PRELIMINARY PHYTOCHEMICAL ANALYSIS FOR
ACTIVE CONSTITUENTS OF Thespisia macrophylla Blume.
INTRODUCTION:

The inhabitants of the area utilize plant wealth for food, medicine and other purposes. Many of the food and medicinal plants contain a variety of chemical substances such as alkaloids, tannins, glycosides, saponins, carbohydrates, flavonoids, sterols, phenols etc. The phytochemical screening of the plants is a preliminary and important aspect for the scientific verification of folklore claim with regard to the utility of plants.

A vigorous search for drugs from medicinal plants during the last century has resulted in the discovery of many active principles from innumerable plant species. A good number of these have been found to be great use in the treatment of diseases and improvement of health. Many a compounds have been adopted by modern pharmacopoeia, after a thorough chemical and therapeutic investigation, yet a large number still needs detailed study.

The crude drugs have been in use in various system of medicine for many centuries, however pharmacological and clinical evaluation of these drugs has to be taken up systematically.

In this country, the population has always used traditional remedies to prevent or to cure diarrhoea but a few of these remedies have been established clinically or studied chemically, in order to identify the active principles (WHO, 1983).

The gastrointestinal and urinary tract infections have been some of the earliest medical problems. Management of diarrhoeal
diseases has been described in Ayurveda using herbal drugs (Sushruta - Samhita). Many of these herbs belong to the Malvaceae family (Deshpande, et al. 1970). In present study also (chapter-4) antibacterial properties were discerned with two test plants of Malvaceae viz. Sida spinosa and Thespesia macrophylla.

With a view to evaluate the active antibacterial phytochemical constituents of the most promising plant samples of present study, further phytochemical isolation and antibacterial evaluation was made.

As is evident from foregoing results (chapter-4), about 35 plant samples were found to show activity against the test bacteria. Among these Terminalia tomentosa (bark), Acacia leucophloea (bark) and Thespesia macrophylla (root) showed comparatively strong antibacterial effects. Further selection of plant sample was made from among these three species T. tomentosa and A. leucophloea are tree species and are sparse in their occurrence, therefore their material (bark) seems difficult to collect in sufficient quantities for their large scale use.

On the other hand Thespesia macrophylla is a middle sized shrub which can be easily managed to grow in large quantities, and any suitable quantity of its roots can be obtained for large scale use. Moreover, on the basis of ethnobotanical survey (chapter-2), T. macrophylla roots were found to be best remedy for bacterial ailments and its use is common among tribes, and villagers of Sagar area.

Interestingly, Thespesia macrophylla has not yet been described in the Flora of Sagar (Anonymous, 1968). Therefore, due to its great ethnobotanical importance and good potentialities for the production of
antibacterial substance (S), *T. macrophylla* was selected for further study. A short account of this plant is as follows:

**Thespesia macrophylla** Blume syn. *T. lampas* Dalz. et Gibs.

**Hindi name**: Janglibhendi; Bankapasi.

**Family**: Malvaceae

**Distribution**: Commonly found in India up to an altitude of c. 1,200 m.; also grown as ornamental shrub. In Sagar district it is rarely found however, in Bahrol forest, it grows in collectable amounts.

**Habit**: A shrub of 3-4 ft. height, springly branched.

**Roots**: Tap roots, dark brown with aromatic smell.

**Leaves**: 3 to 6 inch long, leaf entire or 3 lobed, cordate or truncate at the base, lobes acuminate, finely reticulate veined with black glandular dots on the lower surface, hairy, subglabrous on the upper surface, petiole $1\frac{1}{2} - 3\frac{1}{2}$ in. long, peduncles 3-4 in. long, alternate.

**Stem**: Hairy, rough walled, dark brown to green, long, with node and internode.

**Inflorescence**: Cymose.

**Flower**: Axillary, yellow in colour, at the base (inside) of the petals, colour is dark purple, complete, pentamerous, actinomorphic, hypogynous, pedicillate, pedicels $\frac{1}{2}-\frac{1}{2}$ in. long, involucral bracts 5.

