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## IN VITRO ANTIMICROBIAL SCREENING OF MEDICINAL PLANTS AGAINST CLINICAL AND PHYTOPATHOGENIC BACTERIA AND FUNGI

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### Keywords:

Medicinal,  
Antimicrobial, *S. grandiflora*,  
*N. arbor-tristis*, *B. subtilis*,  
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**ABSTRACT:** The positive results of screening medicinal plants for antimicrobial activity forms primary platform for phytochemical and pharmacological studies in future. The work was undertaken to evaluate the antimicrobial activity of four medicinal plants viz., *Sesbania grandiflora*, *Epiphyllum oxypetalum*, *Nyctanthes arbor-tristis*, and *Vetiveria zizanioides*. Leaves were extracted using water and ethanol as solvents. The antimicrobial activity was assessed against five bacterial and fungal pathogens like *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Enterobacter aerogenes* and *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Fusarium moniliforme*, *Penicillium* sp. Aqueous extracts of *S. grandiflora* was found to be most effective in inhibiting *B.subtilis* ( $23 \pm 0.66$ ) while ethanolic extracts showed *N. arbor-tristis* inhibiting *S.typhi* ( $20 \pm 0.35$ ). Further aqueous extracts of *S. grandiflora* showed considerable effect of inhibition against *Penicillium* sp. ( $22.63 \pm 0.60$ ) whereas ethanol extracts of *S. grandiflora* was most efficient against *Penicillium* sp. ( $24 \pm 0.33$ ). All the other plants extracts were also effectual to remarkable extent. The present investigation has thrown a light on the effect of ethanolic leaf extracts of *S. grandiflora* and *N. arbor-tristis* against the selected microorganisms which can be further subjected to purification which may act as alternative for synthetic compounds in especially antioxidants and antimicrobial drugs.

**INTRODUCTION:** Plants provide a new scaffold for the study of natural products for drug discovery<sup>1</sup>. Herbal medicine practice plays an important role in the primary health care delivery system in most developing countries. For century's man as effectively used various components of plants or their extracts for the treatment of many diseases, including bacterial and fungal infections<sup>2</sup>.

Researchers now are focussing on natural products to develop new molecules in treating new diseases since current treat shows the failure existing antimicrobials in treating the infectious diseases<sup>3,4</sup>.

In the present scenario rising resistance by many microorganisms worldwide towards antimicrobials are in use, it is very important that the actual ingredients having antimicrobial potential needs to be extracted<sup>5</sup>. Plants are forthcoming sources of antimicrobial agents in different countries<sup>6</sup>. Plant based antimicrobials represent a vast untrapped source. The use of plant extracts for medicinal treatment has become popular when people realized that the effective life span of antibiotic is limited and over prescription misuse of traditional

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antibiotics are causing microbial resistance<sup>7</sup>. Reports are available on plant by-products which have antimicrobial properties on pathogenic several bacteria and fungi<sup>8</sup>. Although the mechanism of action and efficacy of herbal extracts in most cases is still needed to be validated scientifically, these preparations mediate important host responses<sup>9</sup>.

Due to profitable efficiency of medicinal plants on biological activities there is a need for isolation of newer biological compounds from plants which serve as novel drugs.

## MATERIALS AND METHODS:

**Collection of Plant Materials:** The fresh leaves of plants namely *Sesbania grandiflora*, *Epiphyllum oxypetalum*, *Nyctanthes arbor-tristis* and *Vetiveria zizanioides* free from disease were collected in and around Mysuru district of Karnataka **Table 1** and **Fig. 1**. The plants were identified and authenticated

from P. G. Department of Botany, Maharani's Science College for Women, Mysuru. The voucher specimen of the plants numbers viz. *S. grandiflora* (MSCWM/ PG/ 2017-125), *E. oxypetalum* (MSCWM/ PG/ 2017-194), *N. arbor-tristis* (MSCWM/ PG/ 2017-227) and *V. zizanioides* (MSCWM/ PG/ 2017-308) have been kept in the department for further studies.

**Test Microorganisms:** The identified pathogenic bacteria *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Enterobacter aerogenes*, and fungi *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Fusarium moniliforme*, and *Penicillium* sp. were obtained from PG Department of Microbiology, Maharani's Science College for Women, Mysuru, Karnataka, India.



**FIG. 1: SELECTED MEDICINAL PLANTS**

**TABLE 1: SELECTED MEDICINAL PLANTS AND THEIR USES**

S. no.	Common name	Botanical name	Family	Parts used	Traditional use
1	Agati	<i>Sesbania grandiflora</i>	Fabaceae	Leaves	Diuretic, laxative, antipyretic
2	Brahmakamala	<i>Epiphyllum oxypetalum</i>	Cactaceae	Leaves	Antibacterial activity, to cure dropsy and cardiac infections
3	Parijata	<i>Nyctanthes arbor-tristis</i>	Oleaceae	Leaves	Antifungal, antibacterial, anthelmintic, anti-inflammatory, hepatoprotective, immuno-potential, anti-pyretic, antioxidant
4	Lavanha grass	<i>Vetiveria zizanioides</i>	Poaceae	Leaves	Mouth ulcer, fever, boil, epilepsy, burn, snakebite, scorpion sting, rheumatism, fever, headache

The bacterial cultures were grown and maintained on Nutrient Broth at 37 °C, while the fungal cultures were maintained on Potato Dextrose Agar slants and incubated at 27 °C for further studies.

