International Immunopharmacology 59 (2018) 157–167

Contents lists available at ScienceDirect

International Immunopharmacology

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

Isolation, identification and characterization of apigenin from *Justicia gendarussa* and its anti-inflammatory activity

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ARTICLE INFO

Keywords:
Atherosclerosis
Toll like receptors
Oxidized LDL
Cyclooxygenase
Human peripheral blood mononuclear cells
Apigenin

ABSTRACT

Inflammatory responses during chronic diseases such as atherosclerosis, cancer etc., are harmful to host organisms. Generally NSAIDs are used to treat against these severe conditions but due to its adverse effects studies are going on with medicinal plants, since they are rich in bioactive compounds. *Justicia gendarussa* is one such plant which has been used as a remedial measure for treating inflammatory diseases since ancient time. Thus the present study involved in the isolation, characterization and identification of apigenin (flavonoid) from this plant and to elucidate its molecular mechanism against inflammation via TLR-NF-κB signaling pathway using ox-LDL induced hPBMCs in in vitro model. Methanolic extract was used for the isolation process and results showed that the F6 fraction collected from ethyl acetate through column chromatography showed 89% paw edema inhibition at a dose of 10 mg/kg in carrageenan induced rats. Purification of F6 by TLC with toluene: chloroform: acetone (8:5:7) and further characterization by ^{1}HNMR indicated the presence of bioactive compound, apigenin. In vitro studies revealed that pretreatment of ox-LDL induced hPBMCs with apigenin (25 μM) significantly ($P < 0.05$) reduced the levels of TLR4, MyD88, TRIF, TRAF6, NF-κB, COX-2, PGE2, IL-1β and TNF-α responsible for generating inflammation and elevated the level of anti-inflammatory cytokine, IL-10. These results indicate the therapeutic efficacy of bioflavonoid apigenin which was isolated from *Justicia gendarussa* against ox-LDL induced inflammation. Therefore apigenin can be treated as a suitable therapeutic agent against inflammatory diseases.

1. Introduction

Medicinal plants and its products have been used as a therapeutic agent in most developing countries for treating diseases. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced for the extraction and development of several drugs and chemotherapeutics from these herbal plants [1] since they accumulate secondary metabolites having medicinal properties. Ethnopharmacology of natural products isolated from herbal plants possess pharmacological and therapeutic efficacy for treating diseases [2]. Since time immemorial, various herbs and their derived compounds have been used as therapeutic agents in the treatment of inflammation and related disorders like rheumatism [3]. Among them *Justicia gendarussa* (Brum. f) is one such plant having pharmacological benefits.

Justicia gendarussa is a rapidly growing evergreen shrub, mostly found in shaded moist areas. The plant has been used in traditional medicines against inflammatory disorders from ancient time but its molecular mechanism is not well elucidated. Moreover this plant have other well-known medicinal properties against various ailments such as antiangiogenic [4], anti-arthritic [5], anti-inflammatory and anti-nociceptive [6], anti-bacterial [7], anti-cancer [8]. This plant got this broad spectrum of activities due to the presence of several biologically active components such as alkaloids, carbohydrates, flavonoids, phenolic compounds, saponins, steroids, and terpenoids [9]. In traditional medicinal system, different parts of *Justicia gendarussa* have been mentioned to be useful in a variety of diseases [10]. A decoction prepared by boiling its plant roots in milk is used for treating chronic rheumatic disorders, dysuria, fever, carbuncles and diarrhea [11].

Inflammation is a beneficial response done by host organisms to an

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[2] Vidya Sabu involved in designing the pictures and tables and wrote the manuscript.
[3] G. Sindhu carried out in vivo experiments.
[4] Arun. A. Rauf participated in discussion.
[5] A. Helen given guidance for conducting experiment and review the manuscript.

https://doi.org/10.1016/j.intimp.2018.04.004
Received 23 October 2017; Received in revised form 23 March 2018; Accepted 4 April 2018