**Bracteoles** 4-8.

**Epicalyx**: Six, free, hairy, green.

**Calyx**: Five sepals, gamosepalous, valvate aestivation, green, hairy, capular, truncate, persistent, teeth subulate.

**Corolla**: 5 petals, polypetalous, twisted aestivation, yellow colour with crimson centre, 3 in. in diameter.

**Androecium**: Stamen $\infty$, monoadelphous, short staminal tube with spreading filament, pollengrains yellow, stamen basifixed.
Gynoecium: Ovary superior, tetra-carpillar y, syncarpous, capit ate stigma, axile placentation.

Fruits: Capsule 1-1 1/2 in. long, ovoid, pointed, 4-5 valved, pilose, 3-4 at the base of stem, one seed in each locule.

Seeds: Black, glabrous, club shaped.

Flowering season: August to September.

Fruiting season: September to October.

Ethnobotanical importance:

The roots and fruits are reported to be employed for gonorrhoea and syphilis. The flowers contain a dye. The floral parts are marketed as a drug. Flowers contain quercetin and some protocatechuic acids. Seeds yield 16.92 percent oil. Gossypol is reported to be present in different parts of the plant as follows (dry basis): seeds, 1.74; flowerbuds, 1.95; leaves, 0.98; roots, 2.75; and stem, 0.16% (Nair, 1961; Perkin & Everest, 1918). Roots of I. macrophylla have also been reported for typhoid fever (Manandhar, 1990), but there are no other records in the literature for the chemistry of its roots. Fruits and roots of I. macrophyll are also used in dysentery by villagers and tribal people of Sagar district.
MATERIALS AND METHODS

Roots of *T. macrophylla* were subjected for their preliminary phytochemical analysis and were then further screened for their broad spectrum antibacterial activity on the following lines:

(1) **Continuous liquid extraction procedure:**

The method of Brain and Turner, (1975); was employed with slight modifications.

(a) **Root powder:**

100 gm. of the air dried root material was finely powdered and packed in the thimble of the soxhlet assembly. It was then successively extracted with organic solvents.

(b) **Preparation of extracts:**

Root powder was defatted with the help of petroleum ether (B.P. 60-80°C) for 48-52 hours. After defatination this was filtered and remaining residue was then extracted with 500 ml, 95% ethanol and after extraction of ethanol soluble constituents, the residue was removed. The filtrate was then solidified. Evaporation of ethanol was done under reduced pressure and the percent yield of ethanol extract was determined. These ethanolic extract samples were kept in refrigerator until used.

For the separation of plant constituents in their respective solvents the ethanolic extract (about dry) was then successively fractionated with various solvents according to the procedure as given in figure No. 7. The colours of different fractions and percent yield of
each fraction were also determined (Table IX). Each successively fractionated sample was concentrated to about dryness and stored in screw cap glass tubes under refrigeration until used. Such semisolid fractions were used (after dilution) for antibacterial efficacy tests.

![Diagram of procedure for successive extraction of roots of *Thespesia maophylla*.](image)

Fig. 7 - Procedure for successive extraction of roots of *Thespesia maophylla*. 
Table IX - Percent yield (in grams) and colour of each extract/fraction

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Extract/Fraction</th>
<th>Colour</th>
<th>Yield (gm) (Per 100 gm. of roots)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Petroleum ether</td>
<td>Yellowish brown</td>
<td>3.20</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>Brownish red</td>
<td>9.20</td>
</tr>
<tr>
<td>3.</td>
<td>Benzene</td>
<td>Red blood</td>
<td>1.90</td>
</tr>
<tr>
<td>4.</td>
<td>Chloroform</td>
<td>Yellowish red</td>
<td>1.25</td>
</tr>
<tr>
<td>5.</td>
<td>Ethyl acetate</td>
<td>Orange red</td>
<td>1.0</td>
</tr>
<tr>
<td>6.</td>
<td>Acetone</td>
<td>Orange red</td>
<td>1.72</td>
</tr>
<tr>
<td>7.</td>
<td>Methanol</td>
<td>Orange red</td>
<td>1.50</td>
</tr>
</tbody>
</table>