### Preparation of Extracts:

**Aqueous Extract:** 10 gm of shade dried, powder of leaf material all selected plant species were macerated with 100 ml of sterile distilled water in a blender for 15 min. The macerate was first filtered through double layered muslin cloth and then filtrate was centrifuged at 4000 rpm for 30 minutes at room temperature. Supernatant was filtered through Whatman no. 1 filter paper and the supernatant was made up to make the final volume one-fourth of original volume which was heat sterilized at 121 °C for 20 minutes. The extract was preserved aseptically in brown airtight bottles and stored at 4 °C for further use.

**Ethanol Extract:** 10 gm of shade dried, powder of leaf material all selected plant species were macerated with 100 ml of ethanol kept on a rotary shaker at 190 - 220 rpm for 24 hrs. The filtrate was first filtered through double layered muslin cloth and then filtrated through Whatman no. 1 filter paper and the supernatant was made up to make the final volume one-fourth of original volume which was heat sterilized at 121 °C for 20 minutes. The extract was preserved aseptically in brown airtight bottles and stored at 4 °C for further use.

### Agar-well Diffusion Method:

**Antibacterial Activity:** Agar-well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts<sup>10, 11</sup>. Nutrient agar plates were swabbed with 24 hr old culture of selected bacteria with sterile cotton swabs.

10 mm wells were made in each NA plates using sterile cork borer. 100 µl of each extract were added to the wells by using micropipette and allowed to diffuse at room temperature for 2 hours. The plates were then incubated at 37 °C, for 24 hours. The antibacterial activity was assayed by measuring the diameter of inhibition zone around the well in millimeter<sup>12</sup>.

**Antifungal Activity:** Potato dextrose agar plates were swabbed with 36 - 48 hour culture of selected fungi with sterile cotton swabs. 10 mm wells were

made in each PDA plates using sterile cork borer. 100 µl of each extract were added to the wells by using micropipette and allowed to diffuse at room temperature for 2 hours. The plates were then incubated at 28 °C, for 48 hours. The antifungal activity was assayed by measuring the diameter of the inhibition zone around the well in millimeter<sup>13</sup>. The experiments were conducted in triplicates using appropriate controls. The antibiotic and antifungal sensitivity test using standard antibiotic (Streptomycin for bacteria and Nystatin for fungi 1mg/ ml) were used as positive control, distilled water for aqueous extracts and ethanol for ethanolic extracts negative control for all the microbial strains.

**Statistical Analysis:** Data from three replicates were analysed for each experiment and analysis of variance (ANOVA) using SPSS Inc.17.0. Significant effects of treatments were determined by F-test ( $P \leq 0.05$ ). Treatment means were separated using Tukey's HSD.

**RESULTS:** The antimicrobial activity of four medicinal plants, viz, *S. grandiflora*, *E. oxypetalum*, *N. arbor-tristis* and *V. zizanioides* were tested against human pathogenic bacteria (*B. subtilis*, *S. aureus*, *E. coli*, *S. typhi* and *E. aerogenes* and fungi (*A. flavus*, *A. niger*, *C. cladosporioides*, *F. moniliforme*, and *Penicillium* sp.) showed varied level of inhibition against the human pathogens.

### Antibacterial Activity of Aqueous Leaves

**Extracts:** Aqueous extracts of *S. grandiflora* exhibited maximum zone of inhibition against *B. subtilis* ( $23 \pm 0.66$ ), followed by *E. coli* ( $21 \pm 0.33$ ) followed by *S. typhi* ( $20 \pm 0.57$ ), *E. aerogenes* ( $20 \pm 0.88$ ) and *S. aureus* ( $19 \pm 0.33$ ). *E. oxypetalum* exhibited maximum zone of inhibition against *B. subtilis* ( $19 \pm 0.00$ ), followed by *S. aureus* ( $17 \pm 0.50$ ), *S. typhi* ( $17 \pm 0.3$ ), *E. coli* ( $15 \pm 0.00$ ), *E. aerogenes* ( $14 \pm 0.50$ ). *N. arbor-tristis* revealed effective zone of inhibition against *S. aureus* ( $18 \pm 0.05$ ) followed by *S. typhi* ( $21 \pm 0.86$ ), *E. coli* ( $20 \pm 0.60$ ), *B. subtilis* ( $20 \pm 0.10$ ), *E. aerogenes* ( $12 \pm 0.64$ ). *V. zizanioides* showed considerable zone of inhibition against *E. areogenes* ( $20 \pm 0.33$ ) followed by *S. typhi* ( $18 \pm 0.57$ ), *S. aereus* ( $14 \pm 0.57$ ), *E. coli* ( $13 \pm 0.00$ ), *B. subtilis* ( $12 \pm 0.66$ )

