INTRODUCTION
There has been an increasing interest worldwide on therapeutics values of medicinal plants. The traditional medicine refers to a board range of ancient, natural Health care practices including folk/tribal practices as well as Ayurveda, Siddha and Unani. These medicinal practices originated from time immemorial and developed gradually, to a Large extent, by relying or based on practical experiences without significant references to Modern scientific principles [1]. These practices incorporated ancient beliefs and were passed on from one generation to another by oral tradition and/or guarded literature. Although herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited and/or improperly used. Therefore these plants drugs deserve detailed studies in the light of modern science. It is estimated that about 7,500 plants are used in local health traditions in, mostly, rural and tribal villages of India [2]. Out of these, the real medicinal value of over 4,000 plants is either little known or hitherto unknown to the mainstream population. The classical systems of medicines such as Ayurveda, Siddha, Amchi, Unani, And Tibetan use about 1,200 plants. A detailed investigation and documentation of plants used in local health traditions and pharmacological evalution of these plants and their taxanomical relatives can lead to the development of invaluable plant drugs for many dreaded diseases. Random screening of plants has not proved economically effective [3]. Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illness or maintain well-being. Countries in Africa, Asia, Latin America use traditional medicine (TM) to help meet some of their primary health care needs. In Africa, upto 80% of the population uses traditional medicine for primary health care. In industrialized countries, adaptations of traditional medicine are termed Complementary or Alternative [3].

MATERIALS AND METHODS
Collection and identification of plant materials
The plant material Phyllanthus amarus were collected from Tirumala hills, Chittoor district. The leaves were identified, confirmed and authenticated by comparing with an authentic specimen by a Botanist Madhav chetty. The plant material were dried under shade, segregated, pulivered by a

To whom correspondence should be addressed:
K. Thamizhvanan
Email: Ktvanan2006@yahoo.co.in

Abstract
Medicinal plants play a key role in the human health. Phyllanthus amarus is an economical plant and possess Anti-microbial activity. About 80% of the world Population relies on the use of traditional medicine which is predominately based on plant materials. The traditional medicine refers to a board range of ancient, natural Health care practices including folk/tribal practices as well as Ayurveda, Siddha and Unani. The results of modified agar well diffusion method showed that prepared CWEPA, HWEPA, EEPA, having Inhibitory effect on the microorganisms which are responsible for the intestinal infections, skin infections and urinary tract infection.

Keywords: Antimicrobial activity, Inhibitory effect, Intestinal infections, Skin infections, Urinary tract infections.
mechanical grinder and passed through a 40 mesh sieve [3].

**Phytochemical studies of Phyllanthus amarus**

**Extraction of the Plant Material**
Twenty grammes (20g) of the pulverized leaves of each plant was decocted with 100ml of cold water left overnight, hot water (100oC) for 5 minutes, and ethanol. Prior to decoction, the leaves were soaked in the extracting solvent for 3 days. The mixture was then filtered and the filtrate evaporated to semi solid mass using a rotary evaporator (Brichi, Germany) and subsequently drying in a beaker on water bath to give a dark resinous mass [4]. The plant extracts from the various solvents were reconstituted using 10%v/v ethanol as solubilising agent at concentration of (10 and 100)mg/ml for antimicrobial activity evaluation.

**Phytochemical Analysis**

**Test for Alkaloids**

**Dragondroff’s test**
To 1 ml of the extract, 1 ml of Dragondroff’s reagent was added; formation of orange red precipitate indicated the presence of alkaloids.

**Wagner’s reagent**
To 1ml of the extract, 2ml of wagner’s reagent was added, the formation of a reddish brown precipitate indicated the presence of alkaloids.

**Mayer’s test**
To 1ml of the extract, 3 ml of Mayer’s reagent was added; the formation of full white precipitate confirmed the presence of alkaloids.

**Test for Flavonoids**

**Shinoda test**
To 1ml of the extract, magnesium turnings were added followed by 1-2 drops of concentrated hydrochloric acid. Formation of red colour showed the presence of flavonoids.

**Test for phlobatannins**
3ml of aqueous extract was added to 2ml of 1%Hcl and the extract was boiled. Deposition of red precipitate was taken as an evidence for the presence of phlobatannins.

**Test for Steroids**

**Salkowski Tests**
Chloroform solution of the extract when shaken with concentrated sulphuric acid and on standing yields red colour.

**Lieberman Burchardt tests**
Chloroform solution of the extract with few drops of Aceticanhydride and one ml of concentrated sulphuric acid from the sides gives reddish ring at the junction of 2 layers.

**Test for Terpenoids**
3ml of organic extract was dissolved in 2ml of chloroform and evaporated to dryness. 2ml of concentrated sulphuric acid was then added and heated for about 2min. Development of greyish colour indicates the presence of Terpenoids.

**Test for Cardiac Glycosides**

**Legal test**
The extract was dissolved in pyridine and Sodium nitroprusside solution was added to make it alkaline. The formation of pink red to red colour showed the presence of glycosides.

**Baljet test**
To 1ml of the test extract 1 ml sodium picrate solution was added and the yellow to orange colour revealed the presence of Glycosides.

**Keller Killiani test**
The extract was dissolved in acetic acid containing traces of ferric chloride and it was then transferred to a test tube containing sulphuric acid. At the junction, formation of a reddish brown colour, which gradually became blue, confirmed the presence of Glycosides.

**Borntrager’s test**
A few ml of dilute HCL was added to 1ml of the extract solution. It was then boiled, filtered and the filtrate was extracted with chloroform. The chloroform layer was then treated with 1ml of ammonia. The formation of red colour showed the presence of Anthraquinone glycosides.