2. Extraction of phytochemical constituents:

The fractionation of various chemical constituent groups from *T. macrophylla* roots was carried out according to the methodology (scheme) adopted by Brain and Turner (1975) with slight modifications as shown in figures 8 to 14 and table X.

```
200 ml.
20 gm plant material + petroleum ether

Defated material + 200 ml. ethanol
 Refluxed for 2 hours then filtered

Filtrate

Added 30 ml. distilled water and concentrated up to 5 ml.

TANNINS EXTRACTIVE

Plant material (Rejected)
```

Fig. 8 - Procedure followed for the extraction of Tannins.
20 gm. Plant Material + 200 ml Petroleum ether
Refluxed for 2 hours then filtered

Filtrate
Plant material
(Rejected)

Residue + 30 ml,
10% (Alc. KOH)
Refluxed for an hour, cooled than
Added 100 ml
Distilled water
Soak in a separating funnel with ether
(Two layers separated.)

Unsaponified matter
Saponified matter
(Rejected)

Extracted twice with 50 ml
solvent ether then combined both
ethereal extracts and concentrated
upto 5 ml.

STEROLS EXTRACTIVE

Fig. 9 - Procedure followed for the extraction of Sterols
Fig. 10 - Procedure followed for the extraction of Glycosides
20 gm. plant material + 200 ml. petroleum ether

Refluxed for 24 hours then filtered

Defated material
+ 200 ml. rectified spirit

Refluxed for 2-4 hours. Filtered

Filtrate

Plant material
(Rejected)

Concentrated upto 10 ml.

10 ml. Extract
+ 25 ml. Ethanol
+ 5 ml. Dil H₂SO₄

Refluxed for 2-4 hours. Then cooled.
Poured in a beaker containing 60 ml. ice cooled distilled water

Filtered

Residue

Filtrate

Concentrated upto 5 ml.

AGLYCONES EXTRACTIVE

SUGARS EXTRACTIVE

Fig. - 11 Procedure followed for the extraction of Aglycones and Free Sugars.
20 gm. plant material + 200 ml. Hydrochloric acids 5%  
Kept for 24 hours then filtered

Filtrate

Plant material

Basified with ammonia solution till smell of NH₃

Precipitate

Residue

Dissolved in chloroform.
Concentrated upto 5 ml.

ALKALOIDS
EXTRACTIVE

Fig. - 12 Procedure followed for the extraction of Alkaloids.
20 gm. plant material + 200 ml Hydrochloric acid 5%

Kept for 36 hours, filtered

Plant material

Filtrate
Rejected

Plant material

washed with dilute ammonia

Plant material
+ 200 ml methanol

Refluxed for 2 hours filtered

Filtrate

Residue
Rejected

Concentrated

Methanolic extract

100 ml. solvent ether

Filtered

Filtrate
Rejected

Residue

Dissolved in 100 ml. methanol

Concentrated up to 5 ml.

SAPONINS EXTRACTIVE

Fig. 13 - Procedure followed for the extraction of Saponins.
Fig. 14 - Procedure followed for the extraction of Flavonoids
Various fractions were also tested (with the help of reagents) for the presence of different chemical constituents like sterols, glycosides, sugars, saponins, alkaloids, flavonoids and tannins by using standard chemical methods commonly employed for the group tests as suggested by Finar (1959), Harborne (1973), and Farnsworth (1966 a); Rosenthaler, (1930); Shellard (1957); Agrawal (1981); Vogel (1958); Robinson (1964) and Saxena (1983).

The constituent groups (extractive) as separated from the plant samples by following the above mentioned schemes are given in Table X.

