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Phytochemical Screening and Profiling of Secondary Metabolites of *Annona Muricata* Bark

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Abstract

Screening of phytochemicals is a precious stair in the detection of bioactive principles present in medicinal plants and may lead to novel drug discovery). In present study aimed to investigate the preliminary phytochemical screening, fluorescence, and spectroscopic characterization in Annona muricata bark. The qualitative analysis of aqueous, hexane, ethanol, hydro-ethanolic and methanol extracts of Annona muricata bark were investigated. Among the various extracts, the hydro-ethanolic extract of Annona muricata bark contains a higher concentration of phytochemicals than other extracts and is used for subsequent studies. Quantitative analysis revealed that the Annona muricata bark has contain significant amount of phenol, flavonoids, saponin, tannin and alkaloids were present. Histochemical analysis further confirmed the presence of phytochemicals in Annona muricata bark. UV-VIS spectral analysis we can confirm that the presence of tannins and flavonoids. FTIR analysis had confirmed the presence of alcohol, phenol, aromatic, carboxylic acid, and aliphatic amines. Results of the study concluded that rich source of phytochemicals in Annona muricata bark is responsible for their therapeutic effects. So, it is recommended as a plant of phytopharmaceutical importance and further studies are needed with these plants to evaluate their pharmacological potentials of the bioactive compounds responsible for their bio-activities and other medicinal values.

Keywords: *Annona muricata bark, Phytochemicals, Histochemicals, Fluroscence, UV visible spectrum, FTIR*

1. Introduction

Natural products especially from plant sources, including species have been investigated for their characteristics and health effects. Plants have designed the basis of classy traditional medicine practices that have been used for thousands of years by people in China, India, and many other countries (Sneader, 2005). Some of the earliest records of the usage of plants are drugs are found in the Artharvaveda, which is the basis for Ayurvedic medicine in India, the clay tablets in Mesopotamis (1700 BCE), and the Eber Papyrus in Egypt (1550 BCE) (Sneader, 2005). Plant chemicals are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds etc. i.e., any part of the plant body may contain active components (Solomon Charles *et al.*, 2013).

Plants are used as medicines in various cultures and serve as a source of many potent drugs due to the presence of certain bioactive compounds for pharmaceutical industries (Patel *et al*., 2015). Plants contain different phytochemicals, also known as secondary metabolites. Phytochemicals are useful in the treatment of certain disorders by their individual, additive, or synergic actions to improve health (Nisha *et al.*, 2011). Phytochemicals are vital in pharmaceutical industry for development of new drugs and preparation of therapeutic agents (Starlin *et al.*, 2019). The development of new drugs starts with identification of active principles from the natural sources. The screening of plant extracts is a new approach to find therapeutically active compounds in various plant species. Phytochemicals such as flavonoids, tannins, saponins, alkaloids, and terpenoids have several biological properties which include

antioxidant, anti-inflammatory, anti-diarrhea, anti-ulcer, and anticancer activities, among others (Mahomoodally *et al*., 2013).

In this research, preliminary phytochemical screening, fluorescence, and spectroscopic characterization have been carried out on *Annona muricata* bark.

2. Materials And Methods

Collection of plant

The bark of *Annona muricata* collected from Alangkuppam, Thirupattur District in the year of October 2022 Tamil Nadu, India. The fresh barks of *Annona muricata* were washed with tap water and shade dried at room temperature (28 ±2 ˚C). The dried barks were powdered by electric blender and used for further experiments.

Analysis of phytochemicals

Qualitative phytochemical analysis

10grams of *Annona muricata* bark powder were used for extraction. Extraction was performed with cold extraction using the maceration method into different solvents such as aqueous, ethanol, methanol, hexane and hydro-ethanolic (ethanol and water (70:30)) for 24 hours using the "intermittent shaking" method to obtain extracts. The extracts were further filtered using Whatman filter No 1 paper and filtrate was used for phytochemical analysis. Preliminary phytochemical screening was carried out by using standard procedure followed by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

Quantitative analysis

The amounts of total phenolic contents of *Annona muricata* bark were determined by the spectrophotometric method of Kim *et al*., (2003) with slight modifications. The total flavonoids assay was conducted according to Katasani (2011). Total flavonoids content was determined by using Aluminium chloride colorimetric method. The total Tannins assay was conducted according to Bajaj and Devsharma (1977) method. Total saponins contents in *Annona muricata* bark materials were estimated by colorimetric methods (Hiai *et al*., 1976). The alkaloid estimated by the method of Fazel Shamsa *et al* (2008) and Mallikarjuna Rao *et al* (2012).

