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Research Article

Comparative Phytochemicals Screening of *Annona Muricata Annona Senegalensis and Annona Squamosa*

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Abstract

GC-MS and qualitative phytochemical screening of ethanol leaf extracts of *Annona muricata Annona senegalensis* and *Annona squamosa* were carried out. Qualitative phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, phenol, saponin, and steroid/triterpenes. Gas-chromatography-mass spectrometry (GC-MS) analysis revealed fifty-six compounds with their fragmentation pattern and molecular weight. GC-MS chromatogram of the ethanolic extract of *A. muricata* showed twenty peaks, indicating the presence of twenty compounds. The identified compounds found in higher quantities include (Z)-2-(henicos-12-en-1-yl)-6-methyl-2H-pyran-4(3H)-one (37.83%), N-Aminopyrrolidine (20.38%), Propanoic acid, 2, 3-dichloro- (5.17%), etc. *A. senegalensis* showed six peaks, representing six compounds, with the highest quantity being ntegerrimine (57.472%). *A. squamosa* showed thirty peaks indicating the presence of thirty compounds with Longifolenaldehde (7.34%) as the compound with the highest concentration. The intrageneric phytochemical characters they share in common in this work presence of flavonoids and saponin. The diagnostic features include the presence of alkaloids in *A. squamosa*, the absence of phenol and tannin in *A. muricata*, and the absence of triterpenes in *A. squamosa*. All these phytochemicals are useful in their identification, classification, and delineation. The observations made in the similarities of characters of the three studied species support the present-day classification of the three species in the same genus (*Annona*).

INTRODUCTION

Phytochemicals are bioactive, naturally occurring chemical substances present in plants that have therapeutic and nutritive properties for humans (Hasler & Blumberg, 1999). They defend plants against infection and injury as well as contribute to the color, fragrance, and flavor of the plant. Phytochemicals are plant chemicals that aid plants resist ecological threats such as contamination, stress, drought, UV exposure, and bacterial assault (Gibson et al., 1998; Mathai, 2000). When consumed as food, they have been found to boost immunity and improve human health (Samrot et al., 2009; Koche et al., 2010). About 4,500 phytochemicals have been identified and categorized based on their defensive mechanisms, physical and chemical properties, and only 350 of these have been thoroughly studied (Koche et al., 2010). Phytochemicals can be present in a variety of foods, including bananas, tomatoes, legumes, whole grains, nuts, beans, mushrooms, herbs, and spices (Mathai, 2000). Phytochemicals may be present in a variety of plant parts, including the stem, leaf, vine, fruit, and seed. Many phytochemicals, especially pigment compounds such as anthocyanins and flavonoids, are concentrated in the outer layers of different parts such as leaves and fruits. Nevertheless, depending on the species' atmospheric growth conditions and other factors, the levels

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of these phytochemicals differ from plant to plant (Rao, 2003). These substances have biological activities including antioxidant activity, antimicrobial action, detoxification, enzyme regulation, immune organ activation, reduction in platelet formation, hormone metabolism modulation, and in anticancer function (Hamburger & Hostettmann, 1991). Horborne (1999) focused on the anti-nutritional properties of certain plant chemicals at the same period. The present review is a brief summary of the extremely diverse phytochemicals present in plants and their varied bioactivities.

The family *Annonaceae* is comprised of a large number of genera and species, most of which are native to the tropical regions, having about 2500 species in with 135 genera (Chatrou et al., 2004; Anuragi et al., 2016). The genus *Annona*, commonly known as the custard-apple genus, consists of some 125 species with some species widely cultivated for their edible fruits and often becoming naturalized beyond their native range of tropical America and Africa (Wagner et al., 2014)

Classification of phytochemical

The exact classification of phytochemicals has not been given so far, because of their diverse forms and structures. Phytochemicals have traditionally been classified as primary or secondary metabolites based on their role in plant metabolism. Sugars, amino acids, proteins, purines, and pyrimidines of nucleic acid, chlorophylls, etc., are examples of primary metabolites. Alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumins, saponins, phenolics, and glucosides constitute secondary metabolites (Ramawat et al., 2009). The report has it that phenolics are the most important and structurally complex plant chemicals among them.

Phenols

Phenols are the largest group of phytochemicals and the most common wide distributed in the plant kingdom (Walton et al., 2003). Phenolics are a family of chemical compounds that comprise a hydroxyl group (OH) that is specifically bound to an aromatic hydrocarbon group. The simplest class of this category of natural compounds is phenol (C6H5OH). They play an essential role as defensive compounds, have many properties that are advantageous to humans, and their antioxidant properties are functional as protective agents against diseases caused by free radicals (Zubeyir et al., 2017). Phenolic and flavonoids have been reported to act as antioxidants to exert antiallergic, anti-inflammatory, antidiabetic, antimicrobial, antipathogenic, antiviral, antithrombotic, and vasodilatory effects and prevent ailments such as cancer, heart problems, cataracts, eye disorders, and Alzheimer's disease (Comunian et al., 2017; Shahidi & Ambigaipalan, 2015; Vodnar et al., 2017). The most important features of flavonoids include their ability to protect against oxidative diseases, activate or inhibit various enzymes that bind specific receptors, and protect against cardiovascular diseases by reducing the oxidation of low-density lipoproteins (Pietta, 2000). Flavonoids, phenolic acids, and polyphenols are the three most common forms of dietary phenolics.

Flavonoids

Flavonoids are the largest group of plant phenols and also the most studied one (Dai & Mumper, 2010). They are polyphenolic compounds that are ubiquitous in nature and occur as aglycones, glucosides, and methylated derivatives. More than 4,000 flavonoids have been recognized, many of which occur in vegetables, fruits, and beverages like tea, coffee, and fruit drinks (Pridham, 1960). Flavonoids appear to have played a major role in successful medical treatments in ancient times, and their use has persisted up to now. Most flavonoids occur naturally associated with sugar in conjugated form and within any one class, may be characterized as monoglycosidic, diglycosidic, etc. The glycosidic linkage is normally located at position 3 or 7 and the carbohydrate unit can be L-rhamnose, D-glucose, glucorhamnose, galactose, or arabinose (Pretorius, 2003). Flavonoids have gained recent attention because of their broad biological and pharmacological activities. Flavonoids have been reported to exert multiple biological activities including anti-microbial, cytotoxic, anti-inflammatory, and anti-tumor activities; but the best-described property of almost every group of flavonoids is the capacity to act as powerful antioxidants (Shirsat et al., 2012; Teiten et al., 2013) which can protect the human body from dangerous free radicals and reactive oxygen species (ROS). Phenolic acids form a diverse group that includes the widely distributed hydroxy-benzoic and hydroxycinnamic acids. The term 'phenolic acids', in general, designates phenols that possess one carboxylic acid functional group. Naturally occurring phenolic acids contain two distinctive carbon frameworks viz. the hydroxycinnamic and hydroxybenzoic structures. Hydroxycinnamic acid compounds are produced as simple esters with glucose or hydroxycarboxylic acids. Plant phenolic compounds are different in molecular structure, and are characterized by hydroxylated aromatic rings (Balsundaram et al., 2006). These compounds have been studied mainly for their properties against oxidative damage leading to various degenerative diseases, such as cardiovascular diseases, inflammation, and cancer. Indeed, tumor cells, including leukemia cells, typically have higher levels of reactive oxygen species than normal cells so they are particularly sensitive to oxidative stress (Mandal et al., 2010).