Table X List of constituent groups extracted from the root samples of T. macrophylla.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Constituent group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Tannins</td>
</tr>
<tr>
<td>2.</td>
<td>Sterols</td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
</tr>
<tr>
<td>4.</td>
<td>Aglycones</td>
</tr>
<tr>
<td>5.</td>
<td>Sugars</td>
</tr>
<tr>
<td>6.</td>
<td>Alkaloids</td>
</tr>
<tr>
<td>7.</td>
<td>Saponins</td>
</tr>
<tr>
<td>8.</td>
<td>Flavones</td>
</tr>
</tbody>
</table>

The presence of the chemical constituent groups extracted was further verified by suitable standard chemical tests as mentioned in Table XI.
Table XI Phytochemical constituents and their detecting reagents employed in the present study.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Plant constituents</th>
<th>Test applied</th>
</tr>
</thead>
</table>
| 1.    | Tannin            | (1) Lead acetate test  
(2) Alc. ferric chloride test  
(3) Gelatin solution test  
(4) Potassium chromate solution Test |
| 2.    | Sterol            | (1) Salkowski reaction  
(2) Liebermann - Burchard reaction |
| 3.    | Glycoside         | (1) Benedict's reagent  
(2) Keller killani test  
(3) Legal test. |
| 4.    | Sugars            | (1) Tollen's reagent test  
(2) Molisch's test  
(3) Benedict's solution test |
| 5.    | Flavonoids        | (1) Shinoda test |
| 6.    | Saponins          | (1) Honey comb - fonn reaction  
(2) Haemolysis test  
(3) Liebermann - Burchard reaction |
| 7.    | Alkaloid          | (1) Mayer's reagent  
(2) Dragendorff's reagent |

(3) Antibacterial activity of various extracts/fractions:

(a) Test bacteria:

Different extracts and fractions as obtained by aforesaid extractions (schemes given in figures 7 to 14) were tested against eight bacterial species (Gram positive and Gram negative both) according to the procedure described in chapter - 4.

The isolation and identification methods for Klebsiella sp., E. coli and Shigella flexneri were the same as described earlier. While other test bacteria viz, Proteus vulgaris, Salmonella typhi, Shigella
dysenteriae, Bacillus subtilis and Staphylococcus aureus were obtained from the Department of Pharmaceutical Sciences, Dr. H.S. Gour Vishwavidyalaya, Sagar (M.P.), for broad spectrum antibacterial screening.

(b) Preparation of culture medium:

To test antibacterial activity, the nutrient agar medium was used. Culture medium was poured in presterilized petriplates. Constituents of medium are mentioned in chapter-4. In aseptic conditions these plates were put on perfect horizontal plane for solidification of medium.

(c) Preparation of filter paper disc:

Discs of 6 mm diameter were prepared by Whatman No. 1 filter paper sheets. These discs were sterilized at 60-80°C for 24 hours.

The test concentration of each of the extracts and fractions which were obtained by the aforesaid extraction procedures were maintained to 1 mg/ml. The stock solution of each extract was prepared in their respective solvents and used for moistening the filter paper discs. These extract moistened discs were used for antibacterial screening by following disc-diffusion method as described earlier (chapter-4). For control sets, discs moistened with solvents were used to note any activity of solvent itself by following the procedures as described by Saxena (1983). It was observed that the used solvents generally produced no activity. All the extracts and fractionates were tested for their antibacterial efficacy against eight test bacteria.

The disc diffusion method (Maruzzella and Henry, 1958) was
followed. After solidification of nutrient agar medium, petriplates were seeded uniformly with the help of rotator and sterilized cotton boll held up by sterilized forceps on the surface of media. Then sterilized discs moistened with 0.05 ml of extracts were placed on the preseeded media of petriplates with the help of alcohol flammed, fine pointed forceps. The disc was pressed firmly into the agar so that it is in full contact with the agar surface. Plates were then placed for an hour in refrigeration for best diffusion of extract. After one hour these plates were placed in incubator at 35 ± 2°C. After 24 hours of incubation period the antibacterial activity of different solvent extracts and fractions of roots were measured in terms of inhibition zones appearing around the filter paper discs. The diameter of inhibition zones was measured as an average of maximum dimension of zones of inhibition around the filter paper disc. All the results were taken in triplicates. The standardized paper disc technique affords a reasonable accurate estimation of susceptibility by the measurement of zones of inhibition produced on an agar streak plate. (Robert and Elvyn 1966). To compare antibacterial efficacy of different solvent extracts, the antibiotic streptomycin was also used as a standard (0.2 mg/ml).
RESULTS AND DISCUSSION:

