

Novel chemical constituents with anti-inflammatory activity from the leaves of *Sesbania aculeata*



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ABSTRACT

From the hexane and ethyl acetate extracts of the leaves of *Sesbania aculeata*, three novel chemical compounds were isolated and fully characterized as compound **1**, (ceramide type); compound **2**, (cerebroside type) and compound **3** as a triterpene acid 3-O- α -L-rhamnopyranoside along with nine known compounds (Tricontanol, Lauric acid, Palmitic acid, Heptadecanoyl-1-tridecanoic acid, β -sitosterol, stigmasterol, poriferasterol glucoside, ononitol and pinitol). The anti-inflammatory potential of all three compounds were evaluated using *in vitro* target based anti-inflammatory activity in LPS-stimulated macrophages. TNF- α is one of the mediators of various chronic inflammatory disorders and treatment of hexane leaf extract (**HL**), Ethyl acetate leaf extract (**EAL**) and compounds **1**, **2** and **3** at a dose of 10 μ g/mL showed significant ($P < 0.001$) inhibition of TNF- α , a pro-inflammatory cytokine. IL-6 was significantly ($P < 0.05$) inhibited by compound **1** and **HL** at a dose of 10 μ g/mL as compared with vehicle treatment. In-vitro cell cytotoxicity study using MTT assay revealed that these compounds were non toxic to the normal cells.

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Introduction

Inflammation is caused by living tissues being subjected to some kind of tissue injury. In turn, the living tissues respond to the injury in a very complex way, depending on its severity. A local response at the site of injury is often associated with the physiological process of inflammation and the damage tissues undergo changes to allow the influx of plasma and phagocytic cells at the place of injury. This early local response of injured tissues is called acute inflammation. Therefore, acute inflammation is a short-term response that usually results in healing. The mechanism involves in the process showed that leukocytes infiltrate to the damaged region, removing the stimulus and repairing the tissues. If acute inflammation persists for a longer period, it changes into chronic inflammation. Chronic inflammation, by contrast, is a prolonged, dysregulated and maladaptive response that involves active inflammation, tissue destruction and attempts at tissue repair. Such persistent inflammation is associated with many chronic human conditions and diseases, including allergy, atherosclerosis, cancer, arthritis and autoimmune diseases. To overcome the challenges of inflammatory disorders, several classes of anti-inflammatory drugs have been used. These include Non-steroidal anti-inflammatory drugs (NSAIDs), Immuno Selective

Anti-Inflammatory Derivatives (ImSAIDs), corticosteroids etc. (Gautam and Jachak, 2009). In general, the mechanism of action of anti-inflammatory drugs were several, they act by interfering with the immunological mechanisms, start cellular activities like phagocytosis or interfere with the formation and release of the chemical mediators of inflammation (Lemke et al., 2008).

Plant derived natural products play a vital role in the area of medicinal chemistry which offers a vast reservoir of secondary metabolites having pharmacological actions on hepatic, cardiovascular, cancer, inflammatory and central nervous system (CNS) disorders of human beings. The majority of secondary metabolites like alkaloids, flavonoids, terpenoids, phytosterols, phenylpropanoids, coumarins, amines, phenols etc., and their other analogues showed potent anti-inflammatory activity and some established themselves as clinical agents for arthritic disease like colchicine alkaloid, some flavonoids also act against formalin induced oedema in the mouse hind paw. Therefore, plant based natural compounds play a significant role in the development of anti-inflammatory drugs in the pharmaceutical industry which can serve as good lead molecules suitable for further modification during the drug development process (Chin et al., 2006).

Sesbania aculeata (Family Fabaceae) has several synonyms like *Sesbania cannabina* and *Sesbania bispinosa* in English, Jayanti and Dhunchi in Sanskrit and Hindi, and most commonly it is called as dhaincha in Oriya. In appearance the plant may be herbs, shrubs

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and soft wooded but short lived trees distributed in the tropical regions of the world. Dhaincha belongs to the legume family and is an ideal green manure crop as it is quick growing, succulent, easily decomposable with low moisture requirements and produces maximum amount of organic matter and nitrogen. Like all legumes, it also fixes atmospheric nitrogen with its root nodules. The herb is soil tolerant and has a bio-fertilizer potential (The Wealth of India, 1972). Chemical analyses of the leaves showed the presence of moisture, protein, fibre and ash. Leaf, stem and fruit gave positive test for alkaloids (William and Schubert, 1961). A mixture of saponins, reported to be present in the seeds, obtained on hydrolysis of oleanolic acid and a neutral sapogenin (Sen and Ray, 1964). Seeds mainly yield fatty oil. The fatty acid composition of the oil is as follows: palmitic acid, stearic acid, oleic acid and linoleic acid. The seed protein consists of mainly amino acids like: isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine (Kapoor et al., 1992). Dhaincha seeds and fibre have been systematically investigated to yield galactomannans, lignins and cellulose (Farooqi and Sharma, 1972; Kapoor et al., 1989; Mazumdar et al., 1973; Salpekar and Khan, 1997). The thiolignins isolated from the wood are composed of guaiacyl, syringyl and p-hydroxyphenylpropane. The root, stem and leaves of *S. aculeata* have nutritional value to the soil, and the preliminary chemical investigation of the plant showed the presence of chemical constituents such as tricontanol, poriferasterol, β -sitosterol glucoside, poriferasterol glucoside, heptadecanoyl-1-tridecanoic acid, 5,12-dihydroxyoctadec-6(Z)-enoic acid, 14,20-dihydroxycos-6(Z)-enoic acid, pinitol (an antidiabetic compound) (Misra and Siddiqi, 2004, 2005) sucrose, 14-hydroxytetradec-11(Z)-enoic acid, 16-hydroxyhexadec-13(Z)-enoic acid and 14-hydroxyoctadec-12(Z)-enoic acid (Misra and Siddiqi, 2005). A new hydroxyl fatty