**Fig. 2 and 3.**

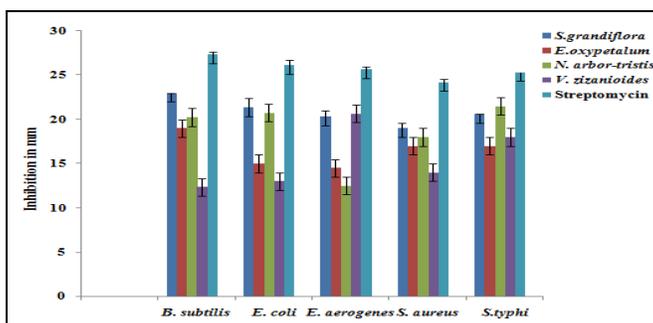


FIG. 2: ANTIBACTERIAL ACTIVITY OF AQUEOUS LEAVES EXTRACTS

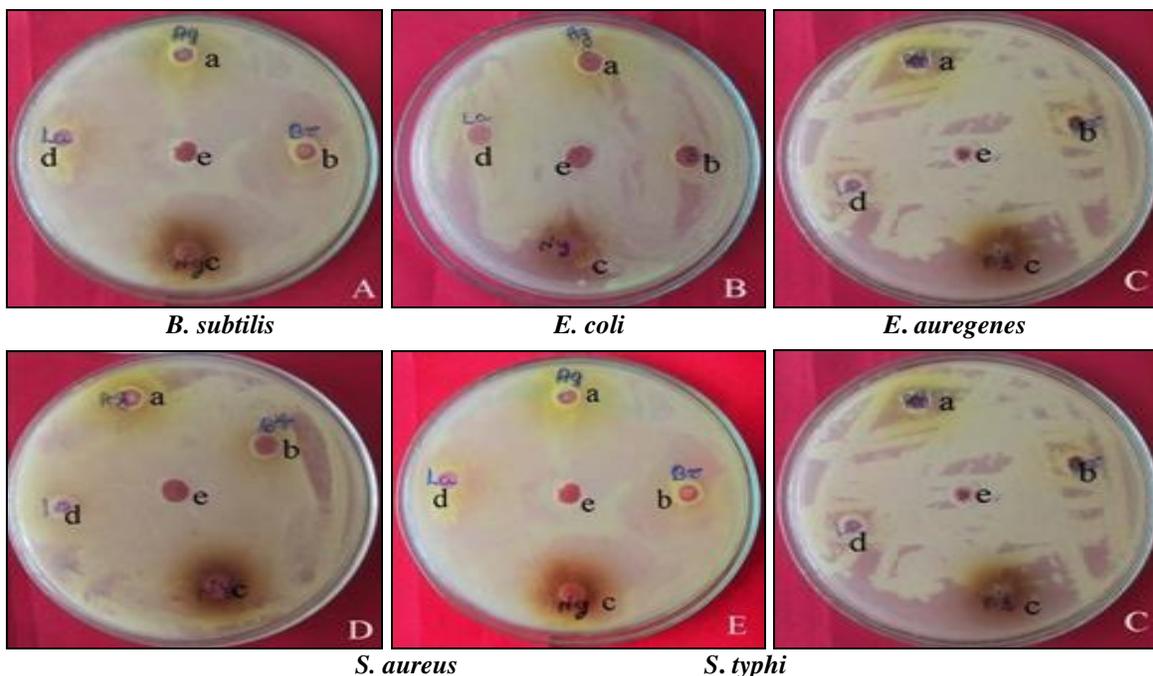


FIG. 3: ANTIBACTERIAL ACTIVITY OF AQUEOUS LEAVES EXTRACTS

a) *S. grandiflora*, b) *E. oxypetalum*, c) *N. arbor-tristis*, d) *V. zizanioides*, e) Negative control (distilled water)

**Antibacterial Activity of Ethanol Leaves Extracts:** Ethanol extracts of *S. grandiflora* showed (18 ± 0.33) zone of inhibition against *B. subtilis* followed by *S. typhi* (15 ± 0.33), *E. aerogenes* (14 ± 0.33), *E. coli* (14 ± 0.33) and *S. aureus* (11 ± 0.33). *E. oxypetalum* exhibited maximum zone of inhibition against *S. aureus* (15 ± 0.00) followed by *E. coli* (14 ± 0.50), *B. subtilis* (14 ± 0.00), *E. aerogens* (12 ± 0.50), and *S. typhi* (12 ± 0.00). Varied level of inhibition was also

observed in *N. arbor-tristis* revealed maximum zone of inhibition against *S. typhi* (20 ± 0.35) followed by *B. subtilis* (17 ± 0.33), *E. aerogenes* (17 ± 0.23), *S. aureus* (16 ± 0.28) and *E. coli* (14 ± 0.25 mm). *V. zizanioides* exhibited significant zone of inhibition against *B. subtilis* (19 ± 0.33) followed by *S. typhi* (18 ± 0.33), *E. aerogenes* (15 ± 0.66), *S. aureus* (15 ± 0.00), and *E. coli* (11 ± 0.33) **Fig. 4 and 5.**

