ABSTRACT

The study consists of evaluation of preliminary phytochemical analysis for the presence of bio-active components against rheumatism from the three shortlisted ethnopharmacologically important plants *Anisomeles malabarica* R.Br., *Clerodendrum serratum* L. and *Atalantia monophylla* DC. Preliminary qualitative chemical tests for different solvent extracts confirmed the presence of carbohydrates, alkaloids, glycosides, tannins, saponins, terpenoids, phenols, oils and fats and proteins and amino acids. Oxidative stress and reactive oxygen species (ROS) plays a major role in the development of various chronic and degenerative diseases like arthritis, cancer and heart diseases. Nitric oxide (NO) is a free radical gas produced by a variety of cells and plays multiple roles in inflammation. i-NOS (inducible nitric oxide synthase) is significant in inflammation because it is induced by a number of pro-inflammatory cytokines, TNF-α being an important one. NO free radicals are also cytotoxic to mammalian cells and tissue damage in large is attributed to its toxic effects. So, in this context, the methanolic extracts of the different parts of the plants *A. malabarica*, *C. serratum* and *A. monophylla* were screened for antioxidant activity by 2, 2-diphenyl, 1-picrylhydrazyl (DPPH) and nitric oxide (NO) radical scavenging assays at various concentrations. Results revealed that the CsA extract exhibited strong DPPH and Nitric Oxide radical scavenging activity with the determined IC$_{50}$ values 13.07 ± 0.34 and 142.30 ± 3.06, whereas the CsR showed satisfactory activity against DPPH with the IC$_{50}$ value of 25.80 ± 0.89 when compared to rest of the extracts tested. It was also observed that the AmA and AmL extracts exhibited good DPPH radical scavenging activity, whereas the AmR extract showed poor antioxidant activity for the DPPH assay. Except CsA, all the other extracts showed poor nitric oxide (NO) radical scavenging assay.
After the initial screening of plants for the presence of various phytoconstituents and evaluating their antioxidant efficacies, the plant extracts were subjected to \textit{in-vitro} anti-inflammatory activity using RAW-32 and L-929 cell lines for the inhibition of LPS induced TNF-\(\alpha\) production. TNF-\(\alpha\) is interesting as TNF-\(\alpha\) is a cytokine and a pro-inflammatory mediator. The primary role of TNF-\(\alpha\) is in the regulation of immune cells. TNF-\(\alpha\) is able to induce apoptotic cell death, induces inflammation, inhibits tumors and viral replication. TNF-\(\alpha\) is found to be a major factor responsible for various inflammatory disorders like arthritis. The best among the six plant extracts from the in-vitro studies were carried forward and were subjected to the \textit{in-vivo} anti-inflammatory investigations using carrageenan-induced paw oedema model in experimental rats. The aerial parts of \textit{A. malabarica} (AmA) and roots of \textit{C. serratum} showed potent anti-inflammatory activity in reducing the inflammation when compared to the standard drug Indomethacin. So, these two extracts were subjected to purification by column chromatography and we were successful in characterizing two compounds, namely Pentadecanoic acid ethyl ester from the aerial parts of \textit{A. malabarica} and \(\beta\)-sitosterol from the roots of \textit{C. serratum} using \(^1\text{H}\)-NMR, \(^{13}\text{C}\)-NMR and IR studies.

Finally the above two isolated compounds were again studied for their anti-inflammatory properties for the inhibition of LPS induced TNF-\(\alpha\) production in RAW-32 cells. \(\beta\)-sitosterol exhibited potent anti-inflammatory activity with by reducing the LPS induced TNF-\(\alpha\) production by 74.86% when compared to the standard drug Dexamethasone, whereas, Pentadecanoic acid ethyl ester exhibited moderate TNF-\(\alpha\) modulatory effect with percentage inhibition value of 25.50%. \(\beta\)-sitosterol is one of the most ubiquitous substances in plant extracts. In the last ten years the biological role of phytosterols in human and animal health has been established, with an emphasis on their \textit{in-vitro} and \textit{in-vivo} immune modulatory activity.