Tannins

Chemically, it is difficult to define tannins since the term encompasses some very diverse oligomers and polymers (Harborne, 1999). It might be said that the tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins

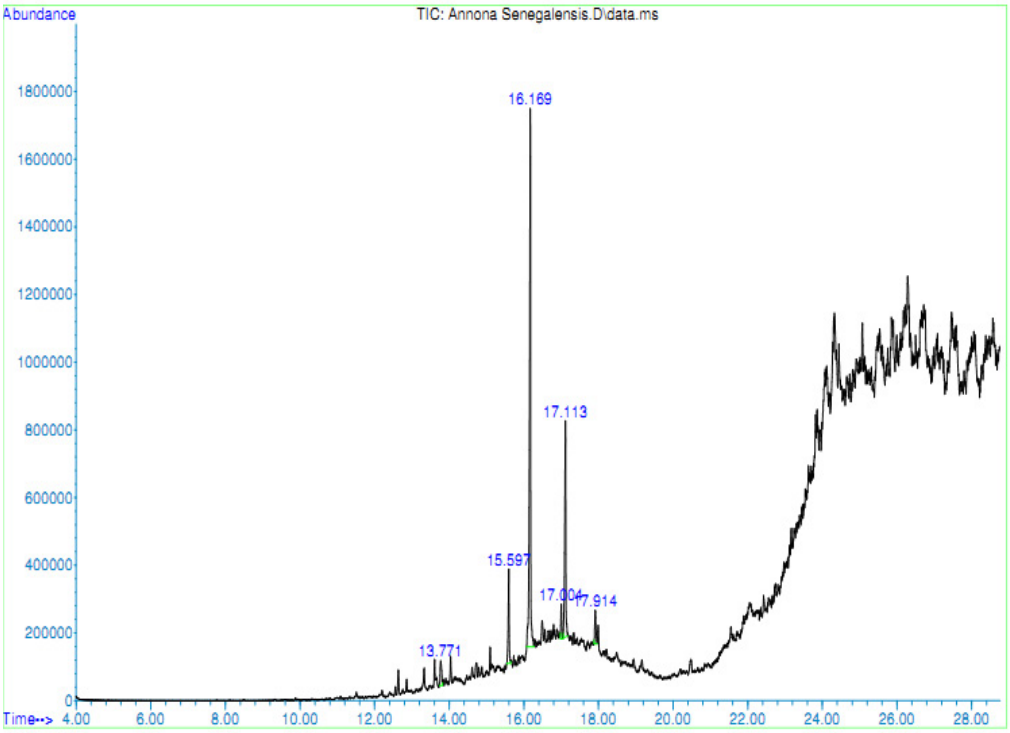

Figure 1. GC-MS Chromatograph of *Annona senegalensis*.

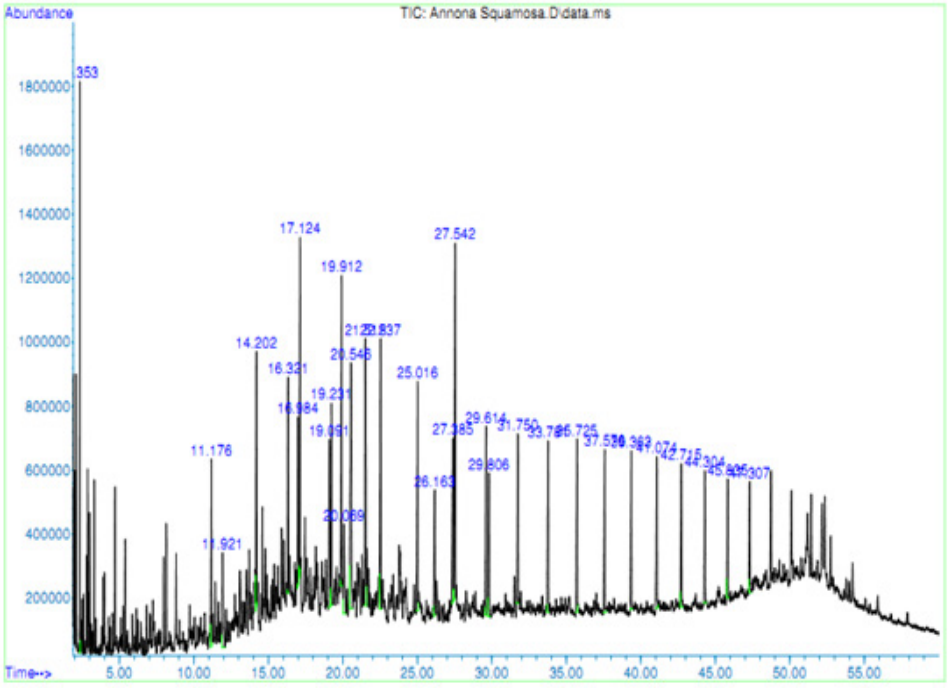

Figure 2. GC-MS Chromatograph of *Annona squamosa*.

(mainly), polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids and minerals (Schofield et al., 2001). Based on their structural characteristics, tannins are into four major groups: gallotannins, ellagitannins, complex tannins, and condensed tannins. Gallotannins- Tannins in which galloyl units or their meta-depsides derivatives are bound to diverse polyol-, catechin-, or triterpenoid units. Ellagitannins are tannins in which at least two galloyl

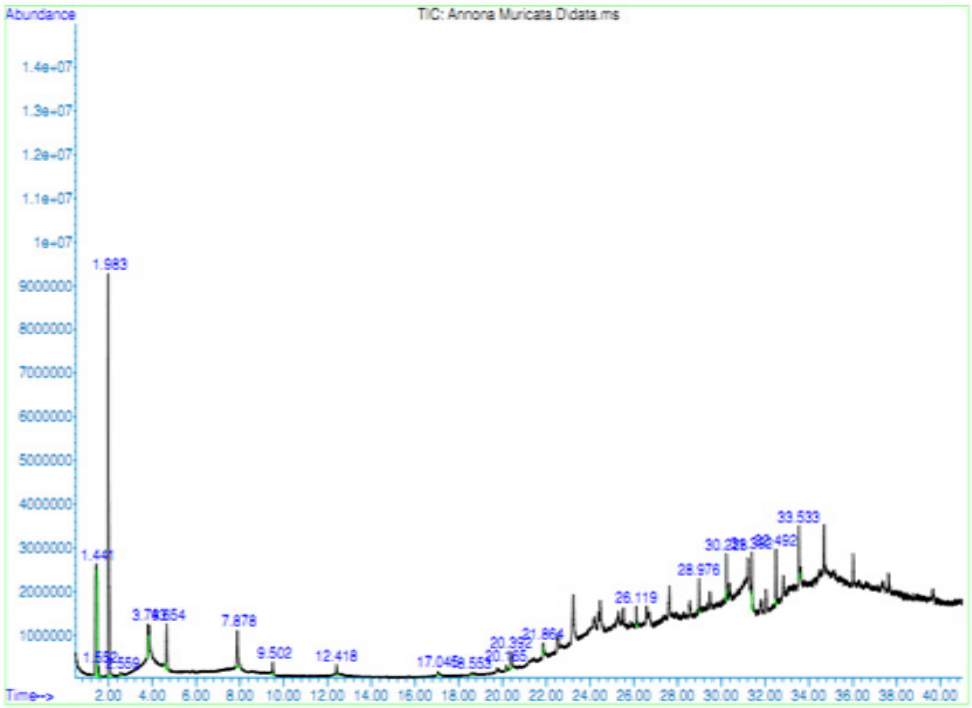

Figure 3. GC-MS Chromatograph of *Annona muricata*.

