CHEMICAL STANDARDIZATION OF SOME ANTI-INFLAMMATORY RASAYANA PLANTS USED IN INDIAN SYSTEM OF MEDICINE

SUMMARY OF THE THESIS SUBMITTED TO UNIVERSITY OF LUCKNOW FOR THE DEGREE OF Doctor Of Philosophy IN CHEMISTRY

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Plants and plant-derived products are part of health care system since ancient human civilization. Natural product remains a prolific source of discovery of new drugs from the ancient Vedic period. Because of its safe use, affordability and easy availability, the majority of world population still rely on plant based medicine. India has long history of management of human health through Ayurveda. Charak and Sushruta had contributed a lot of development in the area of drug discovery. The rich biodiversity of India always have been attracted the attention of researcher, which remained untouched as far as the new drug discovery is concerned.

Being an important ingredient of several Ayurvedic formulation and important part of many folklore & ethnic use, a lot of attention has been given to the phytochemical and biological studies on these three “Vata-category” anti-inflammatory Rasayana plants. Still, there is a great scope to explore the constituent plants in means of their phytochemicals and structure activity relationships (SAR). As many times the plant extracts itself have been made responsible for the activity of particular plant/plant based drug and even some plants are almost unexplored in means of their phytochemical and biological studies. So there is a need to identify the responsible compound/compounds for their biological activity that may be interesting lead towards new drug formulations.

With the above mentioned facts as background, present work was carried out to chemically investigate three “Vata-category” anti-inflammatory Rasayana plants, with the aim to isolate and characterize their chemical constituents, biological activity evaluation of extracts and isolated phytomolecules, development of standardized analytical procedures for quality evaluation of related plant extracts and establishment the quantitative/qualitative structural relationship of isolated phytochemicals. These findings have been summarized in the chapters mentioned below.

**Chapter 1** comprises the details about plants and plant-derived products as a part of health care system especially in Ayurveda since ancient human civilization. It contains the Ayurvedic classification of the plant, their therapeutic claims, current research status of the plant, modern and Ayurvedic approach on inflammation and standardization of herb.

**Chapter 2** comprises aim and objectives of this whole study.
Summary

Chapter 3 discusses the past and present work on the plant *Pluche lanceolata*. *Pluche* species have been used in Indian and Chinese traditional medicine for ancient times to cure several inflammatory conditions specially related to arthritis. A detailed review of literature about the pharmacological & biological activities and its chemical constituents has been mentioned. First part of chapter includes the isolation of 9 compounds from its aerial part and 3 compounds from its root part. Identification of isolated compounds was carried out by different spectral data. Plant has been identified as a new source for 2 molecules: taraxasterol acetate and Plucheachromenonegalactoside (2, 2-dimethyl-7-acetyl-8-β-galactosidechromenone). Extraction and characterization of volatile organic compounds of essential oil of *Pluche* have also been done. Nine major compounds have been identified.

In second part contains analytical method development. For this global fingerprint and marker based high performance thin layer chromatographic methods have been developed. Global fingerprint have been developed using mobile phase 0.5% AcOH in water and 0.5% AcOH in acetonitrile, with gradient elution at a flow rate of 1.0 mL/ min on Phenonemex-C18 column (4.6mm × 250mm, 5μm) column. While, validated HPTLC method includes the simultaneous determination of tarxasterol acetate, taraxasterol and stigmasterol in *Pluche* at 645 nm. The classical and non–classical extraction process and the effect of different parameters, such as microwave energy, sonic power, temperature, varying irradiation time and liquid–solid ratio, on the extraction yield using different designs of experiments i.e. the central composite design (CCD) and Box-Behnken design (BBD), of response surface methodology (RSM) have been performed. Along with extraction, forced degradation studies and kinetic degradation studies of acid-alkaline degradation also have been done.

In third part biological activities of essential oil, plant extracts and pure isolates have been evaluated. Anti-inflammatory activity of plant extracts (methanol, hexane, chloroform, ethylacetate and butanol) and isolated compound i.e. taraxasterol acetate, taraxasterol were performed by inhibition of cyclooxygenase (COX-2), proinflammatory cytokines (TNF-α, IL-6) and nitric oxide synthase (iNOS) and satisfactory results were obtained. Anti-inflammatory activity of isolated taraxasterol acetate, taraxasterol were also performed by inhibition of inflammatory mediators in LPS-stimulated C6 rat glioma cells and were found to be active in neuro-inflammation. This was the first report to find that taraxasterol and its naturally occurring
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acetate derivative from *Pluchea* protect the neuro-inflammation by mean of down regulating the TNF-α, IFN-γ and IL-6 release in C6 rat glioma cell. Taraxasterol acetate along with its active hexane fraction were investigated for antimalarial activity in *P. berghei*-induced murine malaria (*in-vitro, in-vivo*). Safety profile was also developed of *P. lanceolata* for its ethnopharmacological validation. It was found that taraxasterol acetate possessed very good antimalarial activity in *P. berghei*. All extracts and isolates (tarxasterol, taraxasterol acetate) were found to be less active against *Mycobacterium tuberculosis*. All extracts and isolates (tarxasterol, taraxasterol acetate, stigmasterol) were found to be potent for antibacterial activity against MTCC 96 and MRSA 33 strain of Gram-positive bacterium *Staphylococcus aureus*. Essential oil of aerial part of *Pluchea* was found to possess antiacetylcholinesterase activity. All extracts were found to have moderate antioxidant activity.