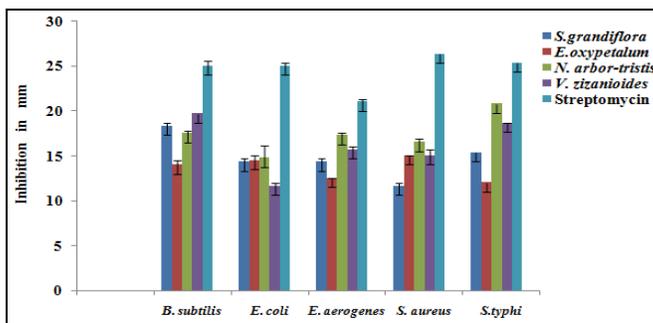
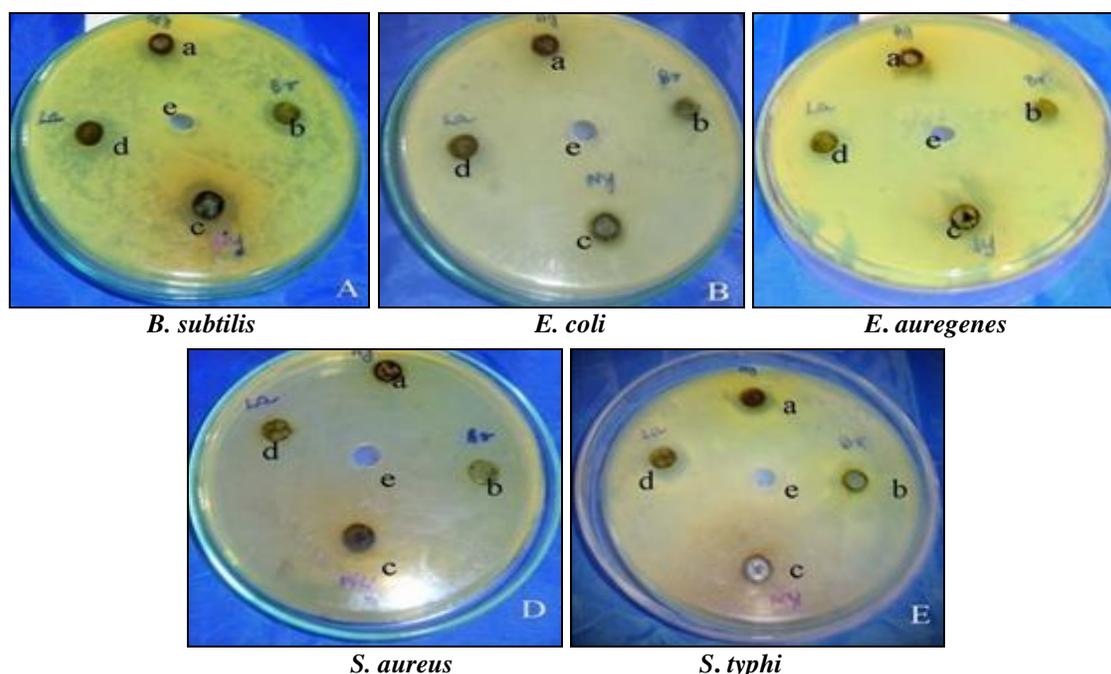


FIG. 4: ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACTS OF LEAVES

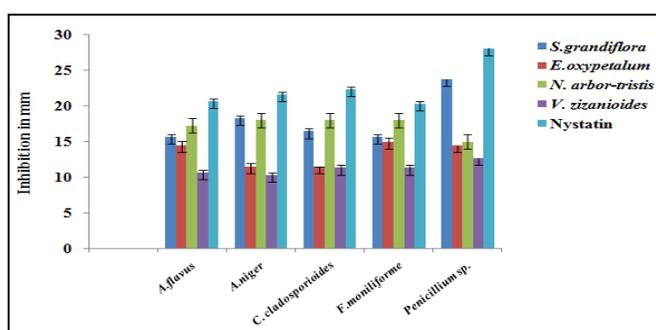


**FIG. 5: ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACTS OF LEAVES**  
 a) *S. grandiflora*, b) *E. oxypetalum*, c) *N. arbor-tristis*, d) *V. zizanioides*, e) Negative control (Ethanol)