units (C–C) are coupled to each other and do not contain a glycosidically linked catechin unit. Complex tannins are tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit. Condensed tannins are all oligomeric and polymeric proanthocyanidin is formed by the linkage of C-4 of one catechin with C-8 or C-6 of the next monomeric catechin. . Phenolic polymers, commonly known as tannins, are compounds of high molecular weight that are divided into two classes viz. hydrolyzable tannins and condensed tannins

Tannins are found commonly in fruits such as grapes, persimmon, blueberry, tea, chocolate legume forages, tree legume like Acacia spp., Sesbania spp., in grasses like sorghum, corn, etc. (Giner-Chavez, 1996). Several health benefits have been recognized for the intake of tannins and some epidemiological associations with the decreased frequency of chronic diseases have been established (Serrano et al., 2009). Recently tannins have attracted scientific interest, especially due to the increased incidence of deadly diseases such as AIDS and cancers. The search for new lead compounds for the development of novel pharmaceuticals has become increasingly important, especially as the biological action of tannin-containing plant extracts has been well documented (Mueller-Harvey, 1999).

Alkaloids

Alkaloids are natural products that contain heterocyclic nitrogen atoms and are always basic in character. The name alkaloids is derived from its 'alkaline' nature and it was used to describe any nitrogen-containing base (Muller-Harvey, 1999). Most alkaloids have a bitter taste. The alkaloid quinine, for example, is one of the bitter-tasting substances known and is significantly bitter at a molar concentration of (1 x 10 - 5) (Mishra, 1989). Alkaloids are so numerous and involve a variety of molecular structures that their rational classification is difficult. However, the best approach is to group them into families, depending on the type of heterocyclic ring system present in the molecule (Krishnan et al., 1983). Classes of alkaloids according to the heterocyclic ring system they contain are listed below. Pyrrolidine alkaloids- These contain a pyrrolidine (tetrahydropyrrole) ring system. For example, hygrine found in leaves of *Erythroxylum spp*. and *Leonotis spp.*

Pyridine alkaloids: These have a piperidine (hexahydropyridine) ring system, for example, coniine, piperine, and isope-lletierine.

Pyrrolidine-pyridine alkaloids: These contains the heterocyclic ring system with pyrrolidine-pyridine. For example myosmine, a nicotine alkaloid found in tobacco (Nicotiana tabaccum).

Pyridine-piperidine alkaloids- This family of alkaloids contains a pyridine ring system joined to a piperidine ring system. For example, anabasine alkaloid from Anabasis aphyllan, Quinoline alkaloids- These have the basic heterocyclic ring system quinoline. For example, quinine

occurs in the bark of the cinchona tree (Manske, 1942; Wiesner et al., 2003; Anjali & Singh, 2016).

Isoquinoline alkaloids: They contain heterocyclic ring system isoquinoline. For example, opium alkaloids like narcotine, papaverine, morphine, and codeine. Alkaloids are significant for the survival of plants because they ensure their protection against micro-organisms (antibacterial and antifungal activities), insects, and herbivores (feeding deterrents) and also against other plants by means of allelopathy (Molineux et al., 1996). The use of alkaloid-containing plants as dyes, spices, drugs, or poisons can be traced back almost to the beginning of civilization. Alkaloids have many pharmacological activities including anti-hypertensive effects (many indole alkaloids), anti-arrhythmic effects (quinidine, spareien), anti-malarial activity (quinine), and anti-cancer actions (dimeric indoles, vincristine, vinblastine). Others alkaloids have stimulant properties such as caffeine and nicotine, and morphine functions as an analgesic (Wink et al., 1998).

Terpenoids

They are natural products that are derived from five-carbon isoprene units. Most of the terpenoids have multi-cyclic structures that differ from one another by their functional groups and basic carbon skeletons. These types of natural lipids can be found in every class of living things and are therefore considered the largest group of naturally occurring secondary metabolites (Elbein et al., 1999). Many of these are commercially useful as flavors and fragrances in foods and cosmetics (Horborne and Tomas-Barberan, 1991). Terpenes are widespread, mainly in plants as constituents of essential oils. Their building block is the hydrocarbon isoprene, CH2=C(CH3)-CH=CH2. Hemiterpenoids consist of a single isoprene unit. The only hemiterpene is the isoprene itself, but oxygen-containing derivatives of isoprene such as isovaleric acid and prenol are also classified as hemiterpenoids. Monoterpenoids-Monoterpenoids have two isoprene units. Monoterpenes may be of two types i.e. linear (acyclic) or type. Some examples of monoterpenes include Geranyl pyrophosphate, Eucalyptol, Limonene, Citral, Camphor, and, Pinene. Sesquiterpenes- Sesquiterpenes have three isoprene units e.g. Artemisinin, Bisabolol, and, Fernesol, cyclic compounds, such as Eudesmol found in *Eucalyptus oil*. Diterpenes- These are composed for four isoprene units. They are derived from geranyl-geranyl pyrophosphate. For example, cembrene, kahweol, taxadiene, and, cafestol. Retinol, retinal, and phytol are the biologically important compounds while using diterpenes as the base (Banskota et al., 2003; Han et al., 2017).

These consist of six isoprene units e.g. Lanosterol and squalene found in wheat germ and olives. Tetraterpenoids contain eight isoprene units which may be acyclic like lycopene, monocyclic like gamma-carotene, or bicyclic like alpha- and beta-carotenes. Among plant secondary metabolites terpenoids are a structurally most diverse group; they function as phytoalexins in plant's direct defense or as signals in indirect defense responses, which involve herbivores and their natural enemies (Mccaskill & Croteau, 1998). Many plants produce volatile terpenes to attract specific insects for pollination. Some are plants less volatile but strongly bitter-tasting or toxic terpenes also protect themselves from being eaten by animals (Degenhardt et al., 2003). In addition, terpenoids have medicinal properties such as anti-carcinogenic (e.g. perilla alcohol and diterpenoid anticancer drug taxol), anti-malarial (e.g. the sesquiterpenoid artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic activity (e.g. glycyrrhizin) (Langenheim, 1994; Dudareva et al., 2004).