Histochemical tests (John Peter Paul, 2014; Gersbach *et al*., 2001).

A small quantity of dried and finely powdered plants sample was placed on a grease free microscopic slide and treated with specific chemicals and reagents and waited for 1-2 minutes. A positive test for histochemical was indicated by the appearance of the appropriate colour change after application of the reagent. Using a light microscope to observe and record any colour changes.

Determination of Fluorescence behavior of *Annona muricata* powder: (Kokashi *et al*., 1957)

Fluorescence analysis of bark powder of *Annona muricata* has been carried out in daylight and under UV light. Florescence analysis of bark powder of *Annona muricata* were carried out by the treatment of different chemical reagents such as H_2O, H_2SO_4, HCl, NH_3, CH_3OH, HNO_3 and NaOH. The powders were observed in normal daylight and under short (245 nm) and long UV light (365 nm).

3. Results and Discussion

Phytochemical screening is a method of bioactive compounds identification that is unknown in plant extracts through qualitative analysis. Phytochemical screening is a preliminary stage in a phytochemical study that aims to provide an overview of the class of compounds contained in plants that are being studied. Phytochemical screening method is done by looking at the color testing reaction using a color reagent (Samuelsson, 2004). Knowledge of the chemical components contained in medicinal plants needs to be studied. This information will be significant for the synthesis of complex active components of chemical compounds contained in medicinal plants (Lavanya *et al*., 2018). Phytochemical screening in medicinal plants, in addition to being used to identify active compounds that are beneficial to the body's health (positive effects of herbal medicines). This causes the phytochemical screening process of medicinal plants to be important before conducting further analysis (Santhi *et al*., 2011; Newman *et al*., 2003).

To determine the character of the bioactive compounds contained in plant extracts, one of the methods that can be used is the fluorescence spectrophotometry method. Characterization using the fluorescence spectrophotometric method can be used to determine the ability of some extracts of medicinal plants in absorbing and releasing ultraviolet radiation. The principle of the analysis of this method is by measuring the absorption of the sample of light energy entering the wavelength of its excitation and releasing the energy of the light at its emission wavelength (Edeoga *et al*., 2005). In present study,

preliminary phytochemical screening, fluorescence, and spectroscopic characterization have been carried out on *Annona muricata* bark.

Qualitative Analysis of Phytochemicals

The qualitative analysis of aqueous, hexane, ethanol, hydro-ethanolic and methanol extracts *Annona muricata* bark were investigated. The results confirm in the presence of tannin, saponin, flavonoids, terpenoids, triterpenoids, alkaloids, anthroquinone, polyphenol, glycoside, coumarins and anthocyanins while steroid was absent in aqueous extract of *Annona muricata* bark. The ethanol, hydro-ethanolic and methanol extracts of *Annona muricata* bark showed the presence of tannin, saponin, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, anthroquinone, polyphenol, glycoside, coumarins and anthocyanins. Hexane extract of *Annona muricata* bark showed the presence of tannin and polyphenol while saponin, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, anthroquinone, glycoside, coumarins and anthocyanins were absent (Table 1). Ozor, Charles Chibuzor, and Okwute, Simon Koma (2023) investigated the crude ethanol extract was subjected to phytochemical screening which showed the presence of tannins, quinones, phenols, terpenoids and saponins. Among the various extracts, the hydro-ethanolic extract of *Annona muricata* bark contains a higher concentration of phytochemicals than other extracts and is used for subsequent studies.

