *Polyporus rubidus* showed in **Plate 4d**. At lower concentration, ethanol and aqueous extracts formed highest zone against *Trichoderma harzianum* and *Polyporus rubidus* respectively showed in **Plate 5c** and **6d.**

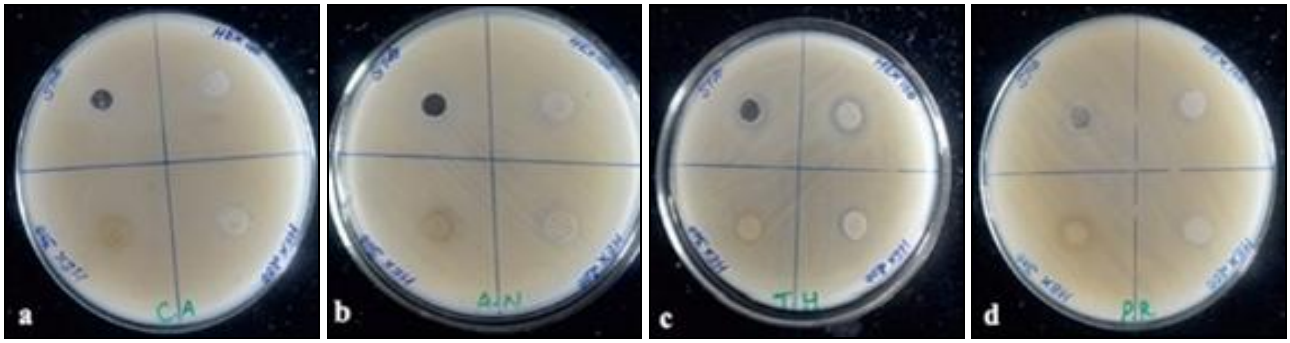


**PLATE 4: ANTIFUNGAL ACTIVITY OF HEXANE EXTRACT**

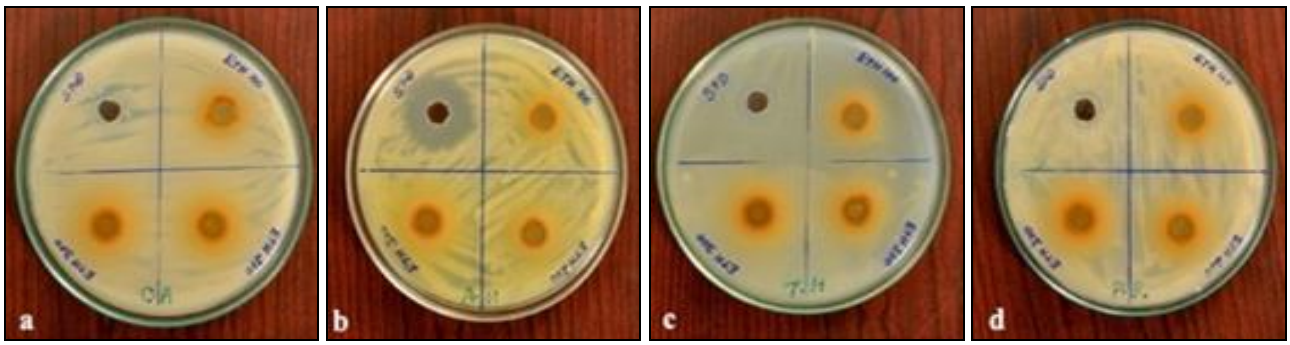


**PLATE 5: ANTIFUNGAL ACTIVITY OF ETHANOL EXTRACT**

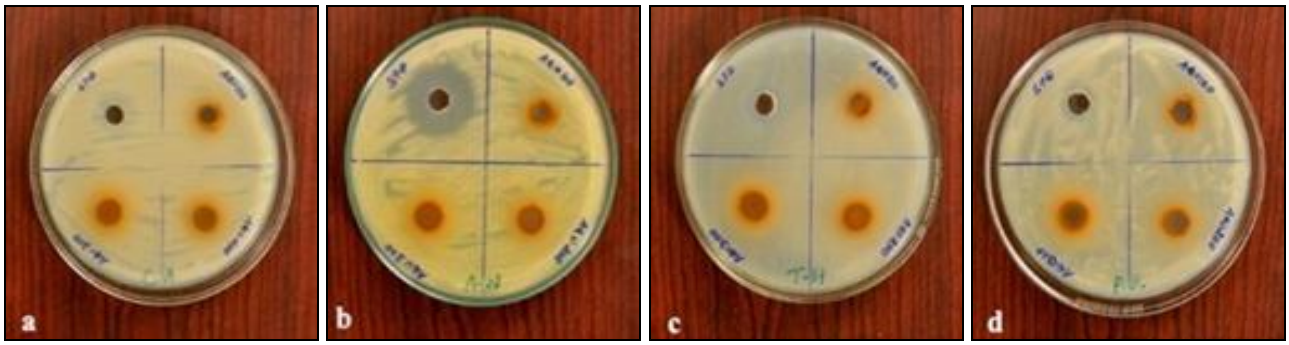


**PLATE 6: ANTIFUNGAL ACTIVITY OF AQUEOUS EXTRACT**

**TABLE 3: ANTI FUNGAL ACTIVITY OF *Z. RUGOSA* PERICARP.**

| S. no. | Microorganisms (fungi) | Zone of growth inhibition in mm | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hexane extract | | | | Ethanol extract | | | | Aqueous extract | | | | |
| | | 100 | 200 | 300 | Std Fluconazole 25 µg/ml | 100 | 200 | 300 | Std | 100 | 200 | 300 | Std | -ve control |
| 1 | *Candida albicans* ATCC 18804 | 11 | 12 | 14 | 11 | 12 | 14 | 15 | 11 | 12 | 12 | 14 | 13 | - |
| 2 | *Staphylococcus* abbreviated ATCC 16404 | 12 | 14 | 15 | 14 | 12 | 14 | 15 | 26 | 12 | 14 | 15 | 26 | - |
| 3 | *T. harzianum* MTCC 3832 | 11 | 13 | 16 | 14 | 15 | 17 | 18 | 12 | 11 | 14 | 16 | 14 | - |
| 4 | *Polyporus rubidus* MTCC 140 | 11 | 13 | 14 | .9 | 12 | 14 | 16 | 16 | 13 | 14 | 15 | 15 | - |

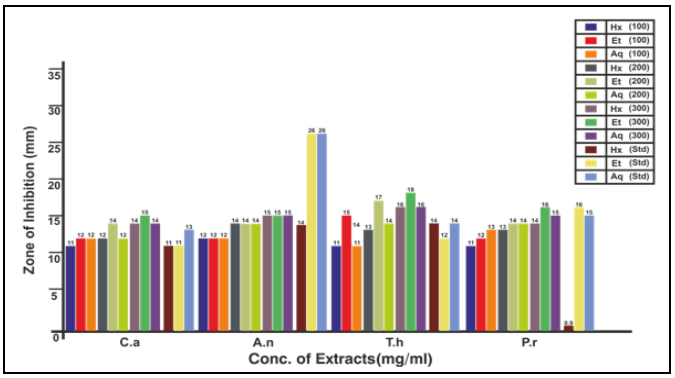


**FIG. 4: ANTIFUNGAL ACTIVITY OF DIFFERENT CRUDE EXTRACTS OF *Z. RUGOSA* LAM. PERICARP**

**DISCUSSION:** The hexane extract of *Z. rugosa* shows MIC at 100 mg/ml for *Bacillus subtilis*, 60mg/ml for *S. aureus* and *S. typhi*, and 80 mg/ml for *E. coli*. The n-hexane and Etoac fractions of aerial parts of *Z. jujuba* showed MIC at low values against *S. typhi* [14]. Among the extracts, aqueous extract only shows the MIC at 40 mg/ml against *S. aureus*. Besides, this extract at 60 mg/ml shows MIC for *E. coli*. The hexane extract at 100 mg/ml shows no activity against *Bacillus subtilis* and forms 11 mm zone against *E. coli*. This is more or less coincide to results of *Z. jujuba, Z. mauritiana* and *Z. nummularia* fruits [15].