Saponin

Most members of this group form stable foam in aqueous solutions such as soap, hence the name 'saponin'. Chemically, saponins, as a group, include compounds that are glycosylated steroids, triterpenoids, and steroid alkaloids. Two main types of steroid aglycones are known, spirostan and furostan derivatives. The main triterpene aglycone is a derivative of oleanane. The carbohydrate part consists of one or more sugar moieties containing glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid glycosidically linked to a sapogenin (aglycone) (Mohan & Daffodil, 2016). Saponins that have one sugar molecule attached at the C-3 position are called monodesmoside saponins, and those that have a minimum of two sugars, one attached to the C-3 and the other at C-22, are called bidesmoside saponins (Lasztity et al., 1998). Many saponins are known to be anti-microbial, inhibit molds, and protect plants from insect attack. Saponins may be considered a part of plants' defense 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 on the membrane-permeabilizing, immunostimulant, hypocholesterolemic, and anti-carcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake, and reproduction in animals. These structurally diverse compounds have also been observed to kill protozoans and molluscs. They are also known to be antioxidants. They also impaired the digestion of protein and the uptake of vitamins and minerals in the gut. They cause hypoglycemia and act as antifungal and antiviral agents (Takechi et al., 1999; Traore et al., 2000).

MATERIALS AND METHODS

Qualitative phytochemical Test

Collection and identification of plant materials

Fresh leaves of *Annona muricata* L., *Annona senegalensis* Pers., and *Annona squamosa* L. were collected from Uturu and Umuahia for identification..

Sample preparation

The fresh leaves were air-dried in the Department of Plant Science of Michael Okpara University of Agriculture Umudike laboratory for two weeks. The dried leaves were pulverized with a blender. 5g portions of powdered samples were separately weighed and dispersed separately in 50 ml of water and ethanol and left for 24 hours. It was then filtered using Whatman No 42 filter paper to obtain the extracts which were used to carry out the analysis.

Preparation of reagents

Wagner's reagent: 3 g of potassium peroxide was weighed and dissolved in 100 mls of distilled water. 1.5 g of potassium iodide was added and followed by the addition of 1g of iodine crystals. The solution was allowed to stand for a few minutes for the crystals to dissolve properly.

30% Ferric chloride solution: 30 g of ferric chloride pellets were dissolved in 100 mls of distilled water and allowed to stand for a few minutes for the pellets to dissolve properly.

10% Acetic acid in ethanol: 10 mls of acetic acid was added to 90 mls of ethanol.

20% Sodium hydroxide: 20 g of sodium hydroxide pellets were mixed with 80mls of distilled water and allowed to dissolve. It was made up to 100mls with distilled water.

20% Aqueous ethanol: 20 mls of ethanol was distilled with 80 mls of distilled water

Phytochemical screening (Qualitative analysis of plant extract)

The chemical test was carried out on the powdered leaf samples of the species Annona using standard procedures to identify the constituents. Tannins, flavonoids, terpenoids, and alkaloids levels were tested for.

Test for alkaloids

1. **Dragendorff's test**: 1mL of the extract was taken and placed into a test tube. Then 1mL of potassium bismuth iodide solution (Dragendorff's reagent) was added and shaken. An orange-red precipitate formed indicates the presence of alkaloids (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016)
2. **Wagner's test**: 1mL of the extract was taken and placed into a test tube. Then 1mL of potassium iodide (Wagner's reagent) was added and shaken. The appearance of a reddish-brown precipitate signifies the existence of alkaloids (Trease & Evans, 1989; Wallis, 1989; Pandey and Tripathi, 2014; Beena et al., 2016).
3. **Mayer's test**: 1mL of the extract was taken and placed into a test tube. Then 1mL of potassium mercuric iodide solution (Mayer's reagent) was added and shaken. The emergence of whitish or cream precipitate implies the presence of alkaloids (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016).
4. **Hager's test**: 1mL of solution of an extract was taken and placed into a test tube. Then 1mL of saturated ferric solution (Hager's reagent) was added and shaken. The formation of a yellow-colored precipitate indicates the existence of alkaloids (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016).

Test for glycosides

1. **Bontrager's test (modified)**: One gram of the crude extract was first weighed, placed into a test tube, and dissolved in 5 mL of dilute hydrochloric acid. Then 5mL of ferric chloride (5%) solution was added. The mixture was shaken and placed in a water bath. Then the mixture was allowed to boil for 10min, cooled, and filtered (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016). Afterward, the mixture was then extracted again with benzene. Finally, an equal volume of ammonia solution was added to the benzene layer. The appearance of pink color indicates the presence of anthraquinone glycosides (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016).
2. **Legals test**: 1mL of an extract was taken, and then an equal volume of sodium nitroprusside was added followed by a few quantities of sodium hydroxide solution and shaken. The formation of pink-to-blood-red precipitate signifies the existence of cardiac glycoside (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016).
3. **Keller–Killiani test**: 2mL of the extract was taken and diluted with an equal volume of water. Then 0.5mL of lead acetate was added, shaken, and filtered. Again, the mixture was extracted with an equal volume of chloroform, evaporated, and dissolved the residue in glacial acetic acid. Then few drops of ferric chloride were added (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016). Again, the whole mixture was placed into a test tube containing 2 mL of sulfuric acid. The emergence of a reddish-brown layer that turns bluish-green implies the presence of digitoxose (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016).

Test for steroids and triterpenoids

1. **Liebermann Burchard's test**: This method is utilized for an alcoholic extract. The extract needs to dry out first through evaporation, then extracted again with chloroform. Add a few drops of acetic anhydrides followed by sulfuric acid from the side of the test tube. The formation of violet to blue-colored ring at the

junction of the two liquids indicated the presence of steroids (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016; Dhawan & Gupta, 2017).

2. **Salkowski's test**: 1mL solution of the extract was taken and 2mL of chloroform was added, shaken, and filtered. A few drops of concentrated sulfuric acid were added to the filtrate, shaken, and allowed to stand. The development of golden-yellow precipitate indicates the presence of triterpenes (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016; Dhawan & Gupta, 2017).

Test for tannins

1. **Gold Beater's skin test**. A Gold Beater's Skin was obtained from Ox skin. The Gold Beater's Skin was soaked in 2% hydrochloric acid and washed with distilled water. Then it was placed in a solution of an extract for 5min and washed with distilled water. Finally, it was placed in 1% ferrous sulfate solution. If the Gold Beater's Skin changes to brown or black tannins are present (Trease and Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016).

2. **Gelatin's test**: 1mL of the extract was taken and placed in a test tube. Then 1% gelatin solution containing sodium chloride is added and shaken. The appearance of a white precipitate indicates the presence of tannins (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016; Dhawan & Gupta, 2017).

Test for flavonoids

1. **Shinoda's test**: 1mL of the extract was taken and placed into a test tube. Then, a few drops of concentrated hydrochloric acid was added followed by 0.5mg of mRimandoium turnings, and shaken. The emergence of pink coloration indicates the presence of flavonoids (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016).

2. **Lead acetate test**: To detect the presence of flavonoids, 1mL of the extract was taken and placed into a test tube. Then few drops of lead acetate were added and shaken. The formation of yellow precipitate signifies the presence of flavonoids (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016).

3. **Alkaline reagent test**: 1mL of the extract was taken and placed into a test tube. Then few drops of sodium hydroxide solution were added and shaken. The emergence of intense yellow color that turns to colorless after adding dilute acid implies the existence of flavonoids (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016; Dhawan and Gupta, 2017).

