Industrial Crops and Products 94 (2016) 610–620

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

In vitro anti-inflammatory and anti-oxidant activities of Sri Lankan medicinal plants

Hettiarachchige Dona Sachindra Melshandi Perera [a], Jayanetti Koralalage Ramani Radhika Samarasekera [a,*], Shiroma Mangalika Handunnetti [b], Ovitigala Vithanage Don Sisira Jagathpriya Weerasena [b]

[a] *Industrial Technology Institute (ITI), 363, Bauddhaloka Mawatha, Colombo 07, Sri Lanka*
[b] *Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, 90, Cumaratunga Munidasa Mawatha, Colombo 03, Sri Lanka*

ARTICLE INFO

Article history:
Received 28 May 2016
Received in revised form 3 September 2016
Accepted 5 September 2016

Keywords:
Medicinal plants
Arachidonate-5-lipoxygenase
Hyaluronidase
Xanthine oxidase
Nitric oxide production
Anti-oxidant

ABSTRACT

The search for novel sources of new anti-inflammatory agents has gained an increasing demand with the rising number of indications of inflammatory mediated diseases. Traditionally, medicinal plant extracts have been used to treat number of diseases mediated by inflammation and still remain as potent sources of new anti-inflammatory agents and antioxidants. Therefore, the present study was undertaken to evaluate the *in vitro* anti-inflammatory and anti-oxidant properties of ten Sri Lankan medicinal plant extracts, traditionally used to treat diseases associated with inflammation.

Arachidonate-5-lipoxygenase (A5-LOX), hyaluronidase, xanthine oxidase enzyme inhibitory assays and nitric oxide (NO) production inhibitory assay in lipopolysaccharide activated RAW 264.7 macrophages were used to evaluate *in vitro* anti-inflammatory activity. Antioxidant activity, total polyphenolic and total flavonoid contents were determined using six standard *in vitro* assays.

The extract of *Murraya koenigii* L.exhibited the highest anti-A5-LOX activity (IC_{50} = 7.83 ± 0.42 μg/mL), while that of *Symplocos cochinchinesis* Lour. showed high anti-hyaluronidase activity (69.35%) along with a high oxygen radical absorbance capacity (ORAC) (1539 ± 31 mg TE/g). The extract of *Calophyllum innophyllum* L. also exhibited high anti-hyaluronidase activity (68.45% at 500 μg/mL), xanthine oxidase inhibitory activity (60.63% at 250 μg/mL), NO production inhibitory activity (35.00% at 500 μg/mL) along with a marked 2,2-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity (IC_{50} = 4.42 ± 0.03 μg/mL) and ferric reducing antioxidant power (FRAP) (8231 ± 5 mg TE/g). Ferrous ion chelating (FIC) activity of plant extracts was found to be low in comparison with the reference standard EDTA-Na_2. Higher total polyphenol content (TPC) and total flavonoid content (TFC) were recorded for the extracts of *Cyanometra cauliflora* L. and *Murraya koenigii* L. respectively. All bio-activities were found to be significant at $p < 0.05$. In the correlations analysis, a high positive correlation was observed between anti-A5-LOX and DPPH free radical scavenging activities. Moderate, positive correlations were observed among NO production inhibitory, xanthine oxidase inhibitory activities, FRAP and ORAC of plant extracts.

This is the first report demonstrating the anti-A5-LOX, anti-hyaluronidase, xanthine oxidase and nitric oxide production inhibitory activities of ethanol extracts of the tested Sri Lankan medicinal plant extracts. The findings of the study support the traditional uses of these plant extracts against inflammatory mediated diseases. Moreover, the extracts, having promising *in vitro* anti-inflammatory and antioxidant properties could be effectively used for pharmaceutical, neutraceutical as well as for cosmaceutical applications.

