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Research Article

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Phytochemical screening and chromatographic identification of acetogenin in *Annona glabra* L. leaves

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Abstract

Annona glabra L. belongs to the family Annonaceae. Annonaceae commonly known as custard apple family with more than 2500 species and 130 genera. Some members of annonaceae have been described as cytotoxic and they are used as folk medicine and some of the tropical countries used in the treatment of tumors and cancers. The study aims to integrate the scientific studies that describe the phytochemical compounds from *Annona glabra* L. leaves by incorporating the advanced methodology. Recently a bioactive compound named acetogenin has aroused considerable interest. Annonaceous acetogenins from annonaceae family are anticipated as a possible source for future antitumor drugs. The acetogenin fractions present in the leaves of *Annona glabra* L. were identified using Kedde's reagent and isolated by column chromatography. *Annona glabra* L. is a rich source of phytocompounds, which can be well exploited in the area of drug discovery and therapeutics.

Keywords: *Annona glabra* L., Phytochemical screening, Annonaceous acetogenin, Thin layer chromatography, Column chromatography.

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Introduction

Nature has been a source of traditional medicine for thousands of years and a splendid amount of modern drugs have been isolated from natural sources (Gavamukulya *et al*., 2017). The term natural products includes a broad range of chemical compounds derived from biological source Hamann (2006). Modern strategies for research in the area of natural products such as bioassay-guided isolation and identification of active compounds from natural sources have evolved quite significantly over the last few decades (Suri *et al*., 2007).

Annona glabra L., popularly known as Pond Apple is a tropical fruit tree in the family Annonaceae. It is a natural introduction to the mangroves of Southern Kerala and has widely spread along backwaters. *Annona glabra* L. is a small, woody tree that grows to 3-6 m high and can reach upto a height of 12m. Great efforts are being made to reverse this trend by screening of medicinal plants from the traditional system of medicine. The members the family Annonaceae family is a rich source of secondary metabolites with high biological activities (Hoang Le Son and Nguyen Thanh Tram, 2013). Medicinal plants and the phytocompounds have received more attention in recent years due to their potential role in preventing many human disease (Bhavani and Rathinavel, 2021). Many active compounds have been found in *Annona glabra* L. recently a novel class of bioactive compounds called annonaceous acetogenins has aroused tremendous interest (Kojima and Tanaka, 2009). Annonaceous acetogenin constitute a unique class of compound C_{35}/C_{37} natural products derived from polyketide pathway widely distributed in Annonaceae family (Gavamukulya *et al*., 2017). Acetogenins possess a broad range of potent biological properties such as cytotoxic, antitumoral, antiparasitic, antimicrobial, immunosuppressive, neurotoxic and pesticidal properties (Castillo *et al*., 2010). The ability to inhibit mitochondrial complex I which is a main gate of ATP production has helped to promote acetogenins as a promising candidates for the development of a new class of antitumor drugs (Degli Esposti *et al*., 1994).

Chromatographic techniques have a major role in the discovery of novel drugs of pharmaceutical and biomedical importance (Sanjeet Kumar *et al*., 2013). Chromatography is a collective term used for the separation of mixtures into their components within a stationary phase and mobile phase. The components are separated based on the differential partitioning between the stationary phase, mobile phase and the molecular weight. Thin Layer Chromatography is used in the identification of compounds to find out the concentration of active substance in the drugs (Sanjeet Kumar *et al*., 2013). Column chromatography is one of the most popular separation techniques for biologically active secondary metabolites from plant sample (Vivek *et al*., 2022). The objective of the work is to identify the acetogenin from *Annona glabra* L. leaves TLC technique and to isolate the acetogenin fraction by using open column chromatography.

Materials and Methods

Sample collection and authentication

Leaves of *Annona glabra* L. were collected locally from Kumarakom, Kerala. Leaves were authenticated by Kerala Forest Research Institute, Peechi, Thrissur, India: voucher specimen number 17686 is deposited at the herbarium. The leaves were washed thoroughly using distilled water, shade dried in open air, homogenised to fine powder and stored in air tight bottles.

Sample preparation

14 g of powdered leaves were extracted with 250 ml 95% ethanolic extract by using soxhlet apparatus. The extracts were concentrated by evaporation of solvents. The concentrated crude extract was used for further analysis.

