

Preliminary Screening of Phytoconstituents and Anti-Bacterial Examination of *Annona muricata* and *Cucumbita maxima* Leaves

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Accepted 14 December 2020

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ABSTRACT

Dried leaves of *Annona muricata* and *Cucumbita maxima* were extracted using chloroform, methanol and hexane solvents. The extracts were analysed for phytoconstituents using standard procedures. A preliminary test was also carried out on the anti-bacterial properties of the different solvent extracts against *S. aureus*, *V. cholera* and *E. coli*. Phytochemical analysis of *Annona muricata* and *Cucumbita maxima* showed the presence of alkaloids, fats and oil, tannins, saponins, flavonoids, terpenoids, steroids and proteins. However, glycosides were not detected in any of the plant extracts and coumarins were not detected in *A. muricata* and flavonoids were not detected *Cucumbita maxima*. The growth of *S. aureus* was inhibited in chloroform and hexane extracts of *A. muricata* and hexane and methanol extract of *C. maxima*. The growth of *V. cholera* was inhibited to varying degrees by the extracts from all the solvents, while *E. coli* was only inhibited in hexane extracts of both plants. The results observed revealed that both plants can serve useful purposes in traditional medicine and can be harnessed for scientific design in drug synthesis and production.

Keywords: Phytochemicals, screening, solvent extracts, *Annona muricata* and *Cucumbita maxima*, anti-bacterial

INTRODUCTION

Researchers have developed a new interest in medicinal plants and have given time and value to different plant-based research and their outcome. Although the application of herbal medicine is age-long, its value has been on the increase because more information on their usefulness to man and the environment are now known. The importance of curative plants has played vital role in the management of illnesses all over the world (Oyewale and Audu, 2005). Different diseases are being treated with medicinal plants on regular basis and also to combat against different contagious infections. Medicinal plants will remain a foremost source of medication and natural products (Halilu, 2006).

The health care of the world population is enormous and it has been estimated that about 80% population is dependent on herbal drugs for their health and therapeutic needs (Kurian, 2010). Plants parts (leaves,

stem, bark, root, etc), which are known and believed to contain bioactive chemicals are used to prepare herbal products (Chintamunnee and Mahomoodally, 2012). The preparations that are obtained from plant parts contain a mixture of different components that are accountable for the management and treatment of ailments. These bioactive medicinal plants have been accepted for the treatment or prevention of a lot of health disorders and contain a lot of natural antioxidants (Rafieian-Kopaei, 2012). Some of these medicinal plants possess curative properties and are active as antimicrobial, anti-cancer and anti-diabetic agents. Thus, making medicinal plants a reliably good source of research for the preparation and synthesis of new drugs.

Medicinal plants are believed to contain different or several chemical compounds which act in synergy and autocatalyze healing reaction to produce a combined

Table 1: Preliminary phytochemical screening of *Annona muricata*.

Phytochemicals	Chloroform	Methanol	Hexane
Alkaloids	+	+	+
Fats & Oil	+	-	+
Glycosides	-	-	-
Tannins	+	-	-
Saponins	+	-	-
Flavonoids	+	+	+
Terpenoids	+	-	+
Steroids	-	+	-
Proteins	+	-	-
Coumarins	-	-	-

effect that exceeds the overall activity of the combined action of individual constituent (Mohamoodally et al., 2013). Still, the collective action of these constituents tends to increase the action of the active metabolite by acceleration or retardation of its absorption in the body. The activities of pathogens within the body cause disease which needs corresponding chemotherapy (Oyewale and Audu, 2005). The study, therefore, was undertaken to qualitatively examine the phytoconstituents and anti-bacterial activity of two medicinal plants, *Annona muricata* and *Cucumbita maxima* Leaves.

MATERIALS AND METHODS

The fresh leaves of *Annona muricata* and *Cucumbita maxima* were harvested from the plant in the morning from farmlands in Obite in Ogbag-Egbema-Ndoni Local Government Area of the Rivers State, Nigeria. The leaves were transported to the Chemistry Laboratory of Ignatius Ajuru University of Education, Port Harcourt. The fresh leaves were washed carefully and placed on clean plastic trays and allowed to air-dry freely. The drying was done until a constant weight was achieved after three consecutive weighings.

The dried leaves were cut into small pieces and then transferred to an electric blender and blended to fine powder. The finely blended leaves were sieved with a 2mm mesh and were subsequently transferred into clean glass bottles and corked. The samples were stored in a cool dry wood cupboard pending time for further analysis.