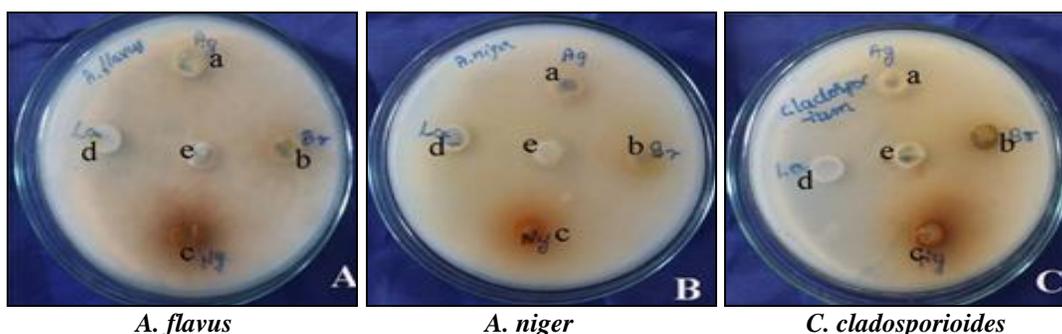
**Antifungal Activity of Aqueous Leaves Extracts:**

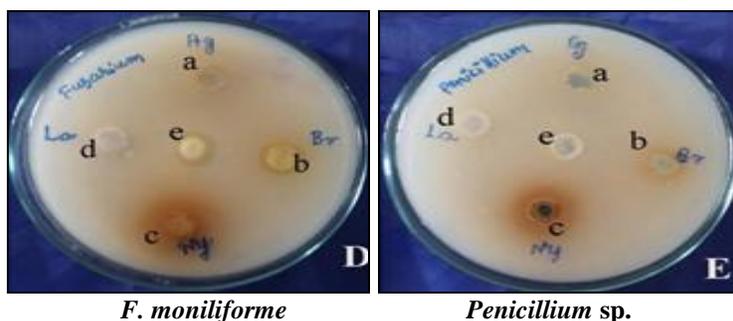
Aqueous extracts of *S. grandiflora* exhibited considerable zone of inhibition against *Penicillium* sp. ( $23 \pm 0.33$ ), followed by, *A. niger* ( $18 \pm 0.33$ ), *C. cladosporioides* ( $16 \pm 0.33$ ) *F. moniliforme* ( $15 \pm 0.33$ ) and *A. flavus* ( $15 \pm 0.66$ ). *E. oxypetalum* exhibited maximum zone of inhibition against *F. moniliforme* ( $15 \pm 0.00$ ), *A. flavus* ( $14 \pm 0.50$ ) *Penicillium* sp. ( $14 \pm 0.50$ ), *A. niger* ( $11 \pm 0.50$ ) and *C. cladosporioides* ( $11 \pm 0.50$ ). *N. arbor-tristis*

showed maximum zone of inhibition against *A. niger* ( $18 \pm 0.67$ ) followed, *F. moniliforme* ( $18 \pm 0.35$ ), *C. cladosporioides* ( $18 \pm 0.22$ ) and *A. flavus* ( $17 \pm 0.75$ ) and *Penicillium* sp. ( $15 \pm 0.60$ ). *V. zizanioides* exhibited maximum zone of inhibition against *Penicillium* sp. ( $12 \pm 0.33$ ), *C. cladosprioides* ( $11 \pm 0.33$ ), *F. moniliforme* ( $11 \pm 0.33$ ) and *A. flavus* ( $10 \pm 0.33$ ) and *A. niger* ( $10 \pm 0.33$ ) **Fig. 6 and 7.**



**FIG. 6: ANTIFUNGAL ACTIVITY OF AQUEOUS LEAVES EXTRACTS**





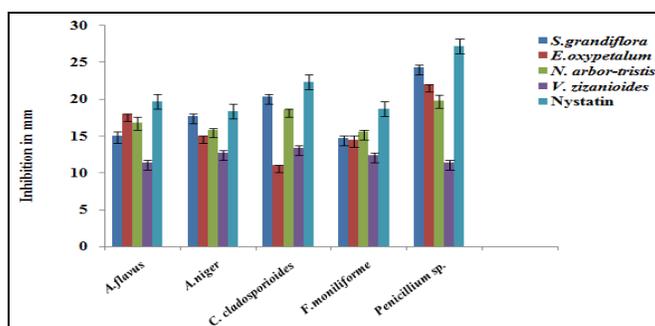
**FIG. 7: ANTIFUNGAL ACTIVITY OF AQUEOUS LEAVES EXTRACTS**

a) *S. grandiflora*, b) *E. oxypetalum*, c) *N. arbor-tristis*, d) *V. zizanioides*, e) Negative control (Distilled water)

