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Antimicrobial and Biochemical Properties of Three Nigerian food Spices; *Piper guineense, Xylopia aethiopica and Monodora myristica*

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ABSTRACT

Piper guineense, Xylopiaaethiopica and Monodoramyristica seeds are among the plant parts commonly used as spices and studied evaluating the proximate composition while mineral parameters were evaluated using Atomic Absorption Spectrophotometer (AAS) methods. Antibacterial activity was assessed using the agar well diffusion method while gas chromatography and mass spectrometry were used for the phytochemical analysis. Results of the study showed that X. aethiopica seeds were high in percentage moisture content (9.60%), crude fiber (9.97%) and crude protein (28.67%) compare to P. guineense and M. myristica while P. guineense was high in percentage lipid (26.00%) and carbohydrate (63.05%). The differences in the moisture content, lipid, crude fibre, crude protein and carbohydrate were statistically significant (P<0-05). M. myristica seeds had a higher amount of copper (49.885mg/kg), potassium (2.835%), calcium (1.570%) and sodium (1.730%) than P. guineense and X. aethiopica seeds, while X. aethiopica seeds had a higher amount of zinc (33.675mg/kg) than M. myristica and P.guineense seeds. P. guineense seeds had a higher amount of iron (655.455mg/kg) than M. myristica and X.aethiopica seeds. The differences in the calcium, potassium, sodium, copper, iron, and zinc were statistically significant (P<0.05). Ethanol extract of X. aethiopica showed higher inhibitory effect (P<0.05) against Staphylococcus aureus with MIC of 3.125mg/ml and MBC 25mg/ml. Among the thirty five compounds detected in piper guineense, thirty two compounds in xylopiaaethiopica and thirty one compounds in Monodoramyristica, the common ones were Lauric anhydride, Dodecanoic acid and Octadecanoic acid. The present study had pontentials for microbial control with Xylopiaaethiopica showing the most inhibitory action.

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INTRODUCTION

Plants have been used by man in the treatment of various diseases throughout the ages (Ebunlomo et al., 2012). Plants have provided a good source of anti-infective agents and phyto-medicines that have been used as natural therapies for maintaining human health (Gislene et al., 2000). Medicinal plants have been valuable sources by which antibacterial agents are procured (Cowan, 1999) for the manufacture of new drugs for human use (Panda et al., 2009). Plant products are important to humans in various ways, and are most commonly used as food, spices and medicines. Antibacterial agents of plant origin have been reported and spices are among those ascertained to have great prospective for use as antibacterial agents, (Arora and Kaur, 1999; Okeke et al., 2001).

Spices are seeds, kernels, fruits, stem, leaves and roots, used in various forms such as fresh, ripe, dried, broken or powdered mainly to contribute to colour, taste, aroma, flavour or preservation of food (Shelef, 1984, Ene-Obong et al., 2015). They can be categorized into tiny or cultivated fruits, nuts, herbs, and leafy vegetables. In Nigeria, some spices are used in small amounts in concocting certain soups which are delicacies and are also used in medicine and religious rituals (Sofowora 1993 and Valko et al., 2007). Spices are the most common plant materials with potential antibacterial properties that are used in foods; and they have been used

traditionally for years by many cultures for preserving foods and to enhance aroma and flavour. Spices are not restricted to flavour but possess dynamic medicinal properties such as antibacterial activity, anticancer, antioxidants and anti-bronchitis. Many spices have antibacterial properties and this explain why spices are mostly used in warm climatic areas which have more infectious diseases (Nanasombat and Lohasupthawee, 2005). The antibacterial property can be entirely inhibitory, cytostatic, total killing or cytocidal. Spices are aromatic or with strong taste, but used in all cultures to enhance the taste of foods. Some of the secondary metabolites that are active against microorganisms fall into groups of compounds generically known as alkaloids, flavonoids, glycosides, tannins and saponins (Shelef, 1984).

The major advantage of using the herbal drug is that there are little or no side effects. Such as side effects of depletion of the normal intestinal flora, bone marrow depression, dysentery, inflammation, damage to the liver and kidney can be overcome by using herbal preparations either as drug (Saha, et al., 2012). Spices have been identified to possess sufficient bioactive antibacterial compounds for medicinal benefits (Shelef, 1984; Papp et al., 2007).