1. Introduction

The search for novel sources of new anti-inflammatory agents has gained an increasing demand with the rising number of indications of inflammatory mediated diseases (Steinhilber and Hofmann, 2014). Inhibitors of arachidonate 5-lipoxygenase (A5-LOX) and hyaluronidase are considered more important as potential targets in drug discovery of mechanism based inhibitors in the treatment of allergic and inflammatory diseases (Riaz et al., 2004; Gonzaílez-Pen˜a et al., 2013). Xanthine oxidase enzyme inhibitors are recognised as important therapeutic agents to treat gouty arthritis, which is mediated by inflammation. Nitric oxide

* Corresponding author.
E-mail address: radhika@iti.lk (J.K.R.R. Samarasekera).

http://dx.doi.org/10.1016/j.indcrop.2016.09.009
0926-6690/

production inhibitors are another group of anti-inflammatory agents, which are capable of suppressing inflammation occurred due to the pro-inflammatory action of nitric oxide.

A5-LOX is a non-heme iron containing dioxygenases enzyme that catalyzes the generation of arachidonic acid metabolites such as leukotrienes (Njenga and Viljoen, 2006). Leukotrienes are liberated from leukocytes, mast cells and other A5-LOX expressing cells upon cell activation and bind to specific receptors to impart the effects. High levels of leukotrienes could be detected in many inflammatory diseases such as bronchial asthma, allergic rhinitis, cardiovascular diseases, rheumatoid arthritis and certain types of cancer (Schneider and Bucar, 2005). The therapeutic significance of anti-leukotriene therapy in asthma has already been confirmed by clinical studies and there is only one A5-LOX inhibitor in the market, zileuton for the treatment of asthma. Due to the undesirable side effects of zileuton, its applicability has been limited. Hence, there is an emerging need for novel A5-LOX inhibitors with clinical efficacy and safety (Steinhilber and Hofmann, 2014).

Hyaluronidase is the hyaluronate hydrolyzing enzyme, which has been recognized to involve in a number of physiological regulatory processes and pathological conditions including inflammation (Gonzaílez-Pen˜a et al., 2013). The *in vivo* activation of hyaluronidase enzyme by metal ions such as calcium, leads to degranulation of mast cells and thereby releases inflammatory mediators (Rahman et al., 2001). Hyaluronate is found in synovial fluid in joints and functions as a viscous lubricating agent. Excessive degradation of hyaluronate by hyaluronidase causing depletion of amount and molecular weight of hyaluronate is observed in rheumatoid arthritis. It has been identified, that hyaluronidase inhibitors are of great importance as ubiquitous regulating agents in regulating metabolism of hyaluronan, thus serving as effective therapeutic agents (Girish et al., 2009).

Xanthine oxidase is a flavoprotien, which catalyses the hydroxylation of hypoxanthine to xanthine and xanthine to uric acid (Sahgal et al., 2009). High levels of uric acid can lead to a metabolic disease called gout, which is a type of arthritis that causes inflammation due to the accumulation of monosodium urate crystals in the lining of joints and synovial fluid (Yumita et al., 2013). Recent data has indicated that, xanthine oxidase enzyme also has a pathogenetic role in various forms of other inflammatory diseases due to the formation of reactive oxygen species during the catalytic function of the enzyme. Therefore, xanthine oxidase inhibitors are recognized as appropriate candidates for various therapeutic indications accompanied by the function of xanthine oxidase enzyme. One such clinically used inhibitor is allopurinol, which is known to impart number of mild to severe side effects. Therefore there is a need for searching new inhibitors of xanthine oxidase, which are devoid of side effects (Pacher et al., 2006).

