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Preliminary Phytochemical Screening and FTIR studies of Soursop (Annona muricata L.) bark

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ABSTRACT

The present study deals with the phytochemical examination, functional group identification of soursop (Annona muricata L.) bark extract were investigated. Qualitative phytochemical analysis of the different solvents like chloroform, methanol, acetone, petroleum ether and ethyl acetate were used for extraction of chemical compounds and the extracts were subjected to phytochemical screening which showed the presence of various phytochemicals. Methanol extract of bark showed the presence of coumarins, glycosides, saponin, tannins, phenols, terpenoids, anthraquinones, steroids, phytosterols, cardiac glycosides, oil and gum mucilage. Minimum number of active constituents was noticed in petroleum ether extract. All extracts of plant bark contains tannin, steroids, phytosterols, gum in the mucilage active constituents whereas alkaloids, flavonoids, carbohydrates, proteins and amino acid were absent in all the extracts. The FT-IR analysis of Annona muricata bark extract was done and the functional groups associated were determined. The absorption peaks observed for 3441.41 cm-1 for intermolecular hydrogen bonding in N-H stretching asymmetric O–H stretching vibration mode corresponds to alcohol and amines.

KEYWORDS : Annona muricata, Phytochemical, FTIR, Functional groups

Introduction

Soursop, botanically known as *Annona muricata* L. is a commercially under-utilized perennial tree species. It has been used as a folkloric herbal medicine in many regions throughout the world . All parts of the soursop (*Annona muricata*) tree are used in natural medicine in the tropic including the bark, leaf, root, fruit and seeds. Different properties and uses are attributed to the different parts of the tree. The bark, leaves are considered sedative and antispasmodic (George and Pamplona, 1999). Flower or flower bud tea is mixed with honey for colds, chest pain and nerve disorders, and the bark and young fruits, which contain tannin, are used to treat diarrhoea and dysentery. The green bark is rubbed on wounds to stop bleeding (Orwa *et al*., 2009). In the Peruvian Amazon, the bark, root and leaves are used for diabetes and as a sedative and antispasmodic. Indigenous tribes in Guyana use a leaf and /or bark tea as a sedative and heart toxic (Pier, 2008). Phytochemicals are known to exhibit a variety of pharmacological actions in human body (Akinmoladun *et al*., 2007). *Annona muricata* are used extensively by local practitioners in therapeutic formulations to treat a variety of human diseases. Phytochemical techniques played a significant role in searching raw materials and resources for pharmaceutical industry. Fourier transform infrared spectrometry (FTIR) can be used to identify the structure of unknown composition or its chemical group, and the intensity of the absorption spectra associated with molecular composition or content of the chemical group(Surewicz *et al*., 1993). At present, particularly in phytochemistry, FTIR has been exercised to identify the concrete structure of certain plant secondary metabolites (Yang *et al*., 2002). Keeping this in view, the present study has been undertaken to investigate the phytochemical constituents present in various extract of *Annona muricata* bark.

Materials and methods

Collection and authentication of the plant material

Fresh bark of *Annona muricata* Linn. (Annonaceae) collected from Coimbatore district in the year 2013 was further authenticated and certified by the taxonomist of Botanical Survey of India (BSI), Coimbatore -641 003, Tamil Nadu.

Chemicals

All the chemicals used were of Analytical Reagent grade obtained from Hi-media, Qualigens, E-Merck and Sigma Aldrich.

Extraction of Sample

Solvent systems used for the extraction were Petroleum ether (40-60°C), chloroform, ethyl acetate, acetone and methanol. Soxhlet and flask extraction procedures were adapted for extraction. 10g grams of powered samples were packed in muslin cloth and used for extraction by soxhlet apparatus at a temperature below the boiling point of each solvent. The filtered dark green extracts were transferred to a 1000 mL round bottom rotating flask. The flask was then connected to the rotary evaporator machine through a clamp. The rotating flask was then heated by partial immersion in a hot water bath at a temperature of 40°C. A typical 70 rpm speed was used for the flask rotation. The rota-evaporated sample was then scrapped using spatula and dried. This crude extract (powder) was stored at -20°C for further use.

Phytochemical screening

Preliminary phytochemical screening of Annona muricata bark extracts was carried out using the standard procedures (Trease and Evans, 1989 and Harborne, 1984).

FTIR Spectroscopic Analysis

Fourier transform infrared (FTIR) spectrophotometer was used to identify the characteristic functional groups in the bark extract. A small quantity (5 mg) of the bark extract was dispersed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min to form a KBr thin disc. The disc was then placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Perkin Elmer 2000 infrared spectrometer. The sample was scanned from 400 to 4000 cm-1 for 16 times to increase the signal to noise ratio. Samples were run in triplicate and all of them were undertaken within a period of one day.

Results and discussion

Phytochemical screening of the bark extract

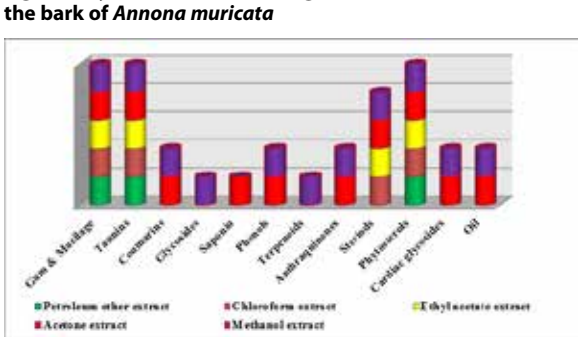
All the solvent extracts were subjected to phytochemical screening and the results are presented in Fig. 1. Qualitative analysis of the bark extract of A. muricata showed the presence of various phytochemicals. Methanol extract of *Annona muricata* contains maximum number of active constituents than other solvent extracts followed by acetone extract. Methanol extract of bark showed the presence of coumarins, glycosides, saponin, tannins, phenols, Terpenoids, anthraquinones, steroids, phytosterols, cardiac glycosides oil and gum mucilage. Minimum number of active constituents was noticed in petroleum ether extract. All extracts of plant bark contains tannin, steroids, phytosterols and gum and mucilage active constituents whereas alkaloids, flavonoids, carbohydrates, proteins and amino acid were absent in all the extracts.