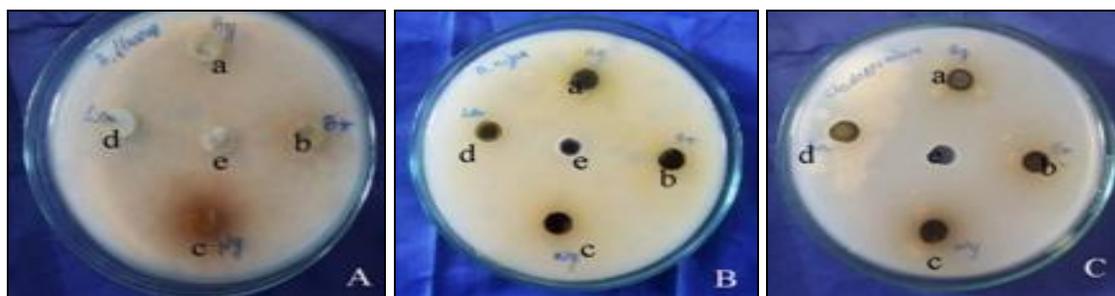
**Antifungal Activity of Ethanol Leaves Extracts:**

Ethanol extracts of *S. grandiflora* exhibited maximum zone of inhibition against *Penicillium sp.* ( $24 \pm 0.33$ ), followed by *C. cladosporioides* ( $20 \pm 0.33$ ), *A. niger* ( $17 \pm 0.33$ mm), *A. flavus* ( $15 \pm 0.57$ ) and *F. moniliforme*, ( $14 \pm 0.33$ ). *E. oxypetalum* exhibited highest zone of inhibition against *Penicillium sp.* ( $22 \pm 0.00$ ) followed by *A. flavus* ( $18 \pm 0.00$ ), *A. niger* ( $15 \pm 0.00$ ), *F. moniliformae* ( $14 \pm 0.50$ ) and *C. cladosporioides* ( $11 \pm 0.00$ ). *N.*

*arbor-tristis* exhibited maximum zone of inhibition against *Penicillium sp.* ( $19 \pm 0.75$ ) followed by *C. cladosporioides* ( $18 \pm 0.17$ ), *A. flavus* ( $16 \pm 0.85$ ), *F. moniliforme*, ( $15 \pm 0.28$ ) and *A. niger* ( $15 \pm 0.25$ ). *V. zizanioides* exhibited maximum zone of inhibition against *C. cladosporioides* ( $13 \pm 0.33$ ) followed by *A. niger* ( $12 \pm 0.33$ ), *F. moniliforme*, ( $12 \pm 0.33$ ), *A. flavus* ( $11 \pm 0.33$ ) and *Penicillium sp.* ( $11 \pm 0.33$ ) **Fig. 8 and 9.**



**FIG. 8: ANTIFUNGAL ACTIVITY OF ETHANOLIC LEAVES EXTRACTS**



*A. flavus*

*A. niger*

*C. cladosporioides*



*F. moniliforme*

*Penicillium sp.*

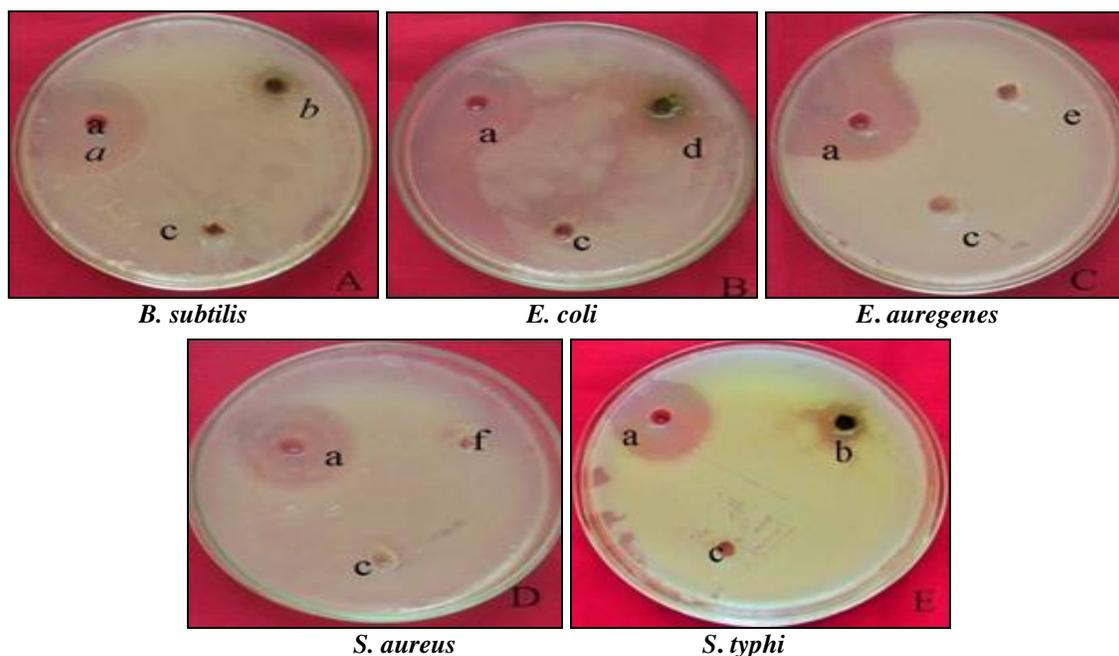
**FIG. 9: ANTIFUNGAL ACTIVITY OF ETHANOLIC LEAVES EXTRACTS**

a) *S. grandiflora*, b) *E. oxypetalum*, c) *N. arbor-tristis*, d) *V. zizanioides*, e) Negative control (Ethanol)