The purpose for the identification of phyto-compounds in plants is to develop therapeutic intended vital part and to remove redundant materials (Waldesch et al., 2003). Proximate composition of the plant materials provides facts of the quantity of the materials favorable in nutrition (Ekanem et al., 2004).The knowledge of the chemical constituents of plants is not only preferable for the finding of medicinal agents but also for unfolding the current origin of valuable productive phyto-compounds for the combination of chemical compounds (Uraku et al., 2015).

Gas chromatography (GC) and mass spectrometry (MS) have over the years been one of the best combinational techniques in the identification of the chemical constituents of various plants. Gas chromatography separates the constituents while mass spectrometry helps to determine the molecular weight of these compounds (Murugan and Mohan, 2014). The Gas chromatography also separates components in a sample while mass spectrometry fragments the components and identifies them on the basis of their mass. Separation in Gas chromatography is based on their boiling points where the substances with higher boiling points come out later and those with lower boiling points come out first. When they are out, they go into the mass spectrometry which identifies them using their mass to charge ratio (Ashish et al., 2014).

Due to resistance of some microorganisms to certain orthodox drugs as well as their side effects, scientists are increasingly becoming involved in the screening of such plants with the aim of establishing their potential antibacterial effects and identifying the compounds responsible for the antibacterial properties (Ndukwe et al., 2005; Aibinu et al., 2007).

Piper guineense belong to the family Piperaceae and cultivated for its fruit. It is known as a herb and spice. They are used for the treatment of cough, bronchitis, intestinal diseases and rheumatism (Essiett and Ibanga, 2012).

Xylopiaaethiopica is an aromatic plant of the Annonaceae family that grows up to 20m high. It is known as a spice. It has been reported in folklore that Xylopiaaethiopica is very potent in curing several ailments including cough, rheumatism and nerve pains as well as in the elimination of blood clots when used to prepare pepper soup for newly delivered mothers (Ekpo et al., 2012).

Monodoramyristica is a flowering plant of the Annonaceae family. It is known as Ehiri or Ehuru (Igbo), Gujiyadanmiya (Hausa), and Ariwo, arigbo, abo lakoshe or evinaghose (Yoruba) (Aboaba et al; 2011). The aromatic seeds are stomachic, tonic and are added to medicines to impart stimulating properties (Achinewhu et al., 1995, Bakarnga-Via et al., 2014, Beare-Rogers et al., 2001).

The seeds of the Piper guineense, Xylopiaaethiopica, and Monodoramyristica are common spices and condiments included in a variety of indigenous Nigerian recipes (Okeke, 1998). In Nigeria it is indicated that the three spices act as stimulants and laxatives, used to smoothen the skin and cure fever, cough and stomach disorders. The results of this study will help in assessing the antibacterial properties of ethanol extracts of Piper guineense, Xylopiaaethiopica, and Monodoramyristica in relation to their ethno-medicinal uses.

A number of spices have been reported in research to exhibit antibacterial activity against different types of microorganisms. This varies widely depending on the type of spices, test medium and microorganism. Besides, some of these claims still need to be authenticated through scientific testing.

LITERATURE REVIEW AND DISCUSSION

Morphological Features and Classification of Piper Guineense

Piper guineense is a spice plant belonging to the family Piperaceae. The plant is native to Western Africa and is cultivated in countries like Nigeria where it is found commonly in the southern part. It is a perennial plant that grows in evergreen rainforest, forest edges, usually in wet places, gallery forest along rocky rivers. It is a spice plant commonly known as African black Pepper. It is also known with different vernacular names in Nigeria: Igbo (Uziza), Yoruba (Iyere) and Ibibios of Akwa Ibom (Odusa) (Isawumi, 1984). They consist of bulbs, flower bud, fruit, seed, and leaves. The plant grow up to 20m tall climbing up bole of trees by means of adventitious roots and is characterized by heart-shaped leaves and oval, alternate, 12cm long. The leaves have a peppery taste, pale greenish in color when fresh and darker green when dried. Flowers are arranged in a spiral along the spine and greenish yellow in color.