Test for phenols

1. **Ferric chloride test:** 1mL solution of an extract was taken and placed into a test tube. Then 1% gelatin solution containing sodium chloride was added and shaken. The formation of bluish-black color indicates the presence of phenols (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016).

2. **Lead acetate test:** 1mL solution of an extract was taken and placed into a test tube. Then 1mL of the alcoholic solution was added, followed by dilution with 20% sulfuric acid. Finally, a solution of sodium hydroxide was added. The formation of red-to-blue color signifies the occurrence of phenols (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016).

3. **Gelatin test:** A solution of plant extract was placed into a test tube followed by 2mL of 1% gelatin solution and shaken. The appearance of a white precipitate indicates the presence of phenols (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016).

4. **Mayer's reagent test (potassium mercuric iodide test):** To a solution of plant extract, 1mL of Mayer's reagent was added in an acidic solution. The manifestation of white precipitate shows the existence of phenolic compounds (Trease & Evans, 1989; Wallis, 1989; Pandey & Tripathi, 2014; Beena et al., 2016).

Test for Saponins

The presence of saponin was determined using the methods stated below.

Libermann Test (Foam Test): If stable, characteristic honeycomb-like froth is obtained, saponins are present.

Burchard test: A white precipitate indicates the presence of saponin.

GC-MS analysis of leaf extracts

The GC-MS characterization of the compounds present in the extract was done at the Federal University of Technology Akure (FUTA). The reference library used was the National Institute of Standards and Technology (NIST) 2011 mass spectral digital library data. The Perkin-Elmer GC Clarus 500 system(Perkin-Elmer Co. Norwalk, CT06859, and USA), interfaced to a Mass spectrometer (GC-MS) equipped with an Elite-1,5 fused silica capillary column measuring 30 mm X 0.25 mm with a film thickness of 0.25 mm composed of 100% dimethyl polysiloxane was used. The GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. The carrier gas used was Helium (99.999%) at a constant flow rate of 0.5ml/min. About 1µm sample injection volume was utilized (Split ratio 10:1); the inlet/injection temperature was maintained at 250 oC, ion

source temperature was 30 0C. The oven temperature was programmed initially at 6 0C for 2min, then increased to 12 0C, then programmed to increase to 28 0C at a rate of 2 0C for 5mins. The total run time was 77 min. The MS transfer line was maintained at a temperature of 30 0C for 5mins. The Mass Spectra were taken at 70 eV; a scanning interval of 0.5 seconds and fragments from 45-415.00 KD.

RESULT

Quantitative phytochemical studies

GC-MS analysis of *A. muricata*, *A. senegalensis,* and *A. squamosa* ethanolic leaf extracts

GC-MS chromatogram of the ethanolic extract of *A. muricata* showed twenty peaks, indicating the presence of twenty compounds. The identified compounds found in higher quantities include (Z)-2-(henicos-12-en-1-yl)-6-methyl-2H-pyran-4(3H)-one (37.83%), N-Aminopyrrolidine (20.38%), Propanoic acid, 2, 3-dichloro- (5.17%), etc. *A. senegalensis* showed six peaks, representing six compounds, with the highest quantity being ntegerrimine (57.472%). *A. squamosa* showed thirty peaks indicating the presence of thirty compounds with Longifolenaldehde (7.34%) as the compound with the highest concentration (Figure 1,2,3).

DISCUSSION

Biologically active plant chemicals other than traditional nutrients that have a beneficial effect on human health have been termed phytochemicals. In the preliminary qualitative phytochemical analysis of *A. muricata A. senegalensis* and *A. squamosa*, they all contained different phytochemicals such as flavoniod, alkaloids, phenol, saponin, tannins etc. This attests to the potential medicinal values of the plant materials. This corresponds with the work of Yahaya et al. (2017). Phytochemical screening of *A. senegalensis* revealed the presence of various secondary metabolites like tannins, flavonoids, saponin and triterpenes. This agrees with the work of Jada et al., (2014; 2015); Afolabi & Afolabi (2003). There is absence of alkaloids and this is in contrast to You et al., (1995). Results of similar evaluations on other medicinal plants have shown that medicinal plants with such bioactive substances are usually potent healing agents. Flavonoids are reportedly strong antioxidant agents with free radical scavenging capacity and as such the extract may be of value in the management of oxidative stress induced diseases. Phenolic and flavonoid compounds have been reported to act as antioxidants to exert antiallergic, anti-inflammatory, antidiabetic, antimicrobial, antipathogenic, antiviral, antithrombotic, and vasodilatory effects and prevent diseases such as cancer, heart problems, cataracts, eye disorders, and Alzheimer's Comunian et al., (2017); Shahidi & Ambigaipalan, (2015); Vodnar et al., (2017). The most important features of flavonoids include their ability to protect against oxidative diseases, activate or inhibit various enzymes bind specific receptors, and protect against cardiovascular diseases by reducing the oxidation of low-density lipoproteins (LDL) (Pietta, 2000). The antioxidant effect of *Annona muricata*, *Annona senegalensis* has been reported. Results of previous studies have shown that flavonoids exhibited an antioxidant effect which compared favourably with that of vitamin C. This may be why *A. muricata, A. senegalensis* and *A. squamosa* leaf extract are good anti-cancer agents (Vanitha et al., 2017). The flavonoids have been reported to exert multiple biological properties including anti-microbial, cytotoxic, anti-inflammatory and anti-tumor activities; but the best-described property of almost every group of flavonoids is the capacity to act as powerful antioxidants (Shirsat et al., 2012; Teiten et al., 2013) which can protect the human body from the dangerous free radicals and reactive oxygen species (ROS). These compounds have been studied mainly for their properties against oxidative damage leading to various degenerative diseases, such as cardiovascular diseases, inflammation and cancer. Indeed, tumour cells, including leukemia cells, typically have higher levels of reactive oxygen species than normal cells so that they are particularly sensitive to oxidative stress (Mandal et al., 2010).

The GC/MS analysis of *A. muricata* revealed twenty compounds. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1,2,3). The prevailing compound is (z) – 2 – (henicos – 12 – en – 1-yl) – 6 – methyl – 2H – pyran – 4 (3H) – one which was present at the concentration of 37.83%. It possess anti-inflammatory and antimicrobial activities. Cytarabine of 4.39% concentration is a cancer medicine that interferes with the growth and spread of cancer cells in the body. It is used to treat certain types of leukemia (blood cancer). This supports the work of Pieme et al. (2014) and Yang et al. (2015) that reported the use of this plant for the treatment of cancer and microbial diseases.

