CHAPTER V

GENERAL SUMMARY AND CONCLUSION
On account of increasing realization about the adverse side effects of many modern synthetic drugs on human health, the attention is now again being focused on traditional remedies. Herbal drugs are gaining renewed importance and therefore, ethnombotany especially dealing with various aspects of ethnomedicinal plants has become the subject of very active interest during past few decades. Unfortunately, however majority of these investigations have not been carried out with modern scientific approach and therefore lack accuracy and authenticity. Thus the present work was planned and carried out with the emphasis on the following aspects:

(1) Ethnombotanical survey of various localities of Sagar and Shahdol districts of M.P.

(2) Collection, identification and preservation of the authentic plant samples.

(3) Screening of selected medicinal plants for their antimicrobial activity.

(4) Phytochemical analysis of most effective plant samples of *Thespesia macrophylla* roots and their antibacterial activity.

An ethnombotanical survey was carried out during March 92 to December 95, in different villages and forest of Sagar and Shahdol districts of M.P. and relevant information about the uses of various plants, for the treatment of
gastrointestinal infections by tribals, villagers, medinemen, herbalists etc., was collected and recorded on the basis of these ethnobotanical records about 86 plants were collected in sufficient quantity and processed for further use in the laboratory. These plants were then identified with the help of herberia and floras. The specimens have been deposited in the departmental herbarium.

All the 86 plants were tested for their antibacterial activity against eight test bacteria viz. Escherichia coli, Bacillus subtilis, Shigella flexneri, Proteus vulgaris, Salmonella typhi, Klebsiella sp., Staphylococcus aureus, and Shigella dysenteriae.

As evident from the data of experimental results, about 69 plants showed positive antibacterial activity. Amongst these, Flacourtia indica (Stem bark), Kydia calycina (Stem bark), Thespesia macrophylla (Root), Murraya koenigii (Leaves), Murraya paniculata (Leaves), Soymida febrifuga (Stem bark), Ougeinia oojeinensis (Stem bark), Acacia arabica (Stem bark), Acacia leucohloea (Stem bark), Acacia nilotica (Stem bark), Acacia senegal (Pod), Terminalia beberica (Fruit), Terminalia tomentosa (Stem bark), Erigeron canadensis (Plant), Plumbago zeylanica (Root), Evolvulus alsinoide (Plant), Phyllanthus niruri (Plant), Cyperus scariosa (Root tuber), etc. appeared to be very remarkable by producing more than 15 mm inhibitory zone against various test bacteria.
Thespesia macrophylla, Kydia calycina, Murraya koenigii, Murraya paniculata, Ougeinia oojienensis, Acacia arabica, Acacia leucophloea, Acacia nilotica, Acacia senegal, Terminalia bekeria, Terminalia tomentosa, Erigeron canadensis, Plumbago zeylanica, Evolvulus alsinoides, Phyllanthus niruri and Cyperus scariosus etc. showed good antibacterial activity against majority of test bacteria.

Interestingly, however a number of plants viz. Shorea robusta, Sterculia urens, Corchorus trilocularis, Triumfetta rotundifolia etc. instead of showing inhibitory activity caused stimulatory effect on the growth of certain bacteria.

About 10 plants were also tested for their antifungal activity against 5 test fungi viz. Aspergillus flavus, Candida albicans, Chrysosporium sp. Colletotrichum capsici and Trichoderma viridae. Kydia calycina, Murraya koenigii and Thespesia macrophylla showed good fungitoxic effect against Colletotrichum capsici. Ougeinia oojienensis and Cyperus scariosus induced significant antifungal activity against Aspergillus flavus. Antifungal activity of Acacia leucophloea was however, found to be more remarkable against Chrysosporium sp.

On the basis of its overall good performance during present screening work earlier observations indicating its great potentially and also because of its more common use against various gastrointestinal infections, Thespesia
Macrophylla was selected for further detailed phytochemical investigations.