FT-IR Spectroscopy analysis

By FT-IR analysis, the effective peak of the *Annona muricata* bark extract was done and the functional groups associated were determined. The effective peaks of the FT-IR spectrum of the samples were compared with that of the standard gallic acid. The absorption peaks observed for 3441.41 cm^{-1} for intermolecular hydrogen bonding in N-H stretching asymmetric O–H stretching vibration mode corresponds to alcohol and amines. The frequency range 1703.67 cm^{-1} corresponds to phenols and carbonyl group. The frequency range 907.03 cm^{-1} corresponds to carbohydrates group (Table 1). Therefore, the FT-IR analysis on *A. muricata* displayed novel phytochemical markers as useful analytical tool to check out not only the quality of the powder but also to identify the medicinally important plant.

Plants belonging to the family Annonaceae are a rich source of bioactive substances. Most of the studies demonstrate the importance of secondary metabolites in drug discovery. The use of phytoconstituents as drug therapy to treat major ailments has proved clinically effective and less toxic than the existing drugs. Tannins are known to possess general antimicrobial and antioxidant activities (Rievere *et al*., 2009). Saponins are a mild detergent used in intracellular histochemistry staining to allow antibody access to intercellular proteins. In medicine, it is used in hypercholestrolaemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss etc. It is also known to have anti fungal properties (Jeeva *et al.,* 2012). Plant steroids are known to be important for their cardiotonic activities, possess insecticidal and antimicrobial properties. Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity. Phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory (Arts *et al*., 2005; Scalbert *et al*., 2005). It suggests that the plants can be used as antimicrobial activity, antioxidant, antiallergic, antiinflammatory, antidiabetic, anticancerciogenic, anticancer agents in the future. Anticonvulsant effect has been shown for the hydro alcoholic fraction of the root bark extract and leaf of *A. senegalensis* in effect on Maximal Electroshock (MES) induced Seizures and Pentylenetetrazole (PTZ) induced seizures when administered orally (Okoli *et al*., 2010; Okoye *et al*., 2010). Oral administration could lead to loss of some pharmacological activities of *A.senegalensis* root bark extracts (Mahomed and Ojewole, 2006). The wound healing activity of alcoholic extract of stem and bark of *Annona muricata* was found to show the marked reduction in area of the wound which was tested in the albino rats which proves their possible use in the healing the wound (Pathak *et al*., 2010). From the FT-IR analysis we conclude that amines, aliphatic and aromatic nitro compounds, bromides, sulfonates, iodides and fluorides are the most commonly detected compounds in the *Annona muricata* bark (Fig.2). Phytochemical screening and FT-IR analytical method is highly rapid, effective, visual and accurate for pharmaceutical research. The phytochemical screening supported by FT-IR analysis confirms the presence of secondary metabolites with potent therapeutic value. The present study indicates that most of the biologically active phytochemicals are present in the methanol extracts of *Annona muricata* bark. Further studies are needed to isolate the active components, which can be used for developing newer drugs in the treatment of diseases for the welfare of mankind.

Fig. 1. Phytochemical screening of the various extracts of the bark of *Annona muricata*



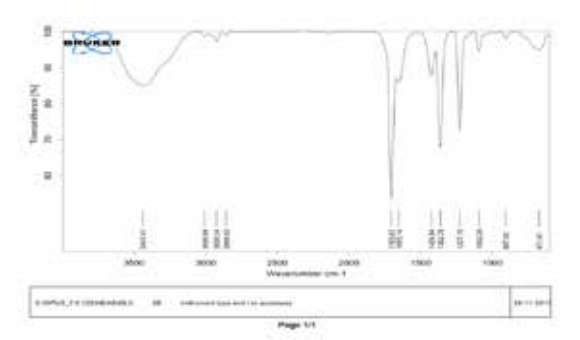


Fig. 2. FT-IR analysis showing functional groups of methanol extract of *A.muricata* bark

Table. 1. FT-IR spectroscopic analysis of methanol bark extract of *A.muricata* showing characteristic absorption peaks at IR range and functional groups

Frequency range (cm^{-1})	Intensity	Type of bond	Functional groups
3441.41	Strong	Intermolecular hydrogen bonding in N-H (s) asymmetric O–H (s) vibration mode	Alcohol, Amines
3006.66	Weak	Asymmetric C–H (s) of aromatic nucleus	Carboxylic aromatic group,
2926.24	Weak	Symmetric C–H (s) of aromatic nucleus, asymmetric C–H (s) vibrations CH_3 and CH_2	Aromatic group, Secondary amines (protein & lipids)
2856.92	Weak	Symmetric C–H (s) vibrations of CH_3 (aliphatic)	Cycloalkanes (Amides)
1703.67	Strong	C=C (s), C=O	Phenols, Carbonyl
1653.14	Strong	N-H (b) vibration	Carbonyl unsaturated ketone amide
1424.94	Strong	CH_2 (b) vibration CH_2-CO	Carbonyl compound in acid
1092.20	Medium	(s) vibration of aliphatic C–O–H	Alkyl amine
907.03	Medium	C–O and C–C (s) vibrations	Carbohydrates

*(s)-stretching; (b)-bending

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