Chapter 4 discusses the past and present work on the plant *Ricinus communis*. *Ricinus* species have been used in Indian and Chinese traditional medicine for ancient times to cure several inflammatory conditions specially related to arthritis. A detailed review of literature about the pharmacological & biological activities and its chemical constituents has been mentioned. First part of chapter includes the isolation of 4 compounds from its root part. Identification of isolated compounds was carried out by different spectral data. Plant has been identified as a new source for 2 molecules: lupeol and erandone (Olean-6-ene-3, 16-dione), in which erandone is found to be the first report in nature. Characterization of volatile organic compounds from hexane fraction of *Ricinus* revealed presence of five major compounds.

In second part contains analytical method development. For this global fingerprint and marker based high performance thin layer chromatographic methods have been developed. Global fingerprint have been developed using mobile phase 0.5% AcOH in water and 0.5% AcOH in acetonitrile, with gradient elution at a flow rate of 1.0 mL/ min on Phenomenex-C_{18} column (4.6mm × 250mm, 5μm) column. While, ELSD based a validated high performance liquid chromatography method included the simultaneous determination of erandone, lupeol, stigmasterol and sitosterol in *Ricinus*. Separation was performed on Phenomenex Luna-C_{18} (4.6 mm × 50 mm, 3μm) (Merck) column, using 1% acetic acid in acetonitrile and 1% acetic acid in water in ratio of 95:5, v/v with isocratic elution at a flow rate of 0.5 mL/min. Along with
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extraction (classical, non-classical), forced degradation studies and kinetic degradation studies of acid-alkaline degradation also had been done.

In third part biological activities of plant extracts and pure isolates have been evaluated. Anti-inflammatory activity of plant extracts (methanol, hexane, ethylacetate and butanol) and isolated compound i.e. erandone, lupeol were performed by inhibition of cyclooxygenase (COX-2), proinflammatory cytokines (TNF-α, IL-6) and nitric oxide synthase (iNOS) and moderate results were obtained. All extracts and isolates (erandone) were found to be inactive against *Mycobacterium tuberculosis*. All extracts were found to be inactive for antibacterial activity against all five strain of Gram-positive bacterium *Staphylococcus aureus*. All extracts were found to have less antioxidant activity.

Chapter 5 discusses the past and present work on the plant *Punica granatum*. A detailed review of literature about the pharmacological & biological activities and its chemical constituents has been mentioned. First part of chapter includes the isolation of 13 compounds from its peel part. Identification of isolated compounds was carried out by different spectral data.

In second part contains analytical method development. For this global fingerprint and marker based high performance thin layer chromatographic methods have been developed. Global fingerprint have been developed using mobile phase 0.5% AcOH in water and 0.5% AcOH in acetonitrile, with gradient elution at a flow rate of 1.0 mL/ min on Phenonemex-C18 column (4.6mm × 250mm, 5µm) column. While, PDA based a validated high performance liquid chromatography method included the simultaneous determination of seven biomarkers *viz.* gallic acid, rutin, ellagic acid, naringin, quercetin, apigenin and kaempferol in *Punica*. Separation was performed on Nova-Pak C18 (3.9 mm × 150 mm, 4µm) (Waters) column, using 1% acetic acid in acetonitrile and 1% acetic acid in water in ratio of 95:5, v/v with gradient elution at a flow rate of 0.8 mL/min. Extraction with different organic solvents by classical and non-classical technique were summarized. Beside this extraction with green solvent (ionic liquids) was also done. Forced degradation studies and kinetic degradation studies of acid-alkaline degradation also had been done.

In third part biological activities of plant extracts and pure isolates have been evaluated. Anti-inflammatory activity of plant extracts (methanol, hexane, chloroform, ethylacetate and butanol)
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were performed by inhibition of cyclooxygenase (COX-2), proinflammatory cytokines (TNF-α, IL-6) and nitric oxide synthase (iNOS) and moderate results were obtained. All extracts and isolates (naringin, kaempferol, ursolic acid and 3-hydroxy ursollic acid) were found to be inactive against *Mycobacterium smegmatis*. All extracts were found to be active for antibacterial activity against all five strain of Gram-positive bacterium *Staphylococcus aureus*. All extracts were found to have very good antioxidant activity.

Chapter 6 discusses in silico analysis of all targeted chemical compounds. Validation of anti-inflammatory and immunomodulatory potential of isolated phytochemical were performed using molecular docking tools. Molecular docking was performed using Computer-Aided Drug Design (CADD) tool.
LIST OF PUBLICATIONS

Research publications part of thesis work

Papers in SCI Journals


4. **Pooja Srivastava**, PV Ajaykumar, Karuna Shanker (2013) Improved specificity of HPTLC determination of triterpenes and sterol by online/offline coupling with DAD, NIR ESIMS and application of Box–Behnken design for optimum extraction variables-solvents (organic × green) and energies (thermal × microwave × acoustic). *Journal of Chromatography-B* (Communicated)


8. **Pooja Srivastava**, Priyanka Trivedi, Dnyaneshwar U Bawankule, Karuna Shanker (2013) Potentiality of Indian plants on inhibition of inducible cyclooxygenase (COX-2), pro-inflammatory cytokines (TNF-α, IL-6) and nitric oxide synthase (iNOS) in lipopolysaccharide (LPS) cultured mouse macrophages. *Asian Biomedicine* (Communicated)


Book/Book Chapter


Papers in Conferences/symposium (presented)


Other Research publications not part of thesis work

Papers in SCI Journals

potential: Stability and pharmacokinetic of withania bioactives. *Food Research International (Under review-First review comment submitted)*


**Papers in Conferences/symposium (presented)**