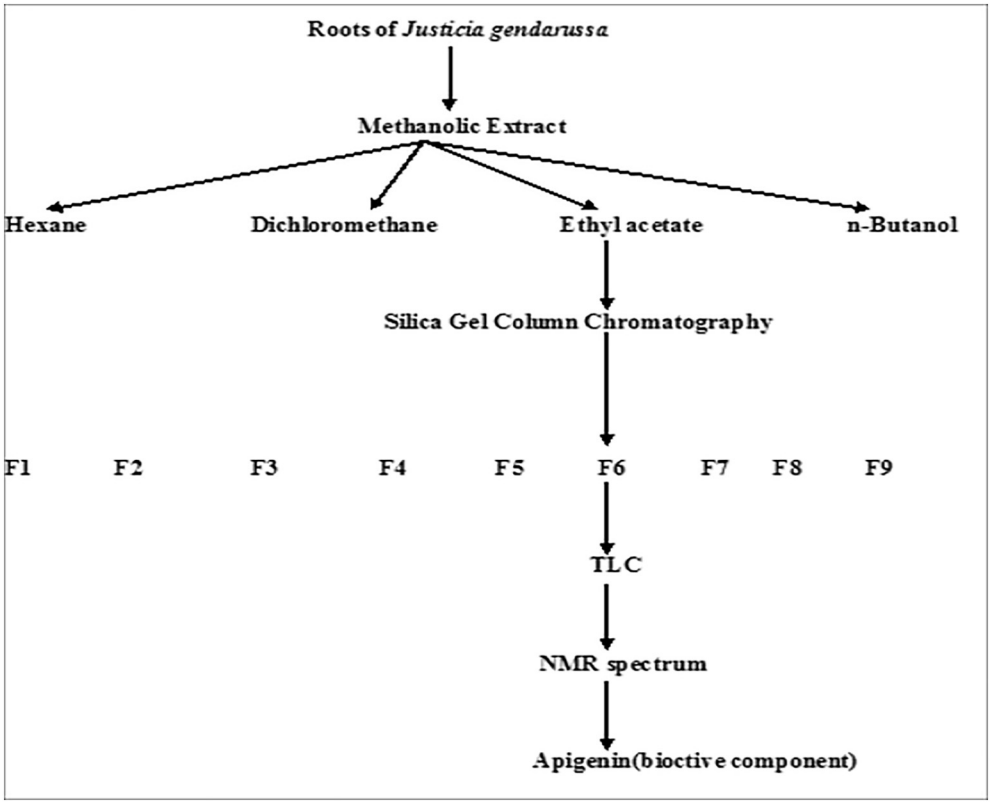

Fig. 1. Isolation of apigenin from roots of *Justicia gendarussa*.

external stimulus. During the time of an inflammatory response, releasing of inflammatory mediators helps to restore the tissue structure and its functions. But if the condition remains for a long period of time it will leads to chronic inflammatory diseases and inflammation in the arterial walls leads to the development of atherosclerosis [12]. Activation of immune cells such as monocytes and macrophages is the pioneer step leading to chronic inflammatory diseases including atherosclerosis [13]. Upon an immunogenic stimulus, inflammatory response initiate through Toll Like Receptors (TLR), since its activation results in the release of various pro-inflammatory cytokines thereby recruits various immune cells to the inflamed areas and cause inflammation. TLRs are membrane receptors on macrophages, dendritic cells; natural killer cells etc. and play a major role in the immune system [14]. Activated TLRs recruits several adapter proteins within the cytosol such as MyD88, TRIF, TRAF, IRAK etc. thereby induce signal transduction pathway by activating subsequent downstream proteins. Interaction of TLRs with adapter proteins results in the activation of transcription factor, NF-κB and initiate specific immune response. Transcription factor, NF-κB plays a major role in TLR signaling pathway to control inflammation. Both MyD88 dependent and independent (via TRIF signaling) pathway can activate NF-κB [15]. This indicates that NF-κB dependent gene requires both TRIF and MyD88 for its activation thereby regulates various genes involved in inflammatory responses [16]. Thus regulation of this transcription factor is an important factor to control severe inflammatory diseases, since activation of this factor leads to the pathogenesis of various diseases such as asthma, rheumatoid arthritis, inflammatory bowel disease [17], cancer [18–20] and atherosclerosis [21].

Previous studies in our laboratory on the methanolic and ethyl acetate fraction of *Justicia gendarussa* root showed potent anti-inflammatory effect in carrageenan and adjuvant/collagen induced arthritic animal [22]. So the present study aimed at the isolation, identification and characterization of bioactive component which is responsible for the antioxidant and anti-inflammatory effect of ethyl acetate fraction of Justicia and also investigates its molecular mechanism of anti-inflammation via TLR-NF-κB signaling pathway using ox-LDL induced hPBMCs. In this study quercetin also a flavonoid is used as standard against apigenin since it has been previously reported that quercetin excerts anti-inflammatory properties against ox-LDL induced hPBMCs via TLR-NF-κB signaling pathway. Hence we used quercetin as standard for conducting experimental studies.

2. Materials and methods

2.1. Plant material

Roots of the herbal plant *Justicia gendarussa* (Brum.f) was used as an experimental material in this study. This plant is commonly called as water willow belongs to the family Acanthaceae. *Justicia gendarussa* were collected from the medicinal garden of Poojapura Research Institute, Trivandrum, Kerala, India. The plant was identified and authenticated by Dr. G. Valsaladevi, Curator, Department of Botany, University of Kerala, Kariavattom (Voucher No. 5797).

2.2. Extraction, identification and characterization of apigenin from roots of Justicia gendarussa

2.2.1. Preparation of Methanolic extract of Justicia gendarussa (JRM)

Roots of *Justicia gendarussa* were collected, cleaned, dried and then powdered. About 50 g of the powdered material was weighed and defatted using petroleum ether (60-80 °C) and then extracted with methanol. This was then dried and used as the Methanolic extract of *Justicia gendarussa* and got a yield of 3.68 g with respect to plant material taken.

2.2.2. Solvent fractionation of JRM

25 g of JRM was weighed and diluted with distilled water. Upon polarity basis, diluted material was partitioned successively using the