**Test for Saponins**
About 1 ml of extract was diluted separately with distilled water to 20 ml, and shaken in a graduated cylinder for 15 minutes. A1% 1cm layer of foam indicated the presence of Saponins [5-7].
IN VITRO ANTI MICROBIAL ACTIVITY

Microbial strains tested

In this study, Micro organisms were selected to cover Gram-positive bacteria and Gram-negative bacteria namely, *Escherichia coli*, *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aureginosa*, *Malassezia furfur*. The tested strains were obtained from Microbiology Laboratory, SVCP, A. Rangampet and Tirupati. The Microorganisms were allowed to grow over night at 37°C in 2% nutrient agar at pH 7. The sensitivity of Microorganisms to the reference [9]. Antibiotic was checked. For this purpose Ciprofloxine was used as reference Antibiotic.

Preparation of inocula

The inocula were prepared by inoculating a loop of each bacterial strain from 24 hours of old culture into a sterile nutrient broth aseptically in the laminar air flow unit. The culture growth was allowed for 24 hours in incubator at 37°C.

Determination of antimicrobial activity

The screening of Antimicrobial efficacy of the various extract of *Phyllanthus amarus* was performed on various Micro organisms by using Agar well diffusion method. The agar plates were prepared by pouring 20 mL of sterile molten Mueller-Hinton (MH) agar (Himedia Lab Pvt. Ltd, Mumbai, India). The bacterial cultures were prepared by adding the seed culture in the autoclaved agar medium followed by pouring into Petri plates. The solid agar medium was gently punctured with the aid of 8mm sterile cork borer to make a proper well. 50µl of Phyllanthus amarus extract (50mg/ml) was added in the pre labelled wells together with reference antibiotic Ciprofloxacin. Here various extract of Phyllanthus amarus served as test and Ciprofloxacin served as standard. The reference Antibiotic was used in the concentration range of 100µg/ml. It was taken care that the sample should be placed at the level of cavity [8]. The diffusion of extract was allowed for 1hr at room temperature on a sterile bench. Then the Petri plates were incubated for 48 hrs at 37°C. After 48 hrs the plates were observed for the presence of inhibition of bacterial growth and that was indicated by clear zone of inhibition of bacterial growth around the wells. The size of Inhibitory zone was measured in mille meters (mm). Minimum Inhibitory Concentration (MIC) was determined.

RESULTS AND DISCUSSION

The results of modified agar well diffusion method (Table 1) showed that prepared CWEPA, HWEPA, EEPA, having inhibitory effect on the microorganisms which are responsible for the intestinal infections, skin infections and urinary tract infection [8]. The Anti-microbial activity of the herbal extract has been comparable to that of market Antibiotic (Ciprofloxacin) [9]. The diameter of Zones of inhibitions was also given in the table 1-5.

<table>
<thead>
<tr>
<th>S. NO</th>
<th>TEST</th>
<th>CWEPA</th>
<th>HWEPA</th>
<th>EEPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Phlobatannins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Cardiac glycosides</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2. Anti-microbial activity of Cold water extract of *Phyllanthus amarus*

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>CWEPA 29±1.0</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>CWEPA 31±1.0</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>CWEPA 27±2.0</td>
</tr>
<tr>
<td><em>Pseudomonas aureginosa</em></td>
<td>CWEPA 30±1.0</td>
</tr>
<tr>
<td><em>Malassezia furfur</em></td>
<td>CWEPA 30±2.0</td>
</tr>
</tbody>
</table>
Table 3. Antimicrobial activity of Hot water extract of Phyllanthus amarus

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>26 ± 1.0</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>24 ± 1.0</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>25 ± 2.0</td>
</tr>
<tr>
<td><em>Pseudomonas aureginosa</em></td>
<td>25 ± 1.0</td>
</tr>
<tr>
<td><em>Malassezia furfur</em></td>
<td>22 ± 2.0</td>
</tr>
</tbody>
</table>
Table 4. Anti-microbial activity of Ethanolic extract of Phyllanthus amarus

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>23±2.0</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>23±1.0</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>23±1.0</td>
</tr>
<tr>
<td><em>Pseudomonas aureginosa</em></td>
<td>25±2.0</td>
</tr>
<tr>
<td><em>Malassezia furfur</em></td>
<td>25±1.0</td>
</tr>
</tbody>
</table>
SUMMARY AND CONCLUSION

In the present study an attempt has been made to explore pharmacognostical and phytochemical parameters besides evaluating anti-microbial activity against microorganisms causing skin infection, intestinal infections and urinary tract infection [10]. The identification of plant material taxonomically and pharmacognostically is important to provide standards and avoid adulteration of drugs. The plants were identified and authenticated by Dr. Madhav chetty, Assistant professor, Head of Botany department, Sree Venkateswara University, tirupathi. The detailed botanical, Pharmacognostical studies with proper authentication of the plants helps in minimizing the adulteration and also for proper identification of the plant [11].

Preliminary phytochemical analysis of the extract showed the presence of these Alkaloids, Flavonoids, Phlobatannins, Steroids, Terpenoids, and Cardiac Glycosides, Anthraquinones and Saponins constituents may be responsible for the healing potential of Skin infections, Intestinal and Urinary tract infection.

Evaluation of anti-microbial activity of *Phyllanthus amarus* against microorganisms causing skin infections, Intestinal and urinary tract infections was done by using agar well diffusion method [12]. After 24 hrs we measured the zone of inhibition to confirm the antimicrobial activity of the prepared *Phyllanthus amarus*.
From the above results it can be concluded that the *Phyllanthus amarus* could effectively fight against microorganisms causing skin infections, Intestinal and Urinary tract infection [14].

**ACKNOWLEDGEMENT**

Nil.

**CONFLICT OF INTEREST**

No interest.

**REFERENCES**