The data on the efficacy of various fractions against 8 test bacteria have been given in Table - XII and Fig. - 15. As indicated in table, total seven solvent fractions of roots of *T. macrophylla* were tested. Various fractions were obtained by successive liquid extraction of the contents of ethanolic extract as the roots were first extracted with ethanol and the ethanol extract was further fractionated by various solvents according to their polarity as described earlier (Material and Method).

Among these acetone and methanolic fractions were found to remain ineffective against all the test bacterial species. Similarly ethylacetate fraction was also found to be ineffective against most of the test bacteria. Only *Salmonella typhi* and *Klebsiella* sp. were inhibited by ethyl acetate fraction (15 and 12 mm. zone of inhibition respectively).

Chloroform fraction was not effective against *Klebsiella* sp. and *Escherichia coli*, while it showed poor or moderate activity against all other bacteria. *Shigella* species were comparatively more sensitive than others. 25 and 18 mm. zones of inhibition were evident in *Shigella flexneri* and *Shigella dysenteriae*, respectively. In case of other bacteria, the zones of inhibition ranged in between 13 to 15 mm, suggesting the poor activity of chloroform fraction.

Benzene fraction was also poorly or moderately effective against all the test bacteria except *E. coli*. This fraction showed slightly more antibacterial activity against a few bacteria than that caused by chloroform fraction. Interestingly, *Shigella flexneri* was more sensitive (26 mm. zone of inhibition) than *Shigella dysenteriae* (17 mm).
### Table - XII Antibacterial activity of different extracts of roots of *T. macrophylla*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of bacteria</th>
<th>Extracts/Fractions</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td>V</td>
<td>VI</td>
<td>VII</td>
</tr>
<tr>
<td>1.</td>
<td><em>Klebsiella</em> sp.</td>
<td>28</td>
<td>32</td>
<td>13</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td><em>Escherichia coli</em></td>
<td>24</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td><em>Shigella flexneri</em></td>
<td>27</td>
<td>37</td>
<td>26</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td><em>Shigella dysenteriae</em></td>
<td>29</td>
<td>38</td>
<td>17</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td><em>Salmonella typhi</em></td>
<td>29</td>
<td>43</td>
<td>25</td>
<td>13</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td><em>Proteus vulgaris</em></td>
<td>29</td>
<td>30</td>
<td>17</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7.</td>
<td><em>Bacillus subtilis</em></td>
<td>38</td>
<td>35</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8.</td>
<td><em>Staphylococcus aureus</em></td>
<td>36</td>
<td>37</td>
<td>15</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

I = Petroleum ether.  
II = Ethanol.  
III = Benzene.  
IV = Chloroform.  
V = Ethyl acetate.  
VI = Acetone.  
VII = Methanol.  
S = Streptomycin (0.2 mg/ml).  
- Data indicate the zone of inhibition in mm, including diameter of disc (6 mm) as average of triplicate plates.  
- Incubation temperature = 35 ± 2°C.  
- Incubation period = 24 hours.
Fig - 15, Antibacterial activity of various solvent extracts/fractions against test bacteria.
TEST BACTERIA:
1 - Klebsiella sp.
2 - Escherichia coli
3 - Shigella flexneri
4 - Shigella dysenteriae
5 - Proteus vulgaris
6 - Salmonella typhi
7 - Bacillus subtilis
8 - Staphylococcus aureus

FIG. 15
Salmonella typhi was also effected to a good extent (25 mm. zone of inhibition).