acid was reported from the seed oil of *S. aculeata*. The structure was established on the basis of spectral data and it was named as (Z)-12-hydroxyoctadec-9-enoic acid (ricinoleic acid) (Parveen and Rauf, 2008). Also a new flavone glycoside, 5,7,8-trihydroxy-6,4'-dimethoxyflavone-7-O- α -rhamnopyranosyl-(1 \rightarrow 4)-O- β -xylopyranosyl-(1 \rightarrow 4)-galactopyranoside, isolated from the stems of *S. aculeata* which showed potent antioxidant activity with the IC₅₀ values of 52.40 μ g/mL and 60.15 μ g/mL, respectively (Satnami and Yadava, 2012).

Therefore, the earlier investigation on the leaves, stem and roots of the plant afforded some biologically active inositol, sterols, flavonoids and lipid derivatives. However, the detailed chemical investigation of the plant has not been done. In this paper, we are going to describe the isolation and structure determination of three novel molecules **1**, **2** and **3** from *S. aculeata*. Moreover, the anti-inflammatory activities of the three new molecules are also reported in this paper.

Results and discussion

Isolation and structure elucidations

As a part of our ongoing efforts to discover structurally novel and bioactive natural compounds from a plant having bio fertilizer potential called *S. aculeata*, we have isolated and identified three novel chemical constituents for the first time from this particular species. The initial screening of hexane and ethyl acetate extracts of the leaves for anti-inflammatory activity showed some potential results and therefore further chemical investigation of both the extracts were carried out. The hexane and ethyl acetate extracts were

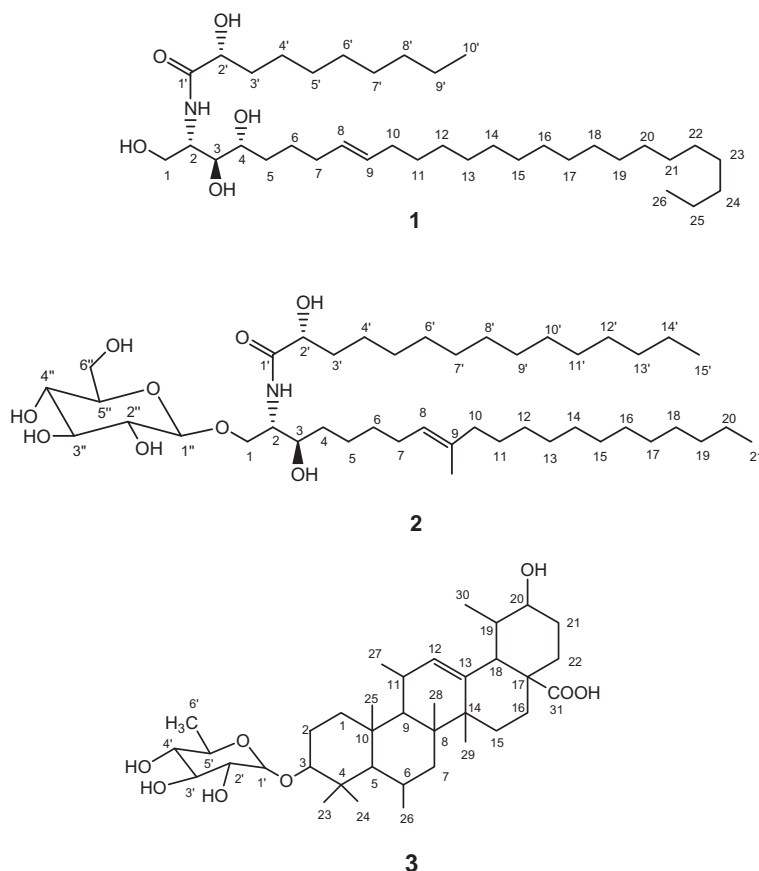


Fig. 1. Chemical structures of compounds 1–3.

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