Among the bacterial strains *Bacillus subtilis* is the most sensitive bacterium for both ethanolic and aqueous extracts. The methanolic crude pulp extract of *Z. mauritiana* at 25µg/ml did not form zone against *Bacillus subtilis*, *S. aureus* and *E. coli* except *S. typhi*. But n-hexane fraction revealed nothing upto 100 mg/ml except *Bacillus subtilis* [16]. Regarding the extracts the aqueous extract shows potent antibacterial activity against both gm +ve and gm -ve bacteria. This similar result also observed in 50% aqueous-ethanol extract of *Z. jujuba* seed [17]. The hexane extract at 200 and 300 mg/ml reveal no inhibition against *S. typhi*.

But this extract exhibits higher activity against the fungal strains *C. albicans*, *A. niger* and *P. rubidus*. Among the fungal strains, *T. harzianum* is most sensitive to all the extracts. Both ethanol and methanol leaf extract of *Z. mauritiana* at higher concentration formed greater zone against *Trichoderma viride* when compared to *Candida albicans*. When compared to methanol extract, the ethanolic extract possessed potential activity [18]. All the extracts at all the concentrations show a similar zone of inhibition against *A. niger*. When compared to fluconazole, all the extracts exhibit more or less similar or wider zone against *C. albicans*, *T. harzianum* and *P. rubidus*. The ethanolic crude extract of *Z. jujuba* fruit exhibited efficient activity against *C. albicans* and *A. fumigatus* [19]. The chloroform and methanol extract of *Z. rugosa* bark revealed good inhibition against *S. typhi*. When compared to methanol extract the chloroform leaf extract of *Z. rugosa* nothing revealed against *S. typhi*. Likewise, chloroform bark and leaves of *Z.oenoplia* also nothing revealed against *S. typhi*. But methanolic bark extract of *Z. oenoplia* did not show that much activity against the fungal strains [20].

**CONCLUSION:** From this study, it is inferred that the aqueous pericarp extract of *Z. rugosa* exhibits efficient antibacterial activity against both $g^+$ and $g^-$ organisms. The ethanolic extract possesses highest antifungal activity followed by aqueous and hexane. All the extracts at all the concentrations formed similar zone against *A. niger*. The ethanolic extract at all the concentrations formed a similar zone against both *A. niger* and *C. albicans*.

Therefore, the awesome chemical constituents present in the pericarp of *Z. rugosa* would help to solve some infectious as well as nutritional deficiency diseases.

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**CONFLICT OF INTEREST:** Nil

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