Scientific classification:

Kingdom – Plantae
Division – Magnoliophyta
Class – Magnoliopsida
Order – Piperales
Family – Piperaceae
Genus – Piper
Species – P. guineense

Phytochemical Constituents and Biological Activities of Piper Guineense

Piper guineense are used as therapeutic agents in minor ailments (Okwu, 2001).Phytochemicals are not vitamins or minerals but are bioactive compound found in plant foods that work with nutrient and dietary fibers to protect against disease. The presence of phytochemicals like alkaloids in seed extract of Piper guineense reveals the possession of medicinal properties within the plant. Alkaloids are used as CNS stimulant and as powerful pain relievers. The flavonoids possess antioxidant, anti-inflammatory, anti-tumor and anti-allergic properties. They are also found to have cholesterol lowering ability. The tannins as primary component are astringent, thus use for the management of diarrhea, dysentery, anti-flammation of the mucous membrane. Saponins have anti-carcinogenic properties. Piper guineense also contains glycosides and are useful in the management of diseases associated with the heart. (Okoye and Ebeledike, 2013).

The extracts of dry fruits of Piper guineense showed improved male reproductive functions on male fertility parameters using adult Sprague dawley rats. It improved sperm motility, sperm function, testicular spermatogenesis and weight and this could be attributed to its androgenic and aphrodisiac properties (Ekanem, et al., 2004).

Morphological Features and Classification of Xylopia Aethiopica

Xylopia is a Greek word meaning "bitter wood" while aethiopica is referring as the origin of the tree. Xylopiaaethiopica is cultivated in West Africa, and belong to Annonaceae family that grows into a tall tree of about 20m high. It is present in rain forests near the coast. It also grows in riverside and fringing forest, and as a pioneer species in arid savanna regions. Leaves of Xylopia aethiopica are always simple and entire. The leaf blades are commonly elliptic, or oblong. Inflorescences in Xylopia aethiopica are axillaries, sometimes arising from the axils of fallen leaves. Flower buds in Xylopiaaethiopica are linear to ovoid. Fruits have a typical aggregate pattern; with each carpel developing into a discrete fruit let called a monocarp of about 0.3cm. The ripe monocarps are longitudinally alone the abaxial surface to expose the seeds (Iwu, et al., 1999).

Xylopiaaethiopica is known by different names in different languages – English (Negro pepper), Yoruba (eru), Igbo (Uda), and Hausa (kimbara) (African Plant Database, 2013).Xylopiaaethiopica is used in African cuisine and traditional medicine. The fruit extract is used as tonic to support female fertility, for allay of childbirth and as a woman rectify after child birth for relief of pains in the ribs, chest, neuralgia and in treatment of boils and skin eruptions (Iwu, et al., 1999).

Scientific classification:
Kingdom – Plantae
Order – Magnoliales
Family – Annonaceae
Genus – Xylopia
Species – X. aethiopica

Pytochemical Constituents and Biological Activities of Xylopia Aethiopica

Phytochemicals as antioxidants play vital roles in human health. Xylopiaaethiopica contain some phytochemicals which exhibit some biological effects as a consequence of their antioxidant activities. The seeds, leaf, bark and root of the Xylopiaaethiopica revealed the presence of alkaloids, saponins, tannins, steroids, flavonoids and glycosides. Alkaloids constituent of Xylopiaaethiopica is known to have anti-pyretic effect. Xylopiaaethiopica is a powerful antibiotic and medicine against malaria as well as a local anesthesia for pain relief. Flavonoids have strong anti-cancer activity, protect against all stages of carcinogenesis and prevent oxidative cell damage. Saponins have wide range of biological properties. They are used to recover homeostasis, have anti-inflammatory and anti-cancer action (Fleischer, 2003).

Xylopiaaethiopica Bark, roots, leaves, fruits, and seeds have all been used in medicines throughout western and central Africa. Xylopiaaethiopica has been used to treat a wide variety of maladies (Fleischer, 2003).