**Antibacterial and Antifungal Sensitivity Test:**

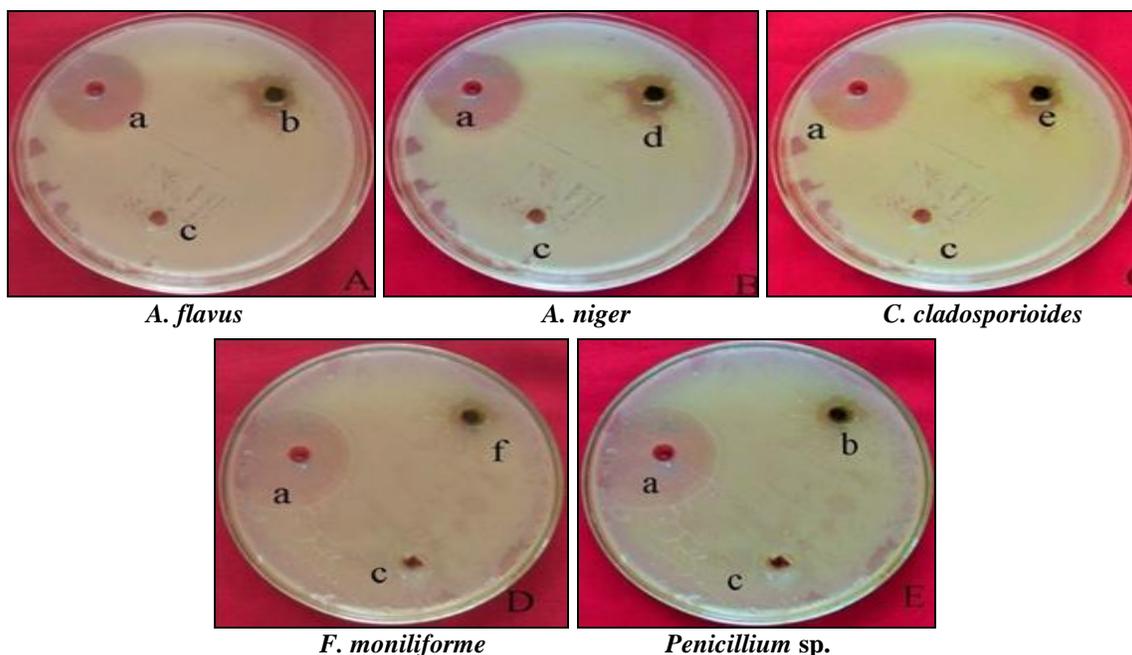
The Antibiotic and antifungal sensitivity test using standard antibiotic streptomycin and Nystatin

showed higher inhibition effect against all test bacterial and fungal strains **Fig. 10** and **11**.



**FIG. 10: ANIBIOTIC SENSITIVITY TEST**

a) Streptomycin (positive control) b) *S. grandiflora*, c) Ethanol (Negative control), d) *E. oxypetalum* e) *N. arbor-tristis* f) *V. zizanioides*



**FIG. 11: ANIFUNGAL SENSITIVITY TEST**

a) *Nystatin* (positive control) b) *S. grandiflora* d) *E. oxypetalum*, e) *N. arbor-tristis*, f) *V. zizanioides*, c) Negative control (Ethanol)

**DISCUSSION:** Medicinal plants offer alternative therapies with spectacular opportunities<sup>14, 15</sup>. Plant derived phytomedicines are cheaper source for treatment and significant accuracy than chemotherapeutic agents<sup>16</sup>. Potential antimicrobial activity is influenced by chloroform and methanol

extracts<sup>17</sup>. Antibiotic resistance has increased largely which has become big problem in therapy which can be overcome through antibiotic resistance inhibition from plants<sup>18, 19</sup>. Plants produce compounds to be safe from various pathogens which are potent against drug resistant

pathogens<sup>20</sup>. Researchers are now focusing on phytomedicines and biologically active compounds derived from plants used for herbal medicines<sup>21, 22</sup>. Phytochemical screening reveals the presence of valuable secondary metabolites, so there is no doubt that this traditional medicinal plant will give clues for the preparation of new drugs. Past some years, there has been a lot of interest in the investigation of natural materials as sources of new antimicrobial agents.

Many reports show the effectiveness of traditional herbs against microorganisms as a result, plants are one of the bases of modern medicines to attain new principles. Synthetic antibiotics are linked with widespread of undesirable effect<sup>23</sup> which reflects the toxicological or pharmacological properties of antibiotic<sup>24</sup> that desires plant derived molecules for microbial infections.