The GC/MS analysis of *A. squamosa* indicated the presence of thirty compounds as presented in (Table 4). The prevailing compound is longifolenaldehyde which was present at the concentration of 7.34%. Longifolenaldehyde which is an essential oil and can be used as aroma in perfummary industries. It is also an intermediate in the synthesis of longifolene which is a constituent of black cumin seed and may exhibit anticancer and antibacterial activities (Hajhashemi et al., 2010). The use of *A. squamosa* as an anti-cancer has been reported by Vanitha et al. (2017). It also indicated the presence of estrone-benzoate (2.57%) which is a plasticizer and also forms polyesters that are used as emulsifying agents and resin intermediates. Octadecanoic acid, ethyl ester (5.59%) is used for the production of detergents, cosmetics, candles, soaps, plastics, photographic

Table 1. Qualitative Test of Phytochemical Compounds present in the Ethanolic leaf extracts of *A. muricata, A. senegalensis,* and *A. squamosal.*

Annona species	**Alkaloids**	**Flavonoids**	**Phenols**	**Saponins**	**Tannins**	**Steroids/triterpenes**
Annona muricata	-	+++	-	++	_	++
Amona senegolensis	-	+++	+	++	+++	++
Annona squamosa	+++	++	++	+	++	-
senegolensis						
Annona squamosa	+++	++	++	+	++	-

++ + = very high concentration

++ = high concentration

+ = low concentration

- = absent

Table 2. GC-MS Phytochemical analysis of *Annona muricata* (Soursop).

S/N	**RT**	**Name of components**		**MW**	**% Report**
1	1.441	N-Aminopyrrolidine	C4H10N2	86	20.38%
2	1.552	N-[2-[N-(p-Methylthiophenyl)sulfamyl]ethyl]-2,4-dihydroxy-3,3-dimethylbutyramide	C15H24N2O5S2	376	0.63%
3	1.983	(Z)-2-(henicos-12-en-1-yl)-6-methyl-2H-pyran-4(3H)-one	C27H48O2	404	37.83%
4	2.559	Pyrrolidine, N-(3-methyl-3-butenyl)-	C9H17N	139	0.32%
5	3.793	1-(3-Methyl-3-phenyl-1,3-dihydroindazol-2-yl)ethanone	C16H16N2O	252	3.00%
6	4.654	Propanoic acid, 2,3-dichloro-	C3H4Cl2O2	142	5.46%
7	7.878	3a,7-Methano-3aH-cyclopentacyclooctene, 1,4,5,6,7,8,9,9a-octahydro-1,1,7-trimethyl-, [3aR-(3aα,7α, 9aβ)]-	C15H24	204	5.17%
8	9.502	2-Amino-3-hydroxypyridine	C5H6N2O	110	1.49%
9	12.418	Dasycarpidan-1-methanol, acetate (ester)	C20H26N2O2	326	1.55%
10	17.045	1-[2-(1,3-Dioxo-1,3-dihydro-isoindol-2-yl)-ethyl]-1H-indole-2,3-dione	C18H12N2O4	320	0.42%
11	18.553	Corynan-17-ol, 18,19-didehydro-10-methoxy-	C20H26N2O2	326	0.25%
12	20.165	Chromone-2-carboxylic acid, methyl ester	C11H8O4	204	0.44%
13	20.392	Gibberellic acid	C19H22O6	346	2.58%
14	21.864	l-Menthone	C10H18O	154	0.78%
15	26.119	3-Pyridinecarboxylic acid, 2,7,10-tris(acetyloxy)-1,1a,2,3,4,6,7,10,11,11a-decahydro-1,1,3,6,9 -pentamethyl-4-oxo-4a,7a-epoxy-5H-cyclopenta[a]cyclopropa[f]cycloundecen-11-yl ester, [1aR-(1aR*,2R*,3S*, 4aR*,6S*,7S*,7aS*,8E,10R*,11R*,11aS*)]-	C32H39NO10	597	1.52%
16	28.976	Butanoic acid, 2-chloro-3-oxo-, methyl ester	C5H7ClO3	150	2.58%
17	30.228	Bicyclo[2.2.1]heptane-1-carboxylic acid, 2-chloro-, methyl ester, endo-	C9H13ClO2	188	2.95%
18	31.392	Methyl glycocholate, 3TMS derivative	C36H69NO6Si3	695	4.31%
19	32.492	Cytarabine	C9H13N3O5	243	4.39%
20	33.533	Triamcinolone Acetonide	C24H31FO6	434	3.97%

Table 3. GC-MS Phytochemical analysis of *Annona senegalensis* (wildsop).

S/N	**RT**	**Name of components**		**MW**	**%Report**
1	13.771	Catechol	C6H6O2	110	3.55
2	15.597	Kaur-16-ene18-oic acid, 13-hydroxy-,methyl ester,(4α)-(±)-	C21H32O3	332	9.074
3	16.169	Ntegerrimine	C18H25NO5	335	57.472
4	17.004	10-Acetoxy-2-hydroxy-1,2, 6a,6b,9,9,12a-heptamethyl-1, 3,4,5,6,6a,6b,7,8,8a,9,10,11, 12,12a,12b,13,14b-octadecahydro -2H-picene-4a-carboxylic acid, methyl este	C33H5205	528	2.979
5	17.113	Paromomycin	C23H45N5O14	615	23.14
6	17.914	Androstan-17-one, 3-hydroxy-, (3α,5β)-	C19H30O2	290	3.784

Table 4. GC-MS Phytochemical analysis of *Annona.*

S/N	RT	Name of components	Formular	MW	% Report
1	2.353	1-Butanol, 3-methyl-, acetate	C7H14O2	130	3.59%
2	11.176	Estrone, benzoate	C25H26O3	374	2.57%
3	11.921	1-Propanol, 3-ethoxy-	C5H12O2	104	1.98%
4	14.202	trans-Cinnamic acid	C9H8O2	148	3.63%
5	16.321	Dodecanoic acid	C12H24O2	200	2.60%
6	16.984	Diethyl Phthalate	C12H14O4	222	2.74%
7	17.124	Myristin, 1,3-diaceto-2-	C21H38O6	386	4.80%
8	19.091	4,4,8-Trimethyl-non-7-en-2-one	C12H22O	182	3.79%
9	19.231	Neophytadiene	C20H38	278	2.92%
10	19.912	,10,14-Trimethyl-pentadec-2-yl nicotinate	C24H41NO2	375	4.55%
11	20.069	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	C20H40	280	2.27%
12	20.546	Docosanoic acid, docosyl ester	C44H88O2	648	6.41%
13	21.512	Octadecanoic acid, ethyl ester	C20H40O2	312	5.59%
14	22.537	[10.8.0]eicosa-1(12),14-diene	C20H34	274	4.27%
15	25.016	Tetratriacontane	C34H70	478	3.76%
16	26.163	Octadecanal	C18H36O	268	2.37%
17	27.385	Hexadecanal	C16H32O	240	3.22%
18	27.542	Longifolenaldehyde	C15H24O	220	7.34%
19	29.614	Hexadecanoic acid, 2,3-bis(acetyloxy) propyl ester	C23H42O6	414	3.14%
20	29.806	Oxalic acid, butyl 2-phenylethyl ester	C14H18O4	250	2.89%
21	31.75	Ergost-5-en-3-ol, (3β)-	C28H48O	400	2.76%
22	33.781	Kauran-18-al, 17-(acetyloxy)-, (4β)-	C22H34O3	346	2.93%
23	35.725	Dicyclooctanopyridazine	C16H24N2	244	3.04%
24	37.576	Erythromycin	C37H67NO13	733	2.90%
25	39.363	Clazolam	C18H17ClN2O	312	2.65%
26	41.074	Cyanazine	C9H13ClN6	240	2.62%
27	42.715	Cholest-5-en-3-ol, 4,4-dimethyl-, (3β)-	C29H50O	414	2.64%
28	44.304	Tetracycline	C22H24N2O8	444	2.15%
29	45.835	Ethanone, 1-(2-aminophenyl)-	C8H9NO	135	1.97%
30	47.307	Benzenesulfonamide, N-[5-(2-methoxyethoxy)-2-pyrimidinyl]-	C13H15N3O4S	309	1.93%

materials and lubricants (Aydin et al., 2005; Hartwig et al., 2003). There is also the presence of erythromycin and tetracycline which are broad spectrum antibiotics.