The powdered plant leaves were extracted using the method described by Obomanu et al (2005). 100 g of each of the powdered plant leaves were extracted using three different extraction solvents (chloroform, methanol and hexane) separately. The extraction was conducted for 48 hrs in a 250 ml beaker which was tightly covered with aluminum foil to prevent loss of solvent through volatility. After 48 hrs, the extraction was stopped and filtration was carried out with a filter paper. The filtrate, which contained the components of interest were put into clean bottles and tightly closed until time for the different phytoconstituents and anti-bacterial tests.

The crude extract of the powdered leaves of *Annona muricata* and *Cucumbita maxima* were qualitatively analyzed to identify alkaloids (Jamuna et al., 2014), fats and oil (Marcus et al., 2019), glycosides (Edori and Ekpete, 2015), tannins (Edori and Dibofori-Orji, 2016), saponins (Makkar et al., 2007), flavonoids (Jia et al., 1999), terpenoids (Yanishlieva, 2001), steroids (Marcus et al., 2019), proteins (Kumar et al., 2013) and coumarins (Sofowara, 1982).

Three different bacterial strains (*E. coli*, *S. aureus*, and *V. cholerae*) were obtained from the Microbiology Department of the University of Port Harcourt Teaching Hospital (UPTH), Choba, Port Harcourt, Rivers State. The strains were cultivated at a temperature of 37°C for 24 hours and thereafter, routine cultures were prepared. Culture of bacterial strains was done according to the method of Liliwirianis et al. (2011). Subsequently, sterile saline water was used to adjust the turbidity of the broth culture prepared for the diverse microbes to a cell concentration of 3.0×10^8 cells/ml.

The microbial activity of the extracts from the plant on the bacterial strains was done according to standard methods (Gulluce et al., 2007; Nna et al., 2019). The zones of inhibition after incubation at 37 °C for 24 hours were observed and recorded appropriately (Dieudonne et al., 2015).

RESULTS AND DISCUSSION

The results of the phytochemicals present in the different solvent extracts of the screened plants *Annona muricata* and *Cucumbita maxima* are shown in Tables 1 and 2. Alkaloids were observed in all the solvent extracts of both plants except in hexane extract of *Cucumbita maxima*. Fats and oil were observed in chloroform extracts of both plants and hexane extract of *Annona muricata*. Glycosides were not detected in all the extracts of both plants. Tannins were present in the chloroform extracts of both plants and methanol extract of *Cucumbita maxima*. Saponins were present in chloroform extract of *Annona muricata* and methanol extract of *Cucumbita maxima*. Flavonoids were identified in all the extracts of *Annona muricata*, but absent in the extracts of *Cucumbita maxima*. Terpenoids were present

Table 2: Preliminary phytochemical screening of *Cucurbita maxima*.

Phytochemicals	Chloroform	Methanol	Hexane
Alkaloids	+	+	-
Fats & Oil	+	-	-
Glycosides	-	-	-
Tannins	+	+	-
Saponins	-	+	-
Flavonoids	-	-	-
Terpenoids	+	+	+
Steroids	+	+	+
Proteins	+	+	-
Coumarins	+	+	-

in the chloroform and hexane extracts of *Annona muricata* and all the extracts of *Cucurbita maxima*. Steroids were only present in methanol extract of *Annona muricata*, but was present in all the extracts of *Cucurbita maxima*. Proteins were present in chloroform extract of both plants and methanol extract of *Cucurbita maxima*. Coumarins were absent in all the extracts of *Annona muricata*, but present in chloroform and methanol extracts of *Cucurbita maxima*.

The presence or detection of any phytoconstituents in plant parts is an important component of traditional curative practice and medicine. These active components are the reasons behind the treatment of many ailments since they have been identified as inhibitors of microbial growth in human systems (Marcus et al., 2019).

The observation of different phytoconstituents in the leaves of *Annona muricata* and *Cucurbita maxima* is in agreement with the observations of different authors who examined different plant parts for phytochemical composition. Chukwudi and Ezeabara (2018) observed the presence of six different phytochemicals in the leaf and stem of *Mimosa invisa*. Tula et al. (2012) observed the presence of eight separate phytochemicals in the leaves, stem and root bark of *Vernonia amygdalina*. The use of plant parts for curative measures is on the increase due to the presence of phytochemicals in them that can serve as antioxidants (Altemimi et al., 2017).