Nitric oxide (NO) is a short-lived intracellular messenger, produced by various cells including macrophages, neutrophils, platelets, endothelium, fibroblasts, neuronal and smooth muscle cells (Patel et al., 1999) NO is excessively generated by inducible nitric oxide synthase (iNOS), a pro-inflammatory enzyme, leading to inflammatory diseases such as asthma, arthritis, neurodegenerative disorders, multiple sclerosis, colitis and psoriasis (Cheenpracha et al., 2010) Thus, inhibitors of iNOS are important candidates for the treatment of inflammatory diseases, accompanied by the excessive production of NO (Yang et al., 2009).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are deleterious by products of metabolic processes cause damage to cellular biomolecules such as proteins, DNA and lipids, leading to oxidative stress (Sen et al., 2010). Oxidative stress is a major causative factor of number of chronic diseases such as cancer, aging, heart diseases, diabetes mellitus gastric problems, immune suppressions, neurodegenerative diseases etc. (Pala and Gürkan, 2008). Antioxidants exert defensive effects against oxidative stress by scavenging free radicals (Valko et al., 2007). Recent research reports have indicated that plant based antioxidants are of great importance as therapeutic agents to combat oxidative stress associated chronic diseases including inflammatory diseases (Njenga and Viljoen, 2006). Moreover, inflammatory processes also involve in the generation of free radicals inid by leucocyte activation (Fantone and Ward, 1982). Hence, screening for antioxidant potential may reveal important information about anti-inflammatory properties of drug candidates (Akula and Odhav, 2008).

Medicinal plants remain as potent sources of new anti-inflammatory agents and antioxidants (Girish et al., 2009). Plant screening leads to discover important biological properties and identify lead compounds, providing the basis for synthesis through which the bioavailability and pharmacokinetic properties could be optimized to improve the therapeutic efficiency of plant compounds (Schneider and Bucar, 2005).

Sri Lanka is considered as a biodiversity hot spot of global importance. Among the native flora of Sri Lanka, there are over 600 species that have been used as medicinal plants in traditional system of medicine. Apart from that, over 900 non-indigenous species have been used in native medicine. The demand for Ayurveda prescriptions and pharmaceutical companies are met by medicinal plants, which are of an immense economic value. In the present study, the authors have mostly attempted to exploit the *in vitro* anti-inflammatory bio activities of less exploited medicinal plants. Ten medicinal plants, *Aleurites moluccana* L., *Bacopa monniera* Linn., *Murraya koenigii* L., *Calophyllum innophyllum* L., *Psidium guajava* L., *Commelina diffusa* Burm., *Spondias dulcis* L., *Cynometra cauliflora* L., *Symplocos cochinchinesis* Lour. and *Melaleuca leucadendra* L., found in Sri Lanka were selected for the present study. Various parts of these plants have been used traditionally to treat number of ailments including inflammatory diseases (Table 1.) ◦previous studies have been reported on *in vitro* anti-inflammatory properties of the ethanol extracts of these Sri Lankan plants using A5-LOX, hyaluronidase and xanthine oxidase enzyme inhibitory activities and nitric oxide production inhibitory activities. The objective of the present study was to evaluate and compare, *in vitro* anti-inflammatory and anti-oxidant activities of ten Sri Lankan medicinal plant extracts traditionally used to treat inflammatory mediated diseases using different mechanism based assay models.

2. Materials and methods

2.1. Chemicals and equipment

Linoleic acid, A5-LOX (soybean), hyaluronidase from bovine testes, xanthine oxidase (bovine milk), xanthine, allopurinol, hyaluronic acid potassium salt from human umbilical cord, fetal calf serum (FCS), Dulbecco's modified Eagle's medium (DMEM), trypsin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), bacterial lipopolysaccharide (LPS), NG-Monomethyl-L-arginine *p*-dimethyl amino benzaldehyde, calcium chloride, sodium hydroxide, sodium borate, folin-ciocalteu reagent, gallic acid, quercetin, 6-hydroxy-2-5-7-8-tetramethylchroman-2-carboxylic acid (trolox), Ethylenediaminetetraacetic acid disodium salt (EDTA-Na_2), tannic acid, baicalein, 2,2-diphenyl-2-picryl-hydrazyl (DPPH), potassium persulphate, 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH), sodium fluorescein, 2,4,6-tripyridyl-s-triazine (TPTZ) and 4,4′-disulfonic acid sodium salt (ferrozine) were purchased from Sigma-Aldrich (USA). All chemicals and reagents used in the experiment were of analytical grade. All the bio-assays were carried out using high throughput 96-well micro-plate readers (Spectra Max