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Anti-ulcer constituents of *Annona squamosa* twigs ☆

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ABSTRACT

Phytochemical investigation of *Annona squamosa* twigs, resulted in isolation and identification of twelve known (**1**–**12**) compounds among them one 1-(4-*β*-*D*-glucopyranosyloxyphenyl)-2-(*β*-*D*-glucopyranosyloxy)-ethane (**11**) is synthetically known but first time isolated from natural sources. Their structures were elucidated using 1D and 2D NMR spectroscopic analysis. The isolated compounds (**2**–**8**, **11**) were evaluated for H^+ K^+-ATPase activity. Three of these compounds (+)-O-methylarmepavine (**2**), N-methylcorydaldine (**3**), isocorydine (**6**) showed promising anti-secretory activity. Activity of these compounds, comparable to the standard drug omeprazole is novel to our finding. Moreover, there is no information accessible regarding the pharmacological effect of *A. squamosa* on the gastrointestinal system. This study is the first of its kind to show the significant anti-ulcer effect of *A. squamosa*. The present study aimed to evaluate the gastroprotective effect of *A. squamosa* (AS) and to identify its active constituents. Anti-ulcer activity was evaluated against cold restraint (CRU), pyloric ligation (PL), aspirin (ASP), alcohol (AL) induced gastric ulcer and histamine (HA) induced duodenal ulcer model and further confirmed through *in vitro* assay of H^+ K^+-ATPase activity and plasma gastrin level. AS and its chloroform and hexane fraction attenuated ulcer formation in CRU, PL, HA model and displayed anti-secretory activity *in vivo* through reduced free, total acidity and pepsin in PL, confirmed by *in vitro* inhibition of H^+ K^+-ATPase activity with corresponding decrease in plasma gastrin level. Cytoprotection of AS was apparent with protection in AL, ASP models and enhanced mucin level in PL.

1. Introduction

Annona squamosa, belongs to the family *Annonaceae* and is commonly known as sugar apple. It is a fruit tree native to Central America and is now cultivated throughout tropics mainly for its edible fruit. The taste of the fruit pulp is sweet because of its high sugar content (58% of dry mass), and it is clear that the fruit pulp contains a high calorie value [1]. This plant is reputed to possess several medicinal properties [2]. Folkloric record reports its use as an insecticide and an anti-tumor agent [3], anti-diabetic [4], anti-oxidant, anti-lipidimic [5], and anti-inflammatory activities due to the presence of cyclic peptides [6]. In addition, the crushed leaves are sniffed to overcome hysteria and fainting spells, and they are also applied on ulcers and wounds. A leaf decoction is taken in case of dysentery [6].

The previous phytochemical investigation of this plant has proved that it has a variety of compounds like acetogenins which are responsible for antifeedant, antimalarial, cytotoxic and immunosuppressive activities [7,8]. Diterpenes isolated from the title plant have anti-HIV principle and anti-platelet aggregation activity [9,10]. The partially purified flavonoids reported from the same source are responsible for antimicrobial

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and pesticidal activities [11]. Some lignans and hydroxyl ketones are also found in this plant [12,13]. The number of alkaloids reported from this plant belongs to different groups such as aporphine [14,15], and benzoquinazoline [7]. The above provided evidences suggest that the plant is known for its various medicinal values, but to the best of our knowledge this plant is not yet, known for its anti-ulcer activity.

Peptic ulcer disease (encircling gastric ulcer and duodenal ulcers) affects a large population of the world. It is now generally agreed that gastric lesions develop when the delicate balance between some gastroprotective (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) and aggressive factors (acid, pepsin, and *Helicobacter pylori*) is lost [16]. Hypersecretion of gastric acid is a pathological condition, which occurs due to uncontrolled secretion of hydrochloric acid from the parietal cells of the gastric mucosa through the proton pumping H^+ K^+-ATPase. Modern approach to this includes proton pump inhibitors, histamine receptor blockers, and drugs that affect the mucosal barrier and prostaglandin analog, but there are reports of development of tolerance, incidence of relapses and side effects on clinical evaluation that make their efficacy arguable. This has been the basis for the development of new anti-ulcer drugs, which includes herbal drugs.