The traditional use of these plants (*Annona muricata* and *Cucurbita maxima*) for curative purposes is supported by the presence of numerous phytoconstituents in them as observed in the different solvent extracts (Adebayo and Ishola, 2009). The curative properties of plants that contain alkaloids and flavonoids are utilized due to their diuretic, anti-inflammatory and analgesic action. Alkaloids containing herbs have been found useful in the treatment of hypertensive headache, cold, high temperature, feverish conditions and lasting Catarrh (Akinnibosun and Edionwe, 2015).

Fats and oil are traditionally used in curative medicine for gastrointestinal tract protection, carminative, anti-vomitive, anti-bacteriological, anti-fungoid, anti-pathological, antiprotozoal, insect repellents, antioxidant, anticancer, antidiabetic and antimutagenic characteristics (Al-Snafi, 2020). They are genuine

sources of energy that aid in growth, offer important fatty acids and vitamins that are readily soluble in fat for the normal function of the human system, enhancement of animal immunity and improvement of the deliciousness of food. On the contrary, fats and oil lead to different body ailments namely; overweightness, coronary thrombosis, diabetics, swelling and cancer and some classes of fats and oil have a toxicological impact on man (Kazeem and Ogunwande, 2012).

Glycosides are organic compounds, which interact with the contraction and relaxation of the heart muscles and thereby may upset the normal functions of the heart. Many of the compounds in this group have been observed to be very toxic (Singh and Rastogi, 1970). Cardiac glycosides are utilized by man in different ways such as coating arrows, as a killer agent, life taking substance, rat poisons, heart stimulants, diuretics and emetics. It is notably used to treat congested heart malfunction and arrhythmia (Singh and Rastogi, 1970; Wang et al., 2008).

Tannins possess anti-cancer, anti-mutagenic and anti-bacterial characteristics which is due to the antioxidant behaviour of tannins. They help in preventing cell damage due to oxidation and peroxidation. Despite these positive health functions, there is however the problem of negative health consequences if consumed at very high levels (Edori and Ekpete, 2015).

Saponins have found utility in the treatment of high levels of cholesterol and glycaemia. They also help to prevent cancerous growth, weight loss and swollen body. Saponins also find use in cough treatment, pain in the upper respiratory part of the human body. Besides, plants rich in saponins are used in the preparation of natural heart tonic, which is also anti-diabetics and anti-fungal (Kamel, 1991). However, some of the saponin compounds may be toxic.

Flavonoids are useful antioxidants that play a preventive role in the body against cancerous growth and deteriorating ailments (Jindal et al., 2012). Additionally, flavonoids represent one of the major plant phytochemicals that are commonly found in different parts of plants that play the role of metabolites (Singh et al., 2007). Some biotic characteristics played by this group of phytochemicals include anti-apoptosis, anti-aging, anti-carcinogenesis, anti-inflammation, anti-

Table 3: Biological Activity of the plant extracts from different solvent against different pathogens.

Bacterial isolate	Chloroform extract		Hexane extract		Methanol extract	
	<i>A. muricata</i>	<i>C. maxima</i>	<i>A. muricata</i>	<i>C. maxima</i>	<i>A. muricata</i>	<i>C. maxima</i>
<i>S. aureus</i>	8.3	R	8.6	8.6	R	8.1
<i>V. cholera</i>	8.2	9.1	12.3	7.9	11.7	9.9
<i>E. coli</i>	R	R	8.8	8.3	R	R

atherosclerosis, protection of heart muscles, enhancement of the functions of endothelial cells, prevention of angiogenesis and propagation of actions of cell (Han et al., 2007). Flavonoids are manufactured by plants to protect them from microbial poisoning, and in-vitro studies have shown their efficacy against different micro-organisms. Their protective capacity against microbes is achieved through the formation of complexes with external cells, proteins and bacterial cell walls (Marjorie, 1996).

Terpenoids are known to fight against microorganisms, fungi, parasites, viral growth, hypersensitive, irregular body movement, hyperglycemia, swollen parts and also regulate immune behaviour (Wagner and Elmadfa, 2003).

Steroids belong to a collection of secondary metabolites that have a variety of structure and biological roles. They are mostly connected with a harmful effect on human well-being. However, they possess several therapeutic uses and different researches are on-going on their applicability in the synthesis, design, discovery and use in health issues (Sultan and Raza, 2015). Anabolic steroids help to build the human immune system in the muscle and other tissues and the growth