Morphological Features and Classification of Monodora Myeistica

Monodoramyristica is a perennial edible plant of the Annonaceae family of flowering plants (Aboaba et al., 2011).The tree grows in the evergreen forests of the sub-Saharan African regions in West Africa: from Liberia to Nigeria, Cameroon and Ghana, as well as in Angola, Uganda and West Kenya. It is commonly known as African nutmeg, calabash nutmeg, Ehiri or Ehuru (Igbo); Gujiyadanmiya (Hause) and Ariwo, arigbo, Abo naghose (Yoruba) (Achinewhu, et al., 1995). The Monodoramyristica tree grows up to 35m in height and 2 m in diameter (Weiss, 2002). The flower is singular, pendant, large and fragrant. The fruit is a smooth berry, green and spherical of 20cm diameter. Inside the fruit the numerous oblongoid, pale brown, 1.5cm long seeds are surrounded by a whitish fragrant pulp (Weiss, 2002).

Scientific classification:
Kingdom – Plantae
Order – Magnoliales
Family – Annonaceae

Genus – Monodora
Species – M. myristica

Phytochemical Constituents Biological Activities of Monodora Myristica

Monodoramyristica seeds revealed the presence of flavonoids, tannins, glycosides, alkaloids, saponins, and steroids. Monodoramyristica seeds possess high amount of copper, potassium, sodium and calcium. The seed extracts of Monodoramyristica have be reported to possess some antibacterial activities which can be employed in the development of novel therapeutic agents against the test organisms such as Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia (Achinewhu, et al., 1995, Bakarncga-Via, et al., 2014, Beare-Rogers, et al., 2001).

PHYTOCHEMICALS

Phytochemicals are chemical compounds that occur naturally in plant and which may have protective or disease preventive properties. These phytochemicals have the ability to modulate diseases such as stroke, diabetes and other diseases (US, FDA, 2002). Phytochemical are derived from the consumption of whole meal and are also available as dietary supplements. Some examples of phytochemicals are tannins, saponins, terpenes, glycosides (US, FDA, 2002).

2.4.1 Alkaloids

Alkaloids are natural plant compounds with a basic character and usually contain one or more nitrogen atom in a heterocyclic ring. They are usually colourless, crystalline, non-volatile solids which are insoluble in water but soluble in ethanol, ether, chloroform and other organic solvents. Only very few liquids are soluble in water. They have a bitter taste and are optically active. Most alkaloids are physiologically active while some are extremely poisonous (Robert, 1998).

2.4.2 Saponins

Saponins are structurally complex amphiphatic glycosides of steroids and triterpenoids that are widely produced by plants (Sparg, et al., 2004) and also by certain marine organisms, such as starfish and sea cucumbers (Tang, et al., 2009; Van Dyck, et al., 2010). Saponins have a wide range of properties, including fungicidal activity, bitterness and sweetness and other industrial applications such as foaming and surface active agents (Takahashi, et al., 2010).

2.4.3 Tannins

Tannins are aromatic compounds containing phenolic groups that bind to and precipitate proteins leading to inhibition of enzymes (Guyot, et al., 1996).The tannin compounds are distributed in many species of plants and growth retardation has been observed in ducks and rats fed on diets containing high tannin in sorghum and legumes (Desphande and Salunkhe, 1982). Tannins have been found to form irreversible system with proline–rich proteins resulting in the inhibition of the cell protein synthesis; as well, herbs that contain tannin are constringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery but this use has not been indicatively found to be due to their antibacterial activities (Shimada, 2006).

2.4.4 Flavonoids

Flavonoids are compounds with structure of a 15-carbon skeleton, which consists of two phenyl rings and a heterocyclic ring. Flavonoids have inhibitory activity against organisms that cause plant diseases (Galeotti, et al., 2008).

2.4.5 Glycoside

Glycoside is a molecule in which a sugar is bound to another functional group through a glycosidic bond (Brito-Arias, 2007).They are used for the treatments of congestive heart failure and cardiac arrhythmias but their relative toxicity prevents them from being used widely (Ambrosy, et al., 2014).

2.5 Importance of Minerals

Minerals are naturally occurring inorganic substances, present in the body and help our body grow, develop, and stay healthy. Minerals are classified as macro (major) or micro (trace) and ultra-trace elements. Calcium, Potassium, sodium and chloride, are the macro elements, iron, copper, cobalt, phosphorus, magnesium, iodine, zinc, manganese, fluoride, chromium, selenium and sulfur are the micro elements while boron, silicon, arsenic and nickel are the ultra-trace elements (Atinmo and Omololu, 1982).