It is evident from Table - XII that the antibacterial effects of benzene and chloroform fractions of roots of Thespisia macrophylla were quite comparable to those of streptomycin (0.2 mg/ml) and even in a few cases these plant extracts were stronger than streptomycin (i.e. benzene fraction against Shigella flexneri and Salmonella typhi, chloroform fraction against shigella flexneri).

Ethanolic and petroleum ether extracts were found to be strong enough in causing significant antibacterial effects against almost all the eight test bacteria. Among these, ethanolic extract was superior than petroleum ether extract.

Petroleum ether extract caused stronger (38-36 mm. zones of inhibition) effects against gram positive bacteria viz. Bacillus subtilis and Staphylococcus aureus than against gram negative test bacterial species (24 to 29 mm. zones of inhibition). Among gram negative bacteria, E. coli was less sensitive (24 mm.) while others were more or less equally sensitive (27 to 29 mm.).

Ethanolic extract of roots of T. macrophylla caused significant antibacterial effects: Almost all the tested bacteria were suppressed. Salmonella typhi was found to be most susceptible where a significant zone of inhibition was evident (i.e. 43 mm.). Next in order of decreasing susceptibility were S. dysenteriae, S. flexneri, Klebsiella sp., Proteus vulgaris and E. coli. In these cases 38, 37, 32, 30 and 25 mm zones of inhibition were evident, respectively. Similarly against two gram positive bacteria also i.e. Staphylococcus aureus and Bacillus
subtilis, the ethanolic extract of T. macrophylla roots was found to produce large zones of inhibition (37 and 35 mm, respectively). These effects were quite high with those caused by streptomycin.

From the overall results on the effects of various extracts it is evident that the roots of this plant probably contain a good number of antibacterial phytochemical constituents and most of these can be extracted out by petroleum ether and ethanol. While some less effective antibacterial substances may also be fractionated out by benzene and chloroform. More extensive studies may explore their chemical nature, isolation, purification and chemical characterization and also their specific and/or broad spectrum activity against bacteria and other microorganisms, both pathogenic and non-pathogenic types. It is however clear from the present study that T. macrophylla roots may be exploited for the isolation of certain strong antibacterial natural substances by using simpler methods and solvent like water, ethyl alcohol and petroleum ether.

This plant material is known to be used for the cure of various bacterial ailments (chapter - 2) by tribals, particular of Sagar region. It seems that the roots cure various bacterial diseases due to the presence of antibacterial phytochemicals in sufficient quantities in them.

Thespisia macrophylla can be grown easily by sowing its seeds at the start of rainy season in organic manured soil. During present study, to procure appropriate quantities of its roots, the plants were grown in botanical garden (Plate No. 21).

In literature, there is no report on the antibacterial efficacy
of roots of this plant. This plant may be of high applied value for the efficient cure of bacterial diseases though detailed evaluation may only explore the possibility of large scale use of this herbal remedy.

For the purpose of separation of various phytochemical constituent groups, further study was made and the effects of isolated constituents viz. sterols, glycosides, alkaloids, flavonoids, aglycones etc. were studied against eight test bacteria.

Table XIII, presents the antibacterial activity of isolated crude phytoconstituents of *T. macrophylla*. Total eight extractives viz. tannins, sterols, glycosides, sugars, aglycones, alkaloids, flavonoids and saponins of this plant were isolated according to the methods (Fig. 8-14), described earlier. Among these extractives, tannins, alkaloids, free sugars and saponins were found to be ineffective against eight test bacterial species. Aglycone extractive showed poor antibacterial activity.

While flavonoids were moderately active against almost all the test bacteria. Similarly, sterol extractive also showed moderate antibacterial effects.

Glycosidal extractive of roots of *T. macrophylla* showed antibacterial efficacy among all the eight test phychemical extractives. It is interesting to note that these extractives were found to show antibacterial efficacy, more or less to the equal extents against various test bactreia, suggesting non specific nature of their activity.