Table 1: Phytochemicals qualitative analysis of bark extract of *Annona muricata*

S. No	Phytochemicals	Extracts				
		Aqueous	Ethanol	Hydro-ethanolic	Hexane	Methanol
1	Tannin	+	++	++	+	++
2	Saponin	++	++	++	-	++
3	Flavonoids	++	++	++	-	++
4	Steroids	-	+	++	-	+
5	Terpenoids	++	++	++	-	++
6	Triterpenoids	++	++	++	-	+
7	Alkaloids	+	++	++	-	++
8	Anthraquinone	+	++	++	-	++
9	Polyphenol	++	++	++	+	++
10	Glycoside	+	+	++	-	++
11	Coumarins	+	+	++	-	++
12	Anthocyanins	+	++	++	-	++

(-) Absent, (+) Present and (++) high concentration

Quantitative Analysis of Phytochemicals

Quantitative analysis revealed that the *Annona muricata* bark has significant amount of phenol (213.17mg/gm), flavonoids (173.54mg/gm), saponin (138.97mg/gm), tannin (107.56mg/gm) and alkaloids (92.66 mg/gm) were present (Table 2).

Table 2 Quantitative analysis of phenol, flavonoid, saponin, tannin and alkaloids content in hydroethanolic extract of *Annona muricata* bark

Name of Extract	Total phenol (Milligrams of Gallic acid (GAE) equivalents per gram)	Flavonoid (Milligrams of quercetin equivalents per gram)	Saponin (Milligrams of Quillaja equivalents per gram)	Tannin (Milligrams of tannic acid equivalents per gram)	Alkaloids (Milligrams of atropine equivalents per gram)
Hydro-ethanolic extract of *Annona muricata* bark	213.17 ± 12.14	173.54 ± 14.91	138.97 ± 9.72	107.56 ± 7.25	92.66 ± 6.48

