Phytochemical And Biological Investigations of Rutaceous plants

CHAKRAVARTHI GUNTUPALLI

A thesis submitted in partial fulfillment of the requirements of the University of Sunderland for the degree of Doctor of Philosophy

November 2008

Abstract

The Rutaceae family consists of 150 genera and 1,500 species, which are herbs, shrubs, and trees. The members of genus Glycosmis and Clausena are aromatic (contain volatile oils) and traditionally used for fever, swollen spleen, digestion, topical infections, skin itch, scabies, boils and ulcers. Accordingly from this family, *Tetractomia roxburghiana*, *Glycosmis calcicola*, and *Clausena excavata* were selected for systematic biological screening to exploit and identify compounds which may serve as subsequent leads for the treatment of skin diseases. While the initial aim of the programme had been to characterise these barely studied plants, the programme was subsequently extended to study their biological activity in order to justify their traditional use as medicines.

During the course of this study, supporting analytical methodologies were used extensively and ultimately the evaluation of these methodologies contributed a significant proportion of the overall research programme. Initially crude extract was subjected to column chromatography and the compounds were isolated by overloading an analytical HPLC column. By the end of the programme, crude extracts were being analysed directly by gradient reversed-phase HPLC with subsequent direct scale up to preparative isolation on a (250 × 22) mm id column.

Analytical reversed phase high performance liquid chromatography (HPLC) of the crude methanolic leaf extracts of *T. roxbhurghina*, *G. calcicola*, and *C. excavata* was carried out in order to qualitatively assess the number of constituents present in each fraction. Separation was achieved by using ACE-5-C18 (250 × 4.6 mm) with a flow rate of 1.5 ml/min, with the UV detection at 254 nm. Semi-preparative and preparative HPLC were

also carried out in order to isolate components of these mixtures. Using spectral analysis, as swertisin, gallic acid, α-asarone and angelicin (furanocoumarin) were identified. In the same way, angelicin was identified from the methanolic leaf extract of *Glycosmis calcicola* and preparative HPLC of the methanolic leaf extract of *Clausena excavata* afforded three compounds, namely 2-(3,4-dimethoxy-phenyl)-5,7-dimethoxy-chromen-4-one, 2-(3,4-dimthoxy-phenyl) 3,7-dihydroxy-5-methoxy-chromen-4-one and 5,7-dihydroxy-2-phenyl-chromen-4-one (chrysin), which were confirmed by spectroscopic methods.

Micellar electrokinetic chromatography (MEKC) was evaluated as a possible high-resolution technique for checking the purity of fractions isolated from preparative RP-HPLC. However it proved more effective to exploit orthogonal (to RP-HPLC) modes of LC by using $–NH_2$ and –SCX ion-exchange HPLC columns and/or, if resolution on analytical RP-HPLC was possible, structural elucidation was carried out using LC-NMR-MS.

With respect to biological activity, a range of procedures that had been established at the University for checking activity against skin diseases was used. Free radical induced lipid peroxidation model has been selected for evaluation of antioxidant activity of the extract. The anti-oxidant activity of these extracts and compounds were assessed by free radical induced lipid peroxidation model. The results indicated that the methanolic leaf extract of *Tetractomia roxburghiana* showed marked anti-oxidant activity whereas methanolic leaf extract of *Glycosmis calcicola* and *Clausena excavata* showed moderate anti-oxidant activity. The $IC_{50\ value}$ of the methanolic leaf extract of *Tetractomia*

roxburghiana was found to be 201.3 µg/ml; whereas those for *Glycosmis calcicola* and *Clausena excavata* were found to be 450.6 µg/ml and 1106 µg/ml respectively.

For all the extracts, no anti-bacterial activity was found against S*taphylococcus aureus*, *Streptococcus pyogenes*, *Propionibacterium acne.* Also no anti-fungal activity against *Candida albicans* was found. A total of three crude methanolic plant extracts, four isolated compounds and eleven semi-purified fractions were tested for *in-vitro* efficacy, using an agar incorporation method to determine the minimum inhibition concentration (MIC), against the dermatophyte species; *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Epidermatophyton floccusum*. The MIC value for crude methanolic extracts of *Tetractomia roxbhurghiana and Glycosmis calcicola* was found to be 62.5 µg/ml and 31.2 µg/ml against *T.rubrum* and *T.mentagrophytes*, whereas the methanol extract of *Clausena excavata* did not show any activity against dermatophytes.

In conclusion, the anti-oxidant activity of *Tetractomia roxburghiana* was found to be comparable with that of propylgallate, which was used as a standard drug thus confirming, as anticipated, that *Tetractomia roxburghiana* might be a good source of anti-oxidant drugs. The extended degree of anti-oxidant activity displayed by methanolic extract of *Tetractomia roxburghiana* could be contributed to the presence of swertisin, gallic acid and angelicin, which are proven anti-oxidants. The anti-fungal activity of *Glycosmis calcicola* could be partly due to the presence of angelicin.

Acknowledgements

I would like to thank my Director of Studies Dr.A.Worsley for his expertise advice and guidance through out the duration of my studies.

I would like to thank Prof. P.W.Groundwater and Dr. R.J. Anderson for their expertise guidance concerning with identification of plant metabolites and providing partial funding of the second year of the research programme.

I would like to thank Dr.W.John Lough for his expert guidance with the analytical technology method development procedures and for providing resources for the biological testing of the plant extracts and metabolites.

I would also like to thank Dr. N .D. Satyanarayan for providing training on the anti-microbial assays.

I would also like to thank my family, and friends for their continuing social, financial and emotional support throughout the period of research.

Lastly but not least I would like to thank my wife Saraswathi for her encouragement and support during this period.

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List of Abbrevations

A.vulgaris	***Acne vulgaris***
BGE	**Back Ground Electrolyte**
BHT	**Butylated Hydroxy Toluene**
C.albicans	***Canida albicans***
CD	**Cyclodextrin**
CE	**Capillary electrophoresis**
C.excavata	***Clausena excavata***
CFU	**Colony Forming Units**
CHL	**Sodium cholate**
CZE	**Capillary Zone Electrophoresis**
DAD	**Diode Array Detector**
DC	**Direct Current**
DCM	**Dichloromethane**
DMSO	**Dimethyl Sulphoxide**
EA	**Ethylacetate**
GATT	**General Agreement on Tariffs and Trade**
RP-HPLC	**Reversed Phase-High Performance Liquid Chromatography**
HPLC	**High Performance Liquid Chromatography**
IRP-LC	**Isocraticreversed phase-High Performance Liquid Chromatography**
LC	**Liquid Chromatography**
MEKC	**Micellar Electrokinetic Chromatography**
MIC	**Minimum Inhibitory Concentration**
MRD	**Maximum Recovery Diluent**
MS	**Mass Spectrometry**
NMR	**Nuclear Magnetic Resonance**
ODS	**OctaDecylSilane**
P.acne	***Propionibacterium acne***
PPM	**Parts-per-million**
SD	**Standard Deviation**

SDA	**Sabouraud Dextrose Agar**
SDS	**Sodium Dodecyl Sulphate**
S.aureus	**Staphylococcus aureus**
S.pyogens	**Streptococcus pyogens**
TBA	**Thiobarbituricacid**
TFA	**Trifluoroaceticacid**
TLC	**Thin Layer Chromatography**
TSA	**Tryptic Soy Agar**
TVAC	**Total Viable Aerobic Count**
WHO	**World Health Organisation**