Calcium has great significance in bone function, nerve transmission and hormonal secretion and play supportive role in bone, teeth and muscle functions. (Van der Velde, et al., 2014).

Potassium helps the cells to produce electrical discharge which are critical for body functions such as heart function (Mikko, et al., 2006). Potassium deficiency disease may result to mental puzzlement, paralysis and muscular weakness (Murray et al., 2000).

Sodium functions in certain metabolic, heart activities, generation of nerve impulses and maintenance of electrolyte balance (Pohl, et al., 2013).

Iron functions as a transport medium for electrons within cells, and serves as oxygen carrier to tissues from the lungs by red blood cell hemoglobin (Mascotti, et al., 1995). Iron is required for making hemoglobin and it is a pro-oxidant which is also needed by microorganisms for proliferation (Galan, et al., 2005). Iron is required for diverse cellular functions, a constant balance between iron uptake, transport, storage, and utilization is required to maintain iron homeostasis (Lieu, et al., 2001).

Zinc serves a major role in immune function, wound healing and as antibacterial agents (McCarthy, et al., 1992). Zinc also play vital role during physiological growth in human body. Some enzymes involved in cell replication and metabolism of macronutrient are dependent on Zinc (Arinola, 2008).

Copper serves in maintaining the strength of the skin, blood vessels, and an antioxidant by neutralizing free radicals and help in preventing some of the damage they cause (Bonham, et al., 2002). Copper stimulates the immune system to fight infections, to repair injured tissues, and to promote healing (Scleiber, et al., 2013). Copper deficiency lead to disease or tissue injury and in the development of severe blood disorders including myelodysplastic syndrome (and in extreme cases death) (Stern, 2010).

2.6 Description of Test Organisms

Pathogens are widely distributed in the environment and may be significant causes of mortality and morbidity in the population (Indu, et al., 2006).

2.6.1 Salmonella Typhi

Salmonella typhi is a gram negative, aerobic, rod-shaped, actively motile, bacterium that causes typhoid fever in humans. Salmonella typhi is acquired by ingestion of contaminated food and water and must get through the gastric pH limit in the stomach prior to attaching in the small intestine (Parry, et al., 2002). Salmonella typhi enters through a supporting layer of loose connective tissue directly under a mucous membrane of the small bowel by perforating the epithelial tissue moderated by the cystic fibrosis transmembrane conductance regulator (CFTR) or through the M-cell, a specialized lymphoid epithelial cell causing hypertrophy of the Peyer's patches (Crump, et al., 2015; Gebert, et al., 1996). Spreading of the bacteria from the Peyer's patches occurs through the lymphatic system and the bloodstream. Cellular reproduction inside the reticuloendothelial system is a mark of the authenticity of the disease (Crump, et al., 2015).They appear grey-white 2-3mm diameter mucoid colonies on blood agar and pale coloured having black centers (H_2S-producing salmonella) on MacConkey agar (Monica, 2000). Salmonella typhi were more resistant to the spice extracts due to the differences in the cell wall composition and structure, especially the polysaccharide and protein outer membrane in the cell wall which limit diffusion of antibacterial agents into the cell wall Agatemor (2009) and Nwinyi, et al., (2009) .

2.6.2 Escherichia coli

Escherichia coli are motile, facultative, anaerobic, Indole positive, lysine decarboxylase positive, gram negative rods that ferment sugars to produce acid and gas (Monica, 2000). Escherichia coli attach or spread through a mucosal surface containing adhesions which are fimbriae that facilitate the establishment of urinary tract infection, sepsis/meningitis and gastro-intestinal infections (Nataro and Kaper, 1998). Due to the polysaccharide and protein outer membrane in the cell wall composition and structure, which limit diffusion of antibacterial agents into the cell wall. Escherichia coli are resistant to the spice extracts Agatemor (2009) and Nwinyi, et al., (2009).

2.6.3 Staphylococcus aureus

Staphylococcus aureus cells are Gram-positive cocci of uniform size, facultative anaerobic, non-motile and do not form, non-capsulate, coagulate positive and Catalase positive (Brown, et al., 2005), lactose fermenting. Staphylococcus aureus produces yellow to cream or white 1-2mm in diameter colonies on blood agar, smaller (0.1-0.5mm) colonies on MacConkey agar (Monica, 2000).Staphylococcus aureus has the ability to produce Staphyloxanthin which is a golden coloured carotenoid pigment which acts as molecules that enable them to achieve attachment to the host cells (Clauditz, et al., 2006).