Phytochemical Screening

The crude plant extracts were used for various biochemical and phytochemical analysis using standard procedures described in (Trease and evans, 1989).

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Identification of acetogenin by using kedde reagent

Annona glabra L. leaves were dried and powdered using mechanical blender. 50g powder was dissolved in 95% ethanol and kept for 5 days. Using a rotary evaporator ethanol was evaporated and the sludge was redissolved in acetone. The solution was filterated by using a Buchner funnel with silica gel 60 on a filter paper. Solvent extraction was carried out using various solvents such as ethanol, ethyl acetate and water. Analytical TLC was carried out on silica gel G as stationary phase and chloroform-methanol (9:1) as mobile phase. The developed plates were sprayed with Kedde's reagent to develop the characterstic color of acetogenin. Kedde's reagent was prepared by mixing equal volumes of 2% solution of 3, 5-dinitrobenzoic acid in ethanol and 5.7% solution of potassium hydroxide in ethanol. The reagent developed a pinkish purple color spot in the TLC plate which indicated the presence of Acetogenin (Luna *et al*., 2007; Vinothini and Lali Growther, 2016).

Isolation of acetogenin by Column chromatography

A chromatography column of 30cm column was used to fractionate the sample. The sample was loaded in the chromatography column packed with silica gel 60 as the stationary phase. Solvents used as elutents were hexane, hexane-chloroform (8:2), hexane-chloroform (1:1) and ethyl acetate. The fractions obtained were rechromatographed on TLC sheet with silica gel G as stationary phase and chloroform methanol (9:1) as mobile phase for further confirmation. The pink colour spot developed on TLC plate by dipping Kedde's reagent confirmed the presence of acetogenin in the separated fraction. (Luna *et al*., 2007; Vinothini and Lali Growther, 2016).

Results and Discussion

Medicinal plants are a rich source of many bioactive natural compounds eliciting many beneficial health benefits to human beings. Traditional medicines support *Annona glabra* L. as sources of novel bioactive drug with a wide range of biological and pharmacological properties (Hien *et al*., 2015).The screening of phytochemicals from the medicinal plants is very important in identifying and characterizing the pharmaceutically important drugs.

Phytochemical screening of ethanolic extracts of *Annona glabra* L. leaves.

The qualitative phytochemical analysis of the *Annona glabra* L. leaf extract exhibited the presence of phenols, flavonoids, steroids, tannins, glycosides and saponins. Phytochemical screening (Table 1) using the ethanolic extracts of leaves of *Annona glabra* L. showed the presence of most of the phytochemicals expect alkaloids and terpenoids. Similar results were showed in the study of ethanolic leaves extract of *Annona glabra* L. conducted by Hoang le son and Nguyen Thanh Tram, 2015.

Table 1: Qualitative phytochemical screening of *Annona glabra* L. ethanolic leaves extracts

Sl.No.	Phytochemical Test	Results
1	Alkaloids	-
2	Flavonoids	+++
3	Saponin	++
4	Steroids	++
5	Phenols	+++
6	Terpenoids	-
7	Tanins	++

(+++: Stronly present); (++: Moderately present); (+: Present); (-: Absent)