The GC-MS analysis of *A. senegalensis* revealed the presence of six compounds as presented in (Table 3). The prevailing compound which was present at the concentration of 57.472 is ntegerrimine which serves as defence mechanism for some organisms. It also contains catechol (3.55%) which is a precursor to pesticides, flavours, fragrances, pharmaceuticals and as a photographic developer (Barner, 2004; Drzyzga, 2003). Catechol skeleton also occurs in a variety of natural products especially the antioxidant (Khalafi & Rafiee, 2010). Paromonycin (23.14%) is an antibiotic that treats only parasitic and bacterial infections. It is used to treat acute and chronic intestinal amebiasis (bowel infection by a parasite in the stomach or bowel). It is also used with other medicines to help lessen the symptoms of hepatic coma (a complication of liver disease) caused by too much natural substance (ammonia) in the body (MFMER, 2021; Vicens & Westhof, 2001). This supports the work of Alawa et al., 2003 and Ruffo et al., 2002 that stated the use of the plant to treat parasitic and bacterial infections.

There were great differences in the GC-MS phytochemical compounds present in the three *Annona* species studied.

The intrageneric phytochemical characters they share in common in this work includes presence of flavonoids and saponin. The diagnostic features includes presence of alkaloids in *A. squamosa*, absence of phenol and tannin in *A. muricata* and absence of triterpens in *A. squamosa*.

CONCLUSION

All these phytochemicals are useful in their identification, classification and delineation. From the observations made

in the similarities of characters of the three studied species supports the present-day classification of the three species in the same genus (*Annona*).

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REFERENCES

Afolabi, F. & Afolabi, O. (2013). Phytochemical Constituents of Some Medicinal Plants in South West, Nigeria. IOSR j appl chem. 4: 76–78.

Alawa, C., Adamu, A., Gefu, J., Ajanusi, O., Abdu, P. et al. *In vitro* Screening of Two Nigerian Medicinal Plants (Veronia amygdalina and Annona senegalensis). For Anthel mantic Activity. Vet Parasitol. 113: 73-81.

Anjali, P.D. & Singh, D. (2016). Quinolone: A diverse therapeutic agent. Int J Pharm Sci Res. 7: 1-13.

Balasundram, N., Sundram, K. & Samman, S. (2006). Phenolic compounds in plant and agri-industrial byproducts: antioxidant activity, occurrence, and potential uses. Food chem. 99: 191-203.

Banskota, A.H., Attamini, F. & Usia, T. (2003). Novel norcassane-type diterpene from the seed kernels of Caesalpinia crista. Tetrahendron Lett. 44: 6879-6882.

Barner, A.B., Bongat, A.F.G., & Demchenko, A.V. (2004). "Catechol" in encyclopedia of reagents for organic synthesis.

Beena P, Rajesh KJ, Arul B (2016). Preliminary phytochemical screening of Cicer arietinum in folklore medicine for hepatoprotection. J innov Pharm biol sci. 3: 153-9.

Barton, D., & Meth-Cohn, O. (Eds.) (1999). Comprehensive natural products chemistry. Newnes.

Comunian, T.A., Ravanfar, R., de Castro, I.A., Dando, R., Favaro-Trindade, C.S. et al. (2017). Improving oxidative stability of echium oil emulsions fabricated by Microfluidics: effect of ionic gelation and phenolic compounds. Food Chem. 233: 125–134.

Dai, J. & Mumper, R.J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecule. 15: 7313- 7352.

Degenhardt, J., Gershenzon, J., Baldwin, I. T. & Kessler, A. (2003). Attracting friends to feast on foes: Engineering terpene emission to make crop plants more attractive to herbivore enemies. Curr Opin Biotechnol. 14: 169–176.

Dhawan D, Gupta J (2017). Comparison of different solvents for phytochemical extraction potential from Datura metel plant leaves. Int J Biol Chem. 11: 17-22.

Drzyzga, O. (2003). Diphenylamine and derivatives in the environment. A review. Chemosphere. 58: 809-818.

Dudareva, N., Pichersky, E. & Gershenzon, J. (2004). Biochemistry of plant volatiles. Plant Physiol. 135: 1893–1902.

Gibson, E., Wardel, J. & Watts, C. J. (1998). Fruit and Vegetable Consumption, Nutritional Knowledge and Beliefs in Mothers and Children. Appetite. 31, 205-228.

Giner-Chavez, B. I. (1996). Condensed tannins in tropical forages. Doctoral Thesis. Cornell University. Ithaca, NY, USA.

Hajhashemi, V., Ghannadi, A. & Hajiloo, M. (2010). Analgesic and anti-inflammatory effects of Rosa damascena hydroalcoholic extract and its essential oil in animal models. Iran J Pharm Res. 9: 163- 168.

Hamburger, M. & Hostettmann, K. (1991). Bioactivity in Plants: The Link between Phytochemistry and Medicine. Phytochemistry. 30: 3864-3874.

Han, B., qingwen, Z. & Ligen, L. (2017). Natural occurring furanoditerpenoids: distribution, chemistry and their pharmacological activities. Phytochem Rev. 16: 235-270.

Harborne, J.B. (1999). An overview of antinutritional factors in higher plants. In: Secondary plants products. Antinutritional and beneficial actions in animal feeding (Eds. Caygill, J.C. and Mueller-Harvey, I) Nottingham University Press, UK. 7-16.

Harborne, J.B. & Tomas-Barberan, F.A. (1991). Ecological Chemistry and Biochemistry of Plant Terpenoids, Clarendon, Oxford.

Hasler, C.M. and Blumberg, J.B. (1999). Phytochemicals: Biochemistry and Physiology. J Nutr. 129: 756S-757S.

Jada, M., Usman, W. & Olabisi, A. (2015). Crude Flavonoids Isolated from the Stem Bark of Annona senegalensis have Antimicrobial Activity. J adv biol. 2: 24–29.

Khalafi, L. & Rafiee, M. (2010). Kinetic study of the oxidation and nitration of catechols in the presence of nitrous acid ionization equilibria. J Hazard Mater, 173: 801-806.

Koche, D., Shirsat, R., Syed I. & Bhadange, D.G. (2010). Phytochemical screening of eight ethnomedicinal plants from Akola District (MS). Int J Pharm Biol. 1: 256 - 259.

Krishnan, R., Chandravadana, M.V., Ramachander, P.R. & Bharathkumar, H. (1983). Inter-relationships between growth and alkaloid production in Catharanthusroseus G. Don. HerbaHungarica. 22: 47-54.