It is clear from the foregoing results that atleast glycoside, flavonoid and sterol constituents of *T. macrophylla* roots need further
Table - XIII Antibacterial activity of various phytochemical constituent extract of roots of *Thespesia macrophylla*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of bacteria</th>
<th>Extractives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tannins</td>
</tr>
<tr>
<td>1.</td>
<td>Klebsiella sp.</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>Escherichia coli</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>Shigella flexneri</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td>Shigella dysenteriae</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>Salmonella typhi</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td>Proteus vulgaris</td>
<td>0</td>
</tr>
<tr>
<td>7.</td>
<td>Bacillus subtilis</td>
<td>0</td>
</tr>
<tr>
<td>8.</td>
<td>Staphylococcus aureus</td>
<td>0</td>
</tr>
</tbody>
</table>

0 = Inactive.
+
++ = Inhibition zone < 10 mm.
+++ = Inhibition zone < 15 mm.
++++ = Inhibition zone > 15 mm.

Incubation temperature = 35 ± 2°C.
Incubation period = 24 hours.
purification, chemical characterization and isolation. The screening of purified samples against many more gram positive as well as gram negative organisms, may provide much more information on the specific and/or non specific antibacterial efficacy of various natural chemicals of this plant.

The record of antibacterial activity of phytochemical constituents of *T. macrophylla* is not available in literature. Thus, it may be the first report for this plant.

The available records of the chemistry of *T. macrophylla* in *Glossary of Indian medicinal plants* is as follows, flowers contain quercetin and protocatechuc acid and in *wealth of India* as gossypol is reported to be present in different parts of the plant (dry basis): seeds, 1.74; flowers buds, 1.95; leaves, 0.98; roots, 2.75 and stems 0.16% (Nair, 1961; Perkin and Everest, 1918).

As stated before, the roots of *T. macrophylla* are used in Sagar district for the cure of dysentery and in available literature it is known to be used for syphilis and gonorrhoea and according to recent reports in typhoid also (Manandhar, 1990).

This plant was collected from the Bahrol forest which is about 11 Km. north east from Sagar city.

Although, it is a wild plant but it can also be cultivated in large scale. From the foregoing results it is evident that the roots of this plant may provide certain good antibacterial natural drugs (s). However, to obtain effective drug with proven efficacy it is necessary to establish it through a number of successful pharmacological and other
aspects and also for the final product through modern scientific and technical procedures. This would certainly enhance the reputation and acceptance of traditional medicines.
Plates showing Thespedia macrophylla plant:

Plate No. 21:

A. A complete plant.
B. A flower.
C. A flowering branch.
D. Floral parts.
E. Fruiting branch.
F. Dry fruits, seeds and small pieces of its roots.
Antibacterial effects of various solvent extracts/fractions of T. macrophylla:

Plate No. 22:

B1. Ethanol: Control (solvent only).
B1. F6: W1: Ethanolic extract against *Shigella dysenteriae*.
B1. F7: Petroleum ether extract against *Shigella dysenteriae*.

Plate No. 23:

B2. Ethanol: (Control).
Plate No. 24:

B3. Ethanol: (Control).

B3. F6 : W1 : Ethanolic extract against *Shigella flexneri*.


B3. F7 : Petroleum ether extract against *Shigella flexneri*.

Plate No. 25:

B4. Ethanol: (Control).


B4. F7 : Petroleum ether extract against *Proteus vulgaris*. 
Plate No. 26:

B3. Ethanol : (Control).

B3. F6. WI : Ethanolic extract against *Staphylococcus aureus*.


B3. F7 : Petroleum ether extract against *Staphylococcus aureus*.

Plate No. 27:

B6. Ethanol : (Control).

B6. F6. WI : Ethanolic extract against *Bacillus subtilis*.


B6. F7 : Petroleum ether extract against *Bacillus subtilis*. 
Plate No. 28:

B7. Ethanol: (Control).
B7. F6. WI: Ethanolic extract against Klebsiella sp.
B7. F7: Petroleum ether extract against Klebsiella sp.

Plate No. 29:

B8. F7: Petroleum ether extract against E. coli.