INTRODUCTION
Herbal medicine – It is also called botanical medicine or phytomedicine-refers to using plants seeds, flowers, roots for medicinal purpose. Herbalism has a long tradition of use of outside of conventional medicine. It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing disease. The medicinal action of plants is unique to a particular plant species, consistent with the concept that the combination of secondary metabolites in a particular plant is taxonomically distinct three medicinal plants description and uses respectively. *Nyctanthes arbor-tristis* is commonly known as Night-flowering Jasmine, Coral Jasmine and Parijat. It is used for its antibacterial, anthelmintic, anti-inflammatory, hepatoprotective, immunopotential, antipyretic, antioxidant and anti-fungal activity. *Nerium oleander* is an evergreen shrub or small tree in the dogbane family Apocynaceae, toxic in all its parts. Used traditionally in treating dermatitis, abscesses, eczema, psoriasis, sores, warts, corns, ringworm, scabies, herpes, skin cancer, asthma, dysmenorrheal, epilepsy, malaria, abortifacients, emetics, heart tonics, and tumor. *Catharanthus roseus* commonly called Madagascar periwinkle is an evergreen shrub or herbaceous plant which exhibits the anti cancer activity due to the presence of vincristine and vinblastine. Here in the present study the above three plants were taken and the antibacterial activity of these plants extracts were studied individually as well as in combination i.e., *Nerium and Nyctanthes combined extract* and *Catharanthus and Nyctanthes combined extract* also was evaluated. The phytochemical constituents were studied by qualitative analysis.

EXPERIMENTAL SECTION
Plant Materials
The leaves of plants *Nerium, Nyctanthes* and *Catharanthus* species were Authenticated by Prof. V. Satyanarayana, Department of Plant Breeding, Bapatla Agricultural College, Bapatla, Andhra Pradesh, India. They were collected from different areas of Guntur, Prakasham districts of Andhra Pradesh, India.

Solvent Extraction
The leaves of *Nerium oleander, Catharanthus roseus* and *Nyctanthes arbor-tristis* were
collected, washed, dried and powdered separately. 50g of dried powder of the leaves was weighed and transferred into a conical flask and it was macerated with sufficient amount of ethanol for about a week days. The whole mixture was filtered and filtrate was collected, concentrated in a china dish on a hot plate till the residue was obtained. The extract was collected, labelled and stored for further experimental use.

Microorganisms
The test organisms used were *E.coli* (ATCC 25922) a Gram –ve strain and *B.subtilis* (ATCC 21332) a Gram +ve strain which were obtained from PG and Research Department of Biotechnology Bapatla College of Pharmacy Bapatla Andhra Pradesh India. The strains were sub-cultured on nutrient agar slants and were incubated for 24 hrs.

Antibacterial activity

**Agar well diffusion method**
Required glass ware was washed and dried in a hot air oven. The sterilized agar medium was transferred into the Petri dishes, was allowed to solidify at room temperature. The selected test organism was spread over the solidified agar with the help of a swab stick. Sterile borer was used to make wells of 8mm diameter. The dilutions of ethanolic extracts of *Nyctanthes arbortristis*, *Nerium oleander* and *Catharanthus roseus* and solutions of combined ethanolic extracts of *Nyctanthes arbortristis* with *Nerium oleander* and *Catharanthus roseus* respectively were poured in the wells with the help of a sterile syringe needle. In each Petri plate a well was prepared for standard i.e., ciprofloxacin 10µg/ml solution. The Petri plates were placed in a refrigerator for 5min to allow diffusion. Later the Petri plates were incubated in inverted position at 37°C for 24 hours in the incubator. After 24hours the zone of inhibition was observed and diameter in mm was measured and recorded.

**Qualitative analysis**
The extracts and crude dried powders of *Nyctanthes arbortristis*, *Nerium oleander* and *Catharanthus roseus* were subjected to qualitative analysis for presence of chemical constituens of *Nyctanthes arbortristis Nerium oleander Catharanthus roseus*.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>COMPONENT</th>
<th>DOSE</th>
<th>Zone of Inhibition (mm)</th>
<th>E.COLI</th>
<th>B.SUBTILIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>STANDARD CIPROFLOXACIN</td>
<td>10 µg/ml</td>
<td>20mm</td>
<td>22mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethanolic extract of <em>Nyctanthes arbortristis</em></td>
<td>500µg/ml</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>750µg/ml</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000µg/ml</td>
<td>2mm</td>
<td>3mm</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Ethanolic extract of <em>Nerium oleander</em></td>
<td>500 µg/ml</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>750µg/ml</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td>1000µg/ml</td>
<td>4mm</td>
<td>5mm</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Ethanolic extract of <em>Catharanthus roseus</em></td>
<td>500 µg/ml</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>750µg/ml</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td>1000µg/ml</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>5.</td>
<td>Combined ethanolic extracts of <em>Nerium oleander and Nyctanthes arbortristis</em></td>
<td>1000µg/ml</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1500µg/ml</td>
<td>8mm</td>
<td>10mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2000µg/ml</td>
<td>12mm</td>
<td>15mm</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Combined ethanolic extracts of <em>Catharanthus roseus and Nyctanthes arbortristis</em></td>
<td>1000µg/ml</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1500µg/ml</td>
<td>3mm</td>
<td>3mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2000µg/ml</td>
<td>2mm</td>
<td>2mm</td>
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</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**
The study of the chemical constituents and the active principles of the medicinal plants have acquired a lot of importance all over the world. The present study includes the antibacterial activity of extracts of leaves of *Nyctanthes arbortristis* in combination with the leaf extracts of *Nerium oleander* and *Catharanthus roseus* separately were performed. Earlier studies on *Nyctanthes arbortristis* indicated that the ethylacetate and chloroform extracts showed significant activity on both Gram +ve and Gram –ve strains. But in present study ethanolic leaf extract showed that the activity
on bacterial strains was significant. But comparably the activity on *B. subtilis* was more than that of *E. coli*.

As the activity obtained for the leaf extract was significant the combined leaf extract of *Nyctanthes arbor-tristis* and *Nerium oleander* were used which showed a synergistic effect i.e., *Nerium oleander* increased the antibacterial activity of the *Nyctanthes arbor-tristis*. While this combination showed synergistic activity the other combination i.e., *Nyctanthes arbor-tristis* and *Catharanthus roseus* showed antagonistic activity. The leaf extract of *Catharanthus roseus* has no antibacterial activity and when used with leaf extract of *Nyctanthes arbor-tristis* it reduced the action of *Nyctanthes arbor-tristis*.

**CONCLUSION**

The Screening of Phytochemical constituents of the plants *Nyctanthes arbor-tristis Nerium oleander Catharanthus roseus* analysis indicated the presence of Carbohydrates, Glicosides, Alkaloids. Out of the Plants the combined ethanolic extract of *Nyctanthes arbor-tristis and Nerium oleander* exhibited significant antibacterial activity of 2000µg/ml Concentration.

**AKNOWLEDGEMENTS**

The authors are thankful to Management Principal of Post Graduate Research Centre Division, Bapatla College of Pharmacy Bapatla Andhra Pradesh India in permitting and providing necessary facilities for carrying out to do the project work.

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