Lacaille-Dubois, M. A. and Wagner, H. (2000). Bioactivesaponins from plants: An update. In: Studies in Natural Products Chemistry; Atta-Ur Rahman, ed. Elsevier Science. Amsterdam. 21: 633-687.

Langenheim, J. H. (1994). Higher plant terpenoids: A phytocentric overview of their ecological roles. J Chem Ecol. 20: 1223- 1280.

Lasztity, R., Hidvegi, M. & Bata, A. (1998). Saponins in food. Food Rev Int. 14: 371-390.

Mandal, S. M., Chakraborty, D. and Dey, S. (2010). Phenolic acids act as signaling molecules in plant–microbe symbioses. Plant Signal Behav. 5, 359-368.

Manske, R.H. (1942). The chemistry of quinolones. Chem Rev. 30: 113-144.

Mathai, K. (2000). Nutrition in the Adult Years. Krause's Food, Nutrition, and Diet Therapy. 271: 274 - 275.

Mccaskill, D. and Croteau, R. (1998). Some caveats for bioengineering terpenoid metabolism in plants. Trends Biotechnol. 16: 349–355.

Mishra, S. N. (1989). Analytical methods for analysis of total alkaloids in root of Withania spp. Proc. All India workshop on M and AP, Faizabad. 492- 95.

Mohan, V.R. Tresina, P.S. & Daffodil, E.D. (2016). Antinutritional factors in legume seeds: characteristics and Determination. Encyclopedia of Food and Health. 16: 211-220.

Molineux, R.J., Nash, R.J. and Asano, N. (1996). Alkaloids: Chemical and Biological Perspectives. 11, Pelletier SW, ed. Pergamon, Oxford; 303.

Mueller-Harvey, I. (1999). Tannins: their nature and biological significance. Secondary plant products: Antinutritional and beneficial actions in animal feeding. 17-39.

Pandey A, Tripathi S (2014). Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. J Pharmacogn phytochem. 2:115-9.

Pieme, C.A., Kumar, S G., Dongmo, M.S., Moukette, B.M., Boyoum, F.F., et al. (2014). Antiproliferative activity and induction of apoptosis by Annona muricata (Annonaceae) extract on human cancer cells. BMC complementary and alternative medicine, 14(1), 1-10.

Pietta, P.G. (2000). Flavonoids as antioxidants. J Nat Prod. 63: 1035 1042.

Pretorius, J.C. (2003). Flavonoids: A Review of Its Commercial Application Potential as Anti- Infective Agents. Current Medicinal Chemistry Anti Infective Agents. 2: 335-353.

Pridham, J.B. (1960). Phenolics in Plants in Health and Disease, Pergamon Press, New York.

Ramawat, K.G., Dass, S. and Mathur, M. (2009). The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. In: Herbal drugs: Ethnomedicine to modern medicine; Springer- Verlag Berlin Heidelberg.

Rao, N. (2003). Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. Asia Pac J Clin Nutr. 12: 9-22.

Ruffo, C.K., Birnie, A. & Tengnas, B. (2002). Edible Wild Plants of Tanzania. Published by Regional Land Management Unit, Nairobi.

Samrot, A., Mathew, A. and Shylee, L. (2009). Evaluation of bioactivity of various Indian medicinal plants- An in vitro study. Intern Med J. 8: 1-6.

Schofield, P., Mbugua, D. M. & Pell, A. N. (2001). Analysis of condensed tannins: a review. Pharm Res. 7: 1089-1099.

Serrano, J., Puupponen-Pimia, R., Dauer, A., Aura, A. & Saura Calixto, F. (2009). Tannins: current knowledge of food sources, intake, bioavailability and biological effects. Mol Nutr Food Res. 53: 310–29.

Shahidi, F. & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects - A review. J Funct Foods. 18: 820–897.

Shirsat, R., Suradkar, S.S. and Koche, D.K. (2012). Some phenolic compounds from Salvia plebeia R. Br. Bioscience Discovery. 3: 61-63.

Takechi, M., Matsunami, S., Nishizawa, J., Uno, C. & Tanaka, Y. (1999). Haemolytic and antifungal activities of saponins or anti-ATPase and antiviral activities of cardiac glycosides. Planta Medical. 65: 585–586.

Teiten, M. H., Gaascht, F., Dicato, M. & Diederich, M. (2013). Anticancer bioactivity of compounds from medicinal plants used in European Medieval traditions. Biochem Pharmacol. 86: 1239-1247.

Traore, F., Faure, R., Ollivier, E., Gasquet, M., Azas, N., et al. (2000). Structure and antiprotozoal activity of triterpenoidsaponins from Glinusoppositifolius. Planta Medical. 66: 368–371.

Trease GE, Evans WC (1989). Textbook of pharmacognosy.13th ed. London, UK; Toronto, Canada; Tokyo, Japan: Bailiere Tindall. p. 200-1.

Vanitha, V., Hemalatha, S., Pushpabharathi, N., Amudha, P. & Jayalakshmi, M. (2017). Fabrication of nanoparticles using Annona squamosa leaf and assessment of its effect on liver (Hep G2) cancer cell line. Materials Science and Engineering. 191.

Vicens, Q. and Westhof, E. (2001). Crystal structure of paromomycin docked into the eubacterial ribosomal decoding A site. Structure. 9: 647-658.

Vodnar, D.C., Călinoiu, L.F., Dulf, F.V., Ştefănescu, B.E., Crişan, G. et al. (2017). Identification of the bioactive compounds and antioxidant, antimutagenic and antimicrobial activities of thermally processed agro-industrial waste. Food Chem. 231: 131–140.

Wallis TE (1989). Text book of pharmacognosy.Delhi, India:CBS Publishers and Distributors. 356-549.

Walton, N.J., Mayer, M.J., & Narbad, A. (2003). Vanillin. Phytochemistry. 63: 505-515..

Wiesner, J., Ortmann, R., Jomaa, H. & Schlitzer, M. (2003). New antimalarial drugs. Angew Chem. 42: 5274-5293.

Wink, M., Schmeller, T. & Latz-Briining, B. (1998). Modes of action of allelochemical alkaloids: Interaction with neuroreceptors, DNA and other molecular targets. J Chem Ecol. 24: 1888-1937.

Yahaya, G., Fred, W. & Hany, A.E. (2017). Annona muricata: Is the natural therapy to most disease conditions including cancer growing in our backyard? A systematic review of its research history and future prospects. Asian Pac J Trop Med. 10: 835-848.

Yang, C., Gundala, S.R., Rao, M., Subrahmanyam, V., Reid, M.D. et al. (2015). Synergistic interactions among flavonoids and acetogenins in Graviola (Annona muricata) leaves confer protection against prostate cancer. Carcinogenesis. 36: 656-665.

You, M., Wickramaratne, D., Silva, G., Chai, H., Chagwedera, T. et al. (1995). Roemerine, an Aporphine Alkaloid from Annona senegalensis that Reverses the Multidrug-Resistance Phenotype with Cultured Cells. J Nat Prod. 58: 598–604.

Zübeyir, H., Şükrü, B. & İlhami, G. (2017). Antioxidant and Antiradical Properties of Selected Flavonoids and Phenolic Compounds. Biochem Res Int. 10.

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