Values are expressed as Mean ± SD for triplicates

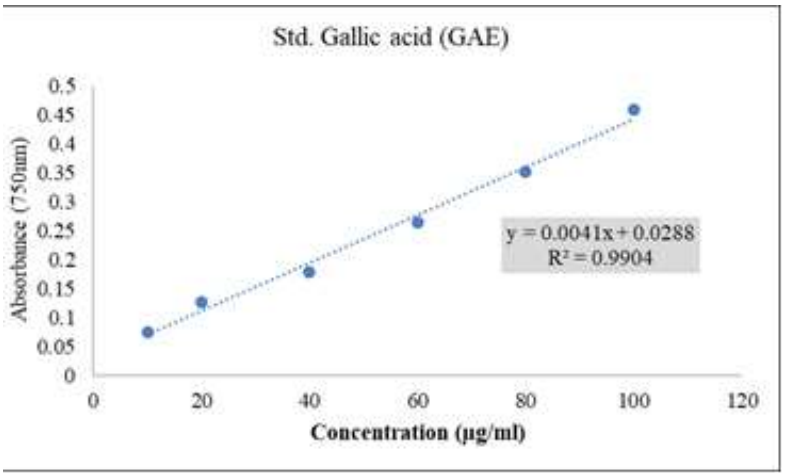


Figure 1 Standard Curve for Phenol using Gallic acid

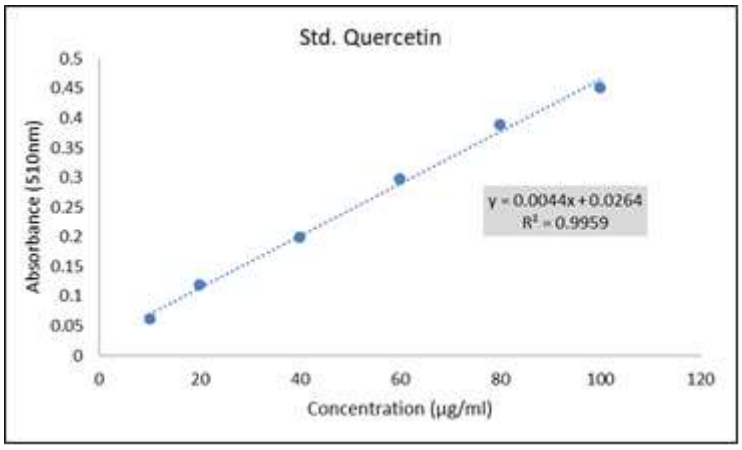


Figure 2 Standard Curve for flavonoid using Quercetin

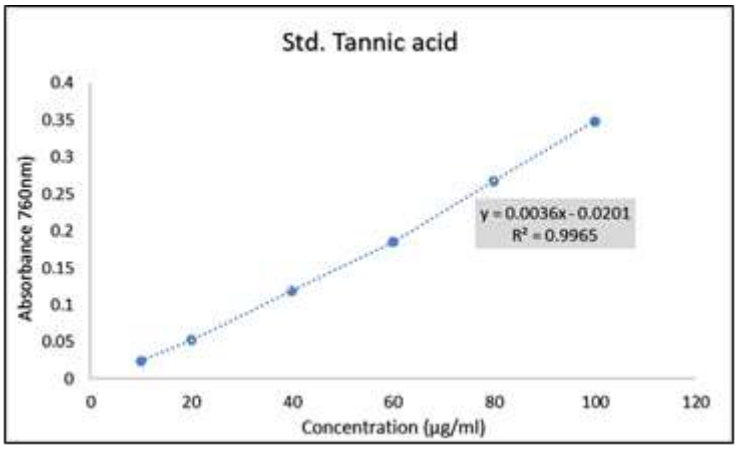


Figure 3 Standard Curve for saponin using Saponin

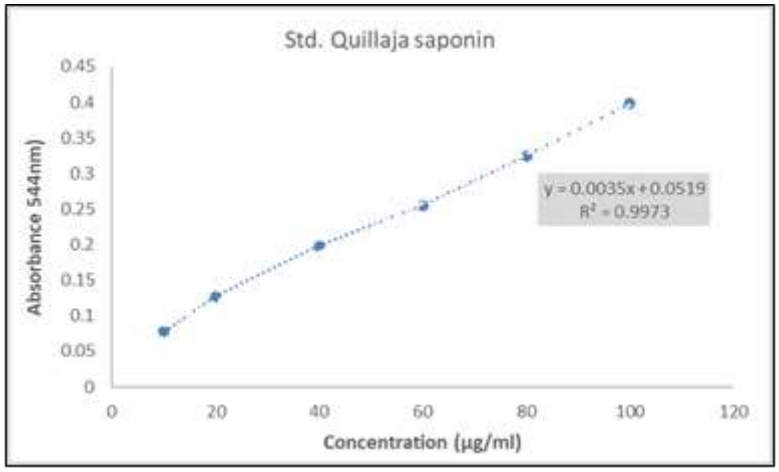


Figure 4 Standard Curve for tannin using Tannic acid

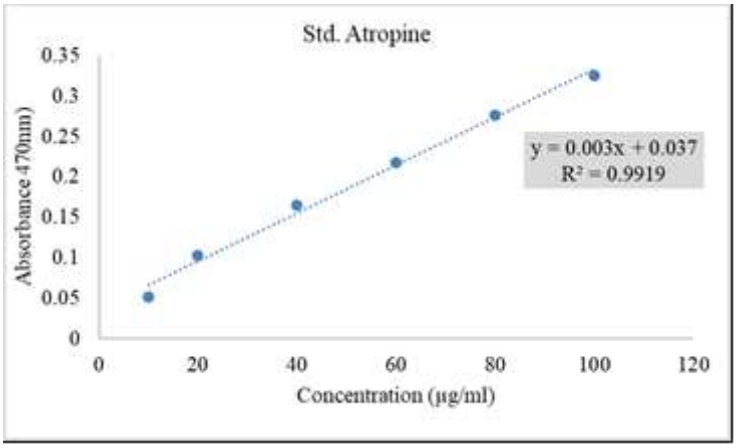


Figure 5 Standard Curve for alkaloids using Atropine

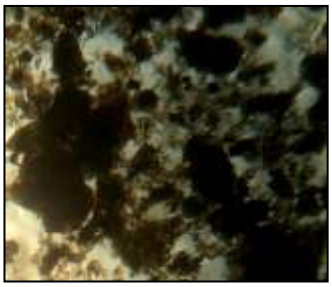
Histochemical Analysis

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues. Histochemistry is devoted to study the identification and distribution of chemical compounds within and between biological cells, using stains, indicators, and light and electron microscopy (Wick, 2012). Histochemical analysis is essential for the study of plant secretory structures whose classification is based, atleast partially, on the composition of their secretion. As each gland may produce one or more types of substances, a correct analysis of its secretion should be done using various histochemical tests to detect metabolites of different chemical classes (Demarco et al., 2017). Histochemical analysis of Annona *muricata* bark powder used to identify bioactive classes of compounds present in tissues. The tannin, flavonoids, alkaloids, steroids, poly- phenol and terpenoids were confirmed by histochemical analysis. This study further supported the qualitative analysis of phytochemicals (Table 3 and Figure 6)