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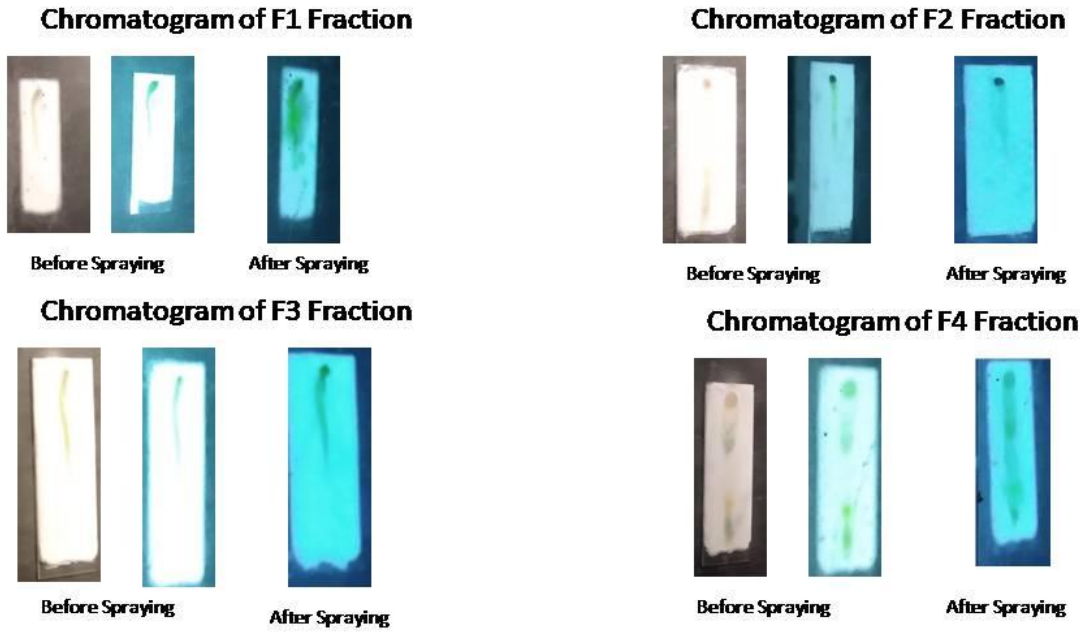
Identification of acetogenin by using kedde's test

All the fractions obtained by column chromatographic seperation were rechromatographed on TLC plate coated with silica gel G as stationary phase and chloroform-methanol (9:1) as mobile phase. The fractions obtained in column chromatography were F1 – H_2O, F2 – H_20 : EtOH (7:3), F3 – H2O : EtOH (1:1), F4 – [EtOH + EtOH : EtOAc (1:1) + EtOAc]. F4 fraction developed a pinkish purple color spot on TLC plate after spraying Kedde's reagent which indicates the presence of acetogenin. F1, F2, F3 fractions did not showed any pink spot which indicate the absence of acetogenin. Similar study was conducted in other species of *Annona* L. by Vinothini and Lali Growther, 2016 which showed the pinkish purple spot in F4 fraction. This indicated the presence of acetogenin, while other fractions were negative.

Fig :1 Chromatogram of *Annona glabra* L. leaves using the fractions F1, F2, F3, F4



Isolation of acetogenin by column chromatography fractions

The F4 fraction which showed the positive result on reaction with Kedde's reagent was further separated by column chromatography. Silica gel 60 used as stationary phase and hexane, chloroform and ethyl acetate solvents were used as the eluents (Table 2). Ethyl acetate solvent fraction showed the presence of acetogenin with Kedde's reagent which forms a pinkish purple, while other solvent fractions have not showed any colour changes. It is confirmed that the ethyl acetate fraction alone contain acetogenin compound. It was further confirmed by using TLC with silica gel G as stationary phase and chloroform – methanol (9:1) as mobilie phase. The studies of Vinothini and Lali Growther, 2016 also showed the presence of acetogenin in ethyl acetate solvent which was confirmed it by rechromatography on TLC.

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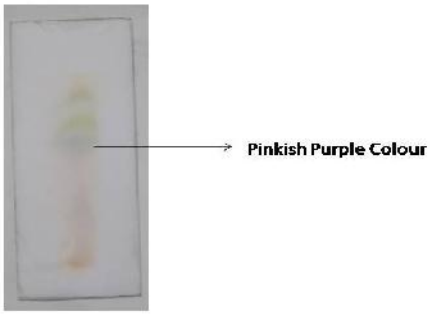
Table 2: Column Chromatogrphy fractions

Sample	Eluent	Color	Kedde test
F4	Hexane	Green	-
	Hexane-chloroform (8:2)	Green	-
	Hexane-chloroform (1:1)	Yellowish green	-
	Ethyl acetate	light green	+

Fig 2: Chromatogram of ethyl acetate fraction



Conclusion

Acetogenins in the *Annona glabra* L. leaves were fractionated by using open column chromatography and identified by using Kedde's reagent. This study shows that acetogenin from *Annona glabra* L. leaves could be fractionated and enriched using the present open column chromatography. The phytochemical compound acetogenin was identified by using Kedde's reagent the presence of the compound was reconfirmed by different chromatographic techniques. The preliminary study form a platform for further pharmacological and therapeutical studies. The therapeutic potential of *Annona glabra* L. leaves can be exploited well in various areas of drug development and discovery.

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