Different levels of inhibition against all the test microorganisms were revealed. It was understood from the present study that the aqueous and ethanolic extracts of *S. grandiflora*, *E. oxypetalum*, *N. arbor-tristis*, *V. zizanioides* contain many phytochemicals. Most of the plants having secondary metabolites act as defenders for external invaders. Each extracts were subjected to explore for their antifungal and antibacterial activities against some pathogenic bacteria and fungi strains.

The various kinds of plants have antibacterial<sup>25</sup> and antifungal activity containing effective phytochemicals. Acetone extracts of *Areca catechu* showed pronounced inhibition against *B. subtilis*, *E. aerogenes*, *K. pneumonia*, *S. epidermidis* and *E. faecalis* and fungi *Candida glabrata*, *C. albicans*, *C. tropicali*, *Aspergillus fumigatus* and *A. niger*<sup>2</sup>.

The outcome of antibacterial activity revealed that aqueous extracts *S. grandiflora* lead to maximum inhibition of *B. subtilis* ( $23 \pm 0.66$ ) and ethanolic leaf extracts of *N. arbor-tristis* which showed maximum inhibitory effect against *S. stypi* ( $20 \pm 0.35$ ) which correlated with the results obtained<sup>26</sup> in inhibiting *B. subtilis* with aqueous extracts of *Strychnos nuxvomica*.

Ethanolic extracts of *N. arbor-tristis* showed considerable inhibiting effect against *S. typhi* which when compared with methanolic leaf extracts showed strongest inhibitory affect against *E. coli*

and *B. subtilis*<sup>27</sup>. Further *N. arbor-tristis* ethanolic extracts were effective against *S. typhi* when compared to methanolic extracts of *Senna alata*<sup>28</sup>, and also in *Nicolaia speciosa*<sup>29</sup> have reported the aqueous extracts of *A. paniculata*. Methanol leaf extract had maximum activity against *S. aureus*<sup>30</sup>. Strongest inhibitory activity of *Nicolaia speciosa* fruit with ethanolic extracts was observed against *E. coli*, *P. Aeruginosa* and *B. cereus*. *Colophospermum mopane*, *S. persica* and *D. cinerea* exhibited antibacterial activity, with methanol extracts performing better than aqueous extracts, justifying use as Ethnoveterinary medicine<sup>31</sup>. Ethanolic extracts of *Punica granatum*, *Syzygium aromaticum*, *Zingiber officinales* and *Thymus vulgaris* were potentially effective against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*<sup>32</sup>. Ethanol extracts gives considerable antibacterial activity compared to ethyl acetate<sup>33</sup> where phenol compounds are more polar than flavonoids,<sup>34</sup>. Strong antioxidant and antibacterial potency with ethanol and methanol extracts is also revealed<sup>14</sup>.

Aqueous extracts of *S. grandiflora* exhibited considerable zone of inhibition against *Penicillium* sp. ( $23 \pm 0.33$ ). Ethanol extracts of *S. grandiflora* exhibited maximum zone of inhibition against *Penicillium* sp. ( $24 \pm 0.33$ ). All the plant extracts revealed varied level of inhibition against the pathogenic micro organisms. The findings of this study suggest that the aqueous and ethanolic leaf extracts of leaves of *S. grandiflora* is found to be most effective and potent source which can be used as alternative antimicrobial compounds. Our antifungal analyses are in agreement with the reports<sup>35</sup> against 15 *Candida* spp with ethanolic extracts of *Allium sativum*, *Azadirachta indica*, *Cordia dichotoma* and *Ocimum sanctum* which resulted in significant inhibition zones. Antifungal activities of some herb and spices have also been reported against *Candida*<sup>36</sup>. Reports of<sup>37</sup> also show antimicrobial activity of the Water extract of *Gymnema sylvestre* fruit and roots were studied. Acetone extracts in inhibition of *A. niger* supportive to our study as examined<sup>38</sup>. Antimicrobial studies showed that the extract has considerable activities against *B. subtilis*, *S. aureus*, *E. coli*, *Klebsiella aerogenes* and *A. niger*.

Our results are in concurrence with <sup>39</sup> and in present agreement using ethanol and water extracts against against *B. subtilis*, *S. aureus*, *E. coli*, *K. aerogenes*, *A. niger* and *P. chrysogenum*. Likewise Methanol and dichloromethane extracts of *Annona squamosa* have showed significant antimicrobial activity against *E. coli*, *K. Pneumonia*, *S. flexneri* and *S. typhi* <sup>40</sup>.

**CONCLUSION:** The results obtained from our pilot study of ongoing research provide and support the use of these plants in traditional medicine. The potential for developing antimicrobials for plants appears rewarding as it leads to development of new drugs which is needed today. Further screening is important to find out the potent compounds within these plants with their entire spectrum of efficacy. However, the current study of *in vitro* antimicrobial activity of some plants forms primary platform for further phytochemical and pharmacological studies.

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**CONFLICT OF INTEREST:** Declared None

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