2.6.4 Klebsiella pneumoniae

Klebsiellapneumoniae are gram-negative, non-motile, capsulated rods and lactose fermenting. Klebsiella pneumoniae are aerobes and facultative anaerobes. Klebsiella pneumoniae can be pathogenic bacteria which can lead to severe bronchopneumonia with lung abscesses, urinary tract infections, and meningitis (Monica, 2000). Klebsiella pneumoniae are easily and rapidly transmitted, mostly in the hospitals due to the nature of procedures that allow easy access of pathogens into the body such as catheters and surgery. Klebsiella pneumoniae produces two important molecules known as Lipopolysaccharide (LPS) and capsular polysaccharide (CPS) that add to their effectiveness and enable them to attach to cells. The Lipopolysaccharide (LPS) in Klebsiella pneumoniae contains lipid A, core, and O-polysaccharide antigen which help them in resisting complement- mediated killing, while the capsular polysaccharide (CPS) in the outer layer of the bacteria controls the influence of surfactant protein D (SP-D)on the bacteria which defends against any pathogens that may invade the lungs resulting in pneumonia. They produce large grey-white mucoid colonies on blood agar, lactose-fermenting mucoid pink colonies on MacConkey agar and yellow mucoid colonies on CLED (Monica, 2000).

CONCLUSION AND RECOMMENDATIONS

The ethanol extract of Xylopiaaethiopica ,Monodoramyristica and Piper guineense seeds possess Alkaloids, Tannins, Flavonoids, Saponins, Steroids, Triterpenes, Glycosides, and Carbohydrate and there was absence of Anthraquinones. Proximate analysis had significant ($p>0.05$) showing Xylopiaaethiopica seeds had highest percentage moisture (9.60%), crude fibre (9.97%), and crude protein (28.67%) compare to Piper guineense and Monodoramyristica seeds while Piper guineense was high in percentage lipid (26.00%) and carbohydrate (63.05%) compare to Xylopiaaethiopica and Monodoramyristica seeds. The mineral analysis had significant ($p>0.05$) showing Monodoramyristica seeds has a high amount of Calcium (1.570%), Potassium (2.835%), Sodium (1.730%) and Copper (49.885mg/kg) than Piper guineense and Xylopiaaethiopica seeds, while Piper guineense seeds have a

high amount of Iron (655.455mg/kg) than Monodoramyristica and Xylopiaaethiopica seeds, while Xylopiaaethiopica seeds have a high amount of Zinc than Monodoramyristica and Piper guineense seeds.

The ethanol extract of Xylopiaaethiopica seeds had MIC and MBC of 12.5mg/ml and 50mg/ml against Staphlococcus aureus while that of M. myristica and P. guineense with MIC of 50mg/ml had no activity on the MBC respectively.

The synergistic effect of ethanol extract of Xylopiaaethiopica and Monodoramyristica had MIC and MBC of 3.125mg/ml and 25mg/ml against Staphylococcus aureus, while that of Monodoramyristica and Piper guineense had MIC and MBC of 12.5mg/ml and 50mg/ml respectively. That of Xylopiaaethiopica + Monodoramyristica + Piper guineense had MIC and MBC of 12.5mg/ml and 50mg/ml respectively.

The Gas Chromatography-Mass Spectrometry analysis (GCMS) had identify thirty two compounds from the ethanol extract of Xylopiaaethiopica seeds, thirty five compounds from Piper guineense and thirty one compounds from Monodoramyristica confirmed based on their peak area retention time and molecular formula. Caryophyllene, β-farnesene, linalool and Germacrene D identify in Piper guineense, Oleic acid, Octadecanoic acid, and n-hexadecanoic acid identify in Monodoramyristica, Dodecanoic acid, Hexadecanoic acid, methyl ester and Methyl-18-methylnonadecanoate identified in ethanol extracts of Xylopiaaethiopica seeds might be responsible for the antibacterial.

The study recommends that, the spices may be used as antibacterial agents. Also, the bioactive compounds identified can be further used in animal studies to determine their toxicological effects.

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