Table 3: Histochemical analysis of *Annona muricata* bark

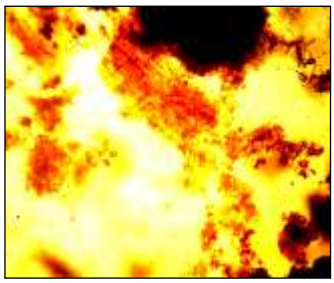
Phytochemical	***Annona muricata* bark**	
	Colour observation	**Results**
Tannin	Dark black	++
Flavonoids	Yellow	++
Alkaloids	Reddish brown	++
Steroids	Light green	+
Poly phenol	Blue	++
Terpenoids	Orange	++

(-) Absent, (+) Present and (++) high concentration

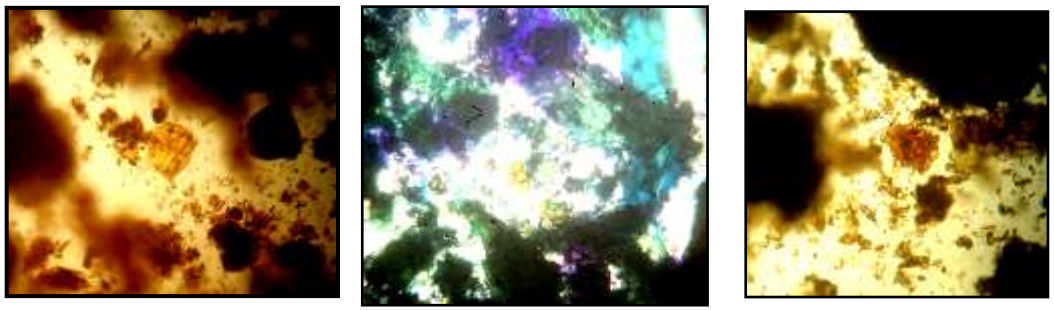


Tannin

Flavonoids



Alkaloids



Steroids **Poly phenol** **Terpenoids**

Figure 6: Histochemical analysis of *Annona muricata* bark

(Arrow mark indicates the respective phytochemical)

Phenolic compounds are famous group of secondary metabolites with wide pharmacological activities. Phenolic acid reduces blood cholesterol, increases bile secretion and lipid levels and antimicrobial activity against some strains of bacteria such as *Staphylococcus aureus* (Gryglewski *et al.*, 1987). Phenolic acid possesses diverse biological activities, for instance, anti-inflammatory, antiulcer, antioxidant (Silva *et al.*, 2007) cytotoxic, anti-spasmodic and anti-depressant activities (Ghasemzadeh *et al.*, 2010).

Most recent researches have focused on the health aspects of flavonoids for humans. Flavonoids have gained recent attention because of their broad pharmacological and biological activities. Flavonoids have been reported to exert various biological property including cytotoxicity, coronary heart disease prevention, hepatoprotective, antimicrobial, antitumor as well as anti-inflammatory activities (Al-Huqail *et al*., 2019). The best-described property of flavonoids is in their capability to act as powerful antioxidants which might shield the form from free radicals and reactive element species. Flavonoids have been reported as enzyme inhibition, anti-inflammatory, oestrogenic, antimicrobial, anti-allergic, vascular activity, antioxidant, and cytotoxic antitumor activity (Havsteen, 2002).

Tannin containing plant extracts are used as astringents, diuretics, against diarrhoea, duodenal and stomach tumours (De Bruyne *et al.*, 1999) and as anti-inflammatory, antiseptic, antioxidant, and haemostatic pharmaceuticals (Dolara *et al.*, 2005). Recently, tannins have attracted scientific interest, especially due to the increased incidence of deadly illnesses such as AIDS and various cancers (Blytt *et al.*, 1988).

Alkaloids are significant in protecting and the survival of plant because they ensure their survival against insects, micro-organisms (antibacterial and antifungal activities) and herbivores (feeding deterrens) and against other plants by means of allelopathically active chemicals (Molyneux *et al.*, 1996). Alkaloids have many pharmacological activities including antimalarial activity (quinine), antiarrhythmic effect (quinidine, spareien), antihypertensive effects (many indole alkaloids) and anticancer actions (dimeric indoles, vincristine, and vinblastine) (Wink *et al.*, 1998). Some alkaloids have stimulant property as caffeine and morphine, nicotine used as the analgesic and quinine as the antimalarial drug (Rao *et al.*, 1978).

Saponins may be considered as part of plants defence systems and as such have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants (Lacaille-Dubois and Wagner, 2000). Saponin mixtures present in plants and plant products possess diverse biological effects when present in the animal body. Extensive research has been carried out into the membrane-permeabilising, immune stimulant, hypo cholesterolaemic, anticarcinogenic hypoglycaemia and to act as antifungal and antiviral properties of saponins (Morrissey and Osbourn, 1999: Traore *et al.*, 2000).

Among plant secondary metabolites, terpenoids are the structurally most diverse group; they function as phytoalexins in plant direct defense, or as signals in indirect defense responses which involves herbivores and their natural enemies (McCaskill and Croteau, 1998). Basically, the terpenoids including the sesquiterpenes (5) of medicinal plants are known to greatly contribute to the therapeutic values such as: anti-hyperglycemic activity, anti-inflammatory activity, anti-parasitic activity, enhancer of skin permeation for many drugs across cell membrane, anti-viral activity, anticancer activity, and antimicrobial activities (Degenhardt *et al.*, 2003; Ramawat *et al*., 2013). Terpenes play an important role as signal compounds and growth regulators (phytohormones) of plants, as shown by preliminary investigations. In addition, terpenoids can have medicinal properties such as anti-carcinogenic (e.g.,

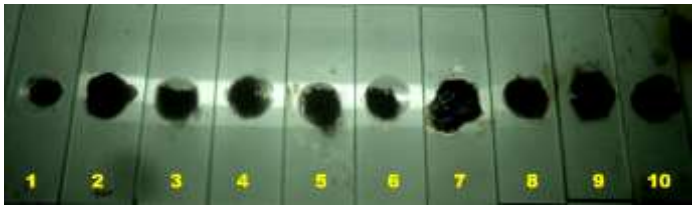
perilla alcohol), antimalarial (e.g., artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic (e.g., glycyrrhizin) activity and the sesquiterpenoid antimalarial drug artimisinin and the diterpenoid anticancer drug taxol (Dudareva *et al.*, 2004).

Fluorescence Analysis

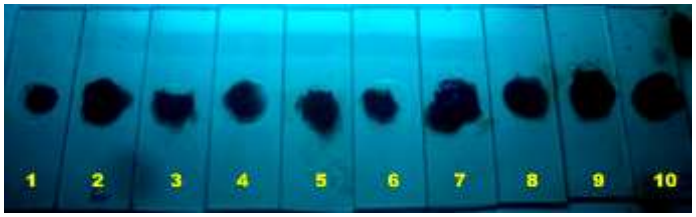
Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products, which do not visibly fluoresce in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation.