For the purpose of separation of phytochemical constituent groups, various extractive of root of T. macrophylla were prepared with pet. ether. The antibacterial activity of isolated constituents including especially, sterols, glycosides, alkaloids, flavonoids, aglycones etc. were tested against the same test bacteria. Among these, glycosidal extractive showed high antibacterial activity while flavonoids, aglycones and sterols were moderately active against the test bacteria. These glycosides, flavonoid and sterol constituents of T. macrophylla roots therefore need further isolation, purification and chemical characterization of active compounds.

For detailed phytochemical analysis of active constituents, the root powder was successively extracted by using various solvent according to their polarity and the extracts were then tested for the antibacterial activity. Of these pet. ether, benzene, chloroform and ethyl acetate extracts/fractions were found to be more remarkable in causing very high antibacterial activity against almost all the eight test bacteria.

As such these fractions/extracts appeared to contain a good number of highly active antibacterial compounds.
Petroleum ether extract:

With the help of column chromatography using pure diethyl ether as solvent, 3a to 3e fractions are collected at the interval of 15 minutes; these fractions showed strong antibacterial activity against almost all the test bacteria; 3c fraction, however showed maximum activity. This was therefore selected for T.L.C. using CHCl₃ : MeOH : H₂O (65:25:4) and Hexane : Ether (4:1) solvent system. T.L.C. plate after spraying with concentrate H₂SO₄ showed 2-3 spot with positive colour of steroids.

This fraction gave a brown gummy mass on evaporation of the solvent with m.p. 180-200°C, and on the basis of spectral analysis data (¹H-NMR, Mass spectrum) and nature of spots on T.L.C., these compounds appeared to remarkable to a series of alkanes (C₁₉⁻C₃₃) and esters (C₄₀⁻C₅₂), β-sitosterol, lupeol and gossypol etc. This fraction therefore could be a mixture of these compounds.

Ethyl acetate fraction:

Ethyl acetate fraction of root of T. macrophylla gave a pale yellow solid as TM-1, which was isolated by TLC of EtOAC fraction with solvent system (EtOAC : Acetone : Acetic acid : H₂O -10 : 3 : 0.7 : 0.7); this was purified by column chromatography over silica gel. The m.p. was found to be 189-90°C (TM-1). Elemental analysis agreed with the molecular formula C_{21}H_{20}O_{11}. The glycosidic nature of the
product TM-1 was evidenced from the molish test obtained after hydrolysis, formation of osazone and $^1$H-NMR spectrum of its acetate. The glycoside gave positive test with magnesium and HCl and sodium amalgam followed by acidification indicating its flavonone or flavone nature (with C$_3$ blocked). The possibility of its being a flavanone glycoside was eliminated as it gave yellow colour with Wilson-boric acid reagent. The chromatographic spot on paper appeared to be deep purple under UV light and turned yellow on fuming with ammonia, further indicating that the C-3 position is substituted.

Hydrolysis with 6% aqueous HCl gave rhamnose and an aglycone characterized as quercetin (II) by its m.p., its Rf value, $^1$H-NMR and MS spectral data as described earlier and also by direct comparison with authentic samples. The sugar was identified as L-rhamnose by Rf value, co-chromatography and by the formation of osazone.

Colour test and MS, $^1$H-NMR spectral data suggested that the sugar residue was attached to the 3-position of the aglycone and this was confirmed by hydrolysis of the methylated glycoside to an aglycone characterised as 3-OH, 3', 4', 5-7-tetramethoxy flavone (III) (quercetin tetra methyl ether), m.p. 194$^0$ which showed a bathochromic shift of 64 nm in band I with AlCl$_3$, confirming the C-3 hydroxyl which was glycosylated in TM-1 has become free. The formation of this partial methyl ether thus proved that the rhamnose is attached to position 3- of the aglycone.
The quantitative estimation of sugar by Somogyi copper micro method showed the presence of 1 mole of glucose per mole of aglycone.

TM-1 was therefore, characterised as quercetin 3-0-rhamnoside (quercetin) (I).

As such on the whole it may be concluded that the roots of Thespesia macrophylla contain a good number of highly active antibacterial compounds. However only one of these active principles could be characterized as QUERCETIN. For complete analysis, the active fractions need further detailed phytochemical investigations.