As a part of our ongoing studies aimed to phytochemically and pharmacologically characterize the title plant, we found that EtOH extract of twigs of *A. squamosa* showed significant protection against cold restraint induced ulcer model in rats. Therefore, we decided to carry out a detailed study aimed to investigate the chemical composition of *A. squamosa*. Chloroform fraction yielded twelve compounds 1-tritriacontanol (**1**) [17], (+)-O-methylarmepavine (**2**) [15], N-methylcorydaldine (**3**) [18], lanuginosine (**4**) [19,20], (+)-anomuricine (**5**) [21], isocorydine (**6**) [15], N-methyl-6,7-dimethoxyisoquinolone (**7**) [22], 6,7-dimethoxy-2- methylisoquinolinium (**8**) [23,24], β-sitosterol (**9**) and β-sitosterol-3-O-β-*D*-glucopyranoside (**10**) [25], 1-(4-β-*D*-glucopyranosyloxyphenyl)-2-(β-*D*-glucopyranosyloxy)-ethane (**11**) [26] and rutin (**12**) [27] (Fig. 1). Among these, compounds **1**, **3**, **5**, **7**, **8** and **11** have been isolated for the first time from the title plant. The known compounds were identified by using spectroscopic methods including, mass, 1D and 2D NMR analysis and also by comparison data already reported in the literature. Thus, the present study aimed to investigate the anti-ulcer activity of ethanolic extract of *A. squamosa* twigs against different experimental gastric and duodenal ulcer models and to identify the active constituents through bioassay-guided fractionation.

2. Materials and methods

2.1. General procedures

Optical rotations were measured on a Perkin-Elmer model 241 digital polarimeter. UV spectra were obtained on a Perkin-Elmer λ-15 UV spectrophotometer. IR spectra were recorded on a Perkin-Elmer RX-1 spectrophotometer using KBr pellets. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX 300MHz NMR spectrometer. ESMS on an Advantage Max LCQ Thermo-Finnigan mass spectrometer and FABMS were carried out on a JEOL SX 102/DA-6000 mass spectrometer. CC was performed using silica gel (230–400 mesh). TLC was carried out on precoated silica gel plates 60

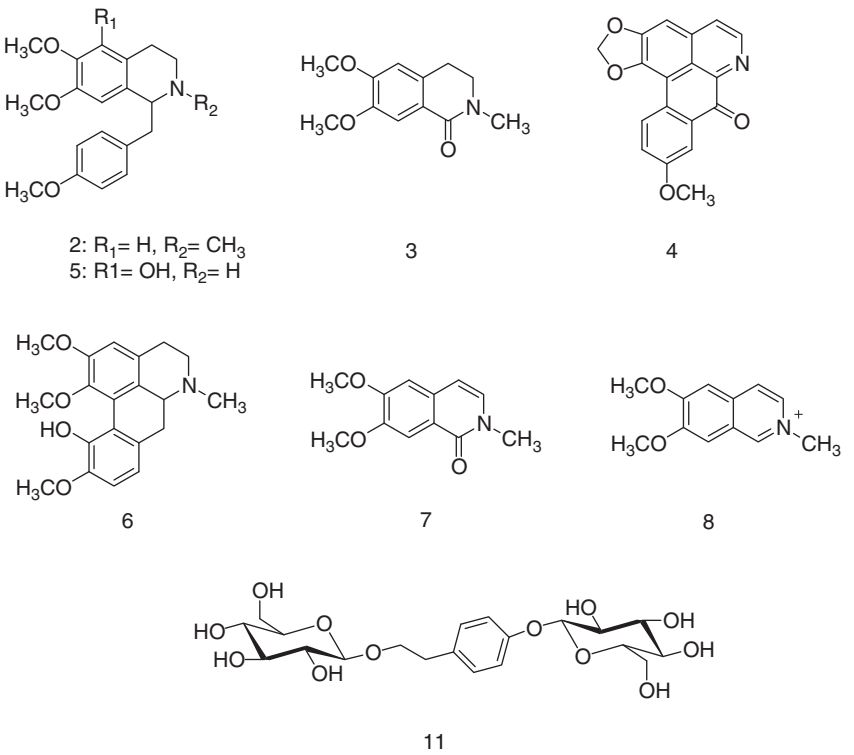

Fig. 1. Structure of chemical constituents isolated from *Annona squamosa*.