Table 4: Fluorescence analysis of *Annona muricata* bark

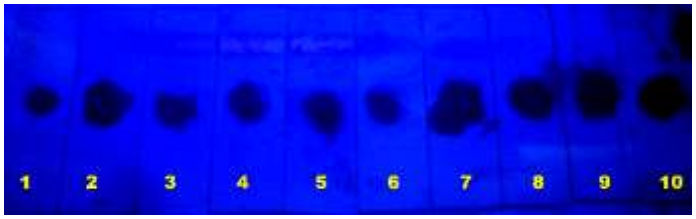
S. No	Test	Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Brown	Brown	Black
2	Plant powder treated with distilled water	Brownish black	Light greenish brown	Black
3	Plant powder treated with Hexane	Brown	Light greenish brown	Black
4	Plant powder treated with Chloroform	Brown	Ash brown	Black
5	Plant powder treated with Methanol	Brown	Ash greenish brown	Black
6	Plant powder treated with Acetone	Brown	Ash brown	Black
7	Plant powder treated with 1N Sodium Hydroxide	Brownish black	Black	Black
8	Plant powder treated with 1N HCL	Brown	Light greenish brown	Black
9	Plant powder treated with H_2SO_4 with equal volume of water	Brownish black	Brown	Black
10	Plant powder treated with NH_3 diluted with an equal volume of water	Yellowish brown	Greenish brown	Black



Visible Light



Short UV Light (254 nm)



Long UV Light (254 nm)

Figure 7: Fluorescence analysis of *Annona muricata* bark

Ultraviolet-visible Spectroscopy Analysis

The absorption spectra of plant constituents can be measured in very dilute solution against a solvent blank using an automatic recording spectrophotometer. For colourless compounds, measurements are made in the range 200 to 400 nanometres (nm); for coloured compounds, the range is 400 to 700 nm. The wavelengths of the maxima and minima of the absorption spectrum so obtained are recorded (in nm) and the intensity of the absorbance (or optical density) at the particular maxima and minima spectral measurements are important in the identification of many plant constituents of crude plant extracts for the presence of particular classes of compound. The UV-visible spectra were performed to identify the compounds containing σ-bonds, π-bonds and lone pair of electrons, chromophores, and aromatic rings (Jain *et al.*, 2016).

The UV-VIS profile of *Annona muricata* bark extract was studied over the 200 to 1100nm wavelength due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 227.95, 281.10, 419.90, 685.25 with the absorption 3.995, 0.272, 0.285 and 0.116 respectively (Figure 8 and Table 5). The UV-VIS spectroscopy revealed the characteristic peaks for different phytochemicals present in the extract. The UV-visible spectra were performed to identify the compounds containing σ-bonds, π-bonds and lone pair of electrons, chromophores, and aromatic rings. In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400nm is a clear indication of the presence of unsaturated groups and heteroatoms such as S, N, O (Njokua *et al.*, 2013). Neha *et al*., (2006) reported that the spectra for phenolic compounds (tannins) and flavonoids lie in the range of 220-290 nm. So, from the phytochemical and UV-VIS spectral analysis we can confirm that the presence of tannins and flavonoids.

