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Original Article

Antifungal and antioxidant activities of organic and aqueous extracts of *Annona squamosa* Linn. leaves

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ABSTRACT

An increasing demand for natural additives has shifted the attention from synthetic to natural antioxidants and antifungal agents. This study was carried out to evaluate the antifungal and antioxidant activities of methanol, chloroform, and aqueous extracts of *Annona squamosa* Linn. leaves. The antifungal activities of all extracts of *A. squamosa* leaves against five different strains of fungi (*Alternaria alternata*, *Candida albicans*, *Fusarium solani*, *Microsporum canis*, and *Aspergillus niger*) were evaluated by the agar well diffusion method and the minimum inhibitory concentration of each extract was assessed by antifungal susceptibility using the broth microdilution method. The antioxidant potential of each extract was determined by free radicals (1,1-diphenyl-2-picrylhydrazyl, nitric oxide, and hydrogen peroxide) scavenging activity and reducing power property of *A. squamosa* leaves. Both organic and aqueous extracts were found to express dose-dependent inhibition against all tested fungi strains in both agar well diffusion and broth dilution methods. The free radical scavenging activity and reducing power property of all extracts were found to be concentration dependent, with the methanol extract exhibiting higher antioxidant activity than the chloroform extract, which was more effective than the aqueous extract of *A. squamosa* leaves. Results of phytochemical analysis of extracts showed the presence of glycosides, saponins, tannins, flavonoids, phenols, etc. The results obtained from *in vitro* studies of antifungal and antioxidant activities clearly suggest that the methanol, chloroform, and aqueous extracts of *A. squamosa* leaves possess antifungal and antioxidant activity.

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1. Introduction

In modern times, the use of herbal products has significantly increased in the developed countries as well as in several other countries. According to a World Health Organization estimate, 80% of the world's population presently uses herbal medicine for some aspect of primary health care [1]. Many of the species are used in traditional medicines for the treatment of a variety of diseases [2]. During the past several years, there has been an increase in the incidence of fungal infections due to the rise in immunocompromised population (e.g., organ transplant recipients and patients with cancer or human immunodeficiency virus infection/acquired immune deficiency syndrome). This fact coupled with the resistance to antibiotics and with toxicity during prolonged treatment with several antifungal drugs has been the reason for an extended search for newer drugs to treat opportunistic fungal infections. Infectious diseases, particularly skin and mucosal infections, are common in most of the tribal inhabitants due to lack of sanitation, potable water, and awareness of hygienic food habits. An important group of these skin pathogens is the fungi, among which dermatophytes and *Candida* spp. are prominent. Antimicrobial properties of certain Indian medicinal plants were reported based on folklore information and few studies were carried out on the inhibitory activity of these plants against certain pathogenic fungi [3].

Annona is the second largest genus of flowering plants in the family Annonaceae after *Guatteria*. *Annona squamosa* Linn., one of the important medicinal plants, commonly called "custard apple," is a well-known plant of this family. It has been reported to possess a wide variety of pharmacological activities and is used in traditional applications [4]. *A. squamosa* is cultivated throughout India, America, Brazil, Southern Florida, and West Indies mainly for its edible fruits [5]. The plant *A. squamosa* Linn. is commonly called custard apple (English), sharifa (Hindi), sitappalam (Tamil), sita phalamu (Telugu), and sitaphala (Kannada) [4]. *A. squamosa* is a small tree that grows up to 3–8 m, with broad, irregularly spreading branches of light brown bark having thin leaves that occur singly, measuring 5–17 cm in length and 2–6 cm in width. Flowering (greenish yellow flowers on a hairy, slender 2-cm long stalk) occurs during the period from spring to early summer and flowers are pollinated by nitidulid beetles. The round or heart-shaped greenish yellow, ripened aggregate fruit is pendulous on a thickened stalk. The pulp of the fruit is white-tinged yellow, edible, and sweetly aromatic. Each carpel contains an oblong, shiny and smooth, dark brown to black, 1.3–1.6-cm long seed (Fig. 1) [6]. Extensive biological research was carried out on this plant because of the presence of valuable annonaceous acetogenins in various parts of the plant, which are traditionally used for the treatment of many ailments [5]. So far, there are no systematic studies on the *in vitro* antifungal activity of the methanol, chloroform, and aqueous extracts as well as on the antioxidant effect of the chloroform extract of *A. squamosa* leaves. Therefore, this investigation was carried out to evaluate the antifungal and antioxidant potential of three different extracts of *A. squamosa* leaves.

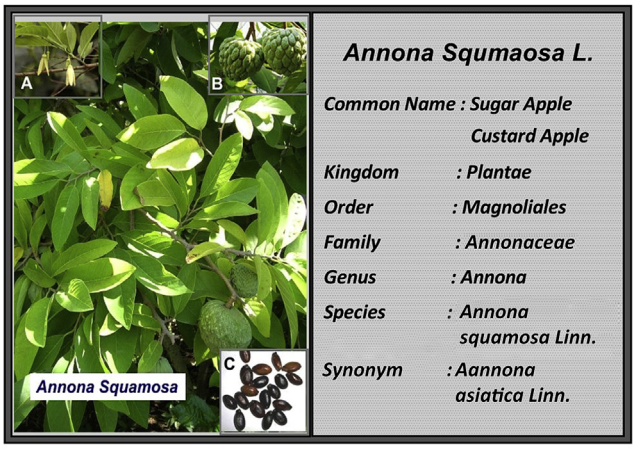

Fig. 1 – *Annona squamosa* Linn. plant: (A) flowers; (B) fruits; (C) seeds [7–10].

2. Materials and methods

2.1. Chemicals and solvents

All the chemicals used in this study were of analytical reagent grade from SD Fine Chem Ltd. (Mumbai, Maharashtra, India), whereas, sabouraud 4% glucose agar and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were from Sigma-Aldrich (Bangalore, Karnataka, India).

2.2. Collection of plant material

The leaves of *A. squamosa* were collected from fields of Dharwad, Karnataka, India, in February 2010. The leaves were identified and authenticated at the herbarium of the Botany Department of the Gulbarga University (voucher specimen HGUG No. 0019), Karnataka, India. After identification, the plant material was processed for extraction procedure.

2.3. Preparation of the plant extract

The leaves of *A. squamosa* were thoroughly cleaned with water to remove dust particles and shade-dried at room temperature and reduced to coarse powder using a mechanical mixer. The powder was subjected to extraction by maceration using water, chloroform, and methanol to obtain their respective extracts. To 75 g of the powdered drug, 750 mL of solvent (water or chloroform or methanol) was added and stirred occasionally. The mixture was filtered on the 8th day, and the solvent was evaporated at 40°C to obtain a solid mass [11]. The percentage yields of water, chloroform, and methanol extracts were found to be 8.7% w/w, 10.1% w/w, and 12.6% w/w, respectively, which are stored in refrigerator (−40°C) until further use.

2.4. Preliminary phytochemical screening

All extracts were subjected to preliminary qualitative phytochemical screening to detect the presence of glycosides, alkaloids, amino acids, flavonoids, carbohydrates, saponins, phenols, steroids, and tannins by standard methods [12,13].