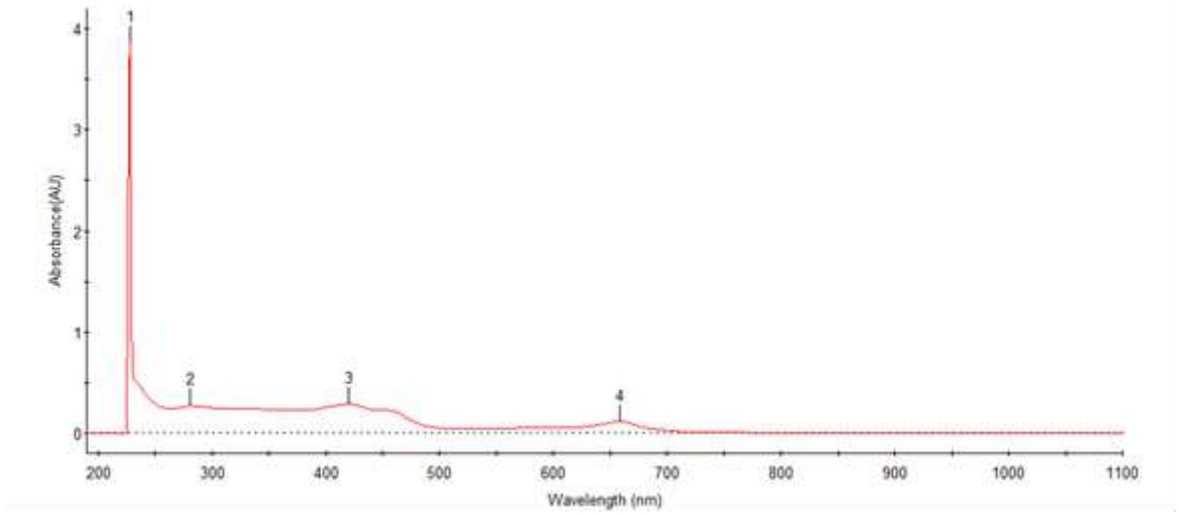


Figure 8: UV-Visible spectrum analysis of *Annona muricata* bark extract

Table 5: UV-Visible spectrum analysis of *Annona muricata* bark extract

Peaks	Wavelength (nm)	Absorbance (AU)
1	227.95	3.995
2	281.10	0.272
3	419.90	0.285
4	685.25	0.116

FTIR analysis

The FTIR spectrum was used to identify functional groups of the active components present in plant samples based on the peak's values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peak's ratio. The results of FTIR peak values and functional groups were represented in figure 9 and table 6. In the current investigation involving *Annona muricata* bark, the results of FTIR analysis had confirmed the presence of alcohol, phenol, aromatic, carboxylic acid, and aliphatic amines.

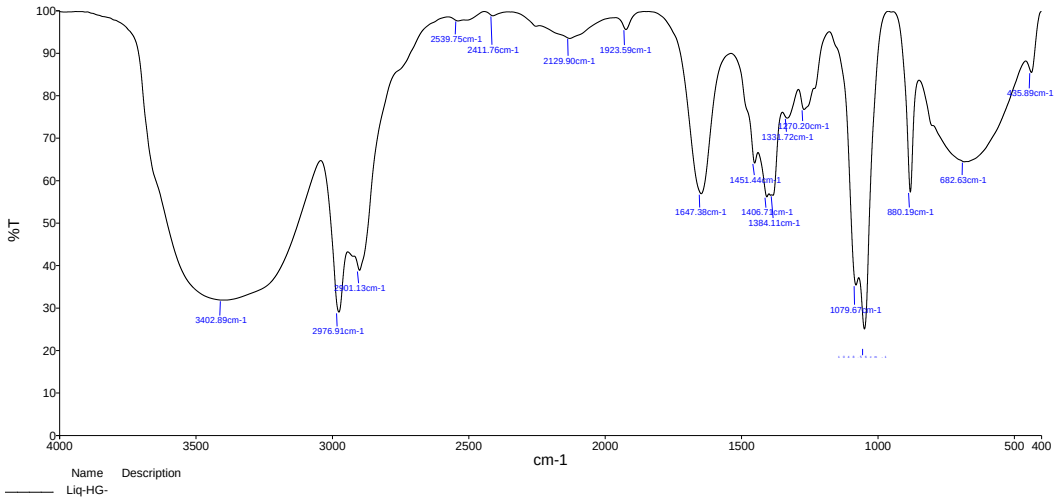


Figure 9: FTIR spectrum analysis of *Annona muricata* bark extract

Table 6: FTIR spectrum analysis of *Annona muricata* bark extract

Frequency cm^{-1}	Bond	Functional group
3402.89	O-H stretch, H-bonded	Alcohols, Phenols
2976.91, 2901.13	O-H stretch	Carboxylic acids
2129.90	-C≡C- stretch	Alkynes
1647.38	-C=C- stretch	Alkenes
1451.44, 1406.71	C-C stretch (in-ring)	Aromatics
1331.72, 1270.20	C-N stretch	Aromatic amines
1079.67, 1048.56	C-N stretch	Aliphatic amines
880.19, 682.63	C-H “loop”	Aromatics

4. Conclusion

This investigation has given preliminary information to determine the chemical composition of *Annona muricata* bark using qualitative, quantitative, histochemical, fluorescence, UV-VIS, and FTIR techniques. In the present study, *Annona muricata* bark have shown to have various secondary metabolites. The phytochemical constituents which contribute the activities like antimicrobial, antioxidant, anticancer, hypercholesterolemic, anti-inflammatory, and other activities. Hence, the presence of phytochemicals is responsible for their therapeutic effects. So, it is recommended as a plant of phytopharmaceutical importance and further investigation is required for possible development of novel drugs using some of the bioactive compounds found in *Annona muricata* bark.

Conflict of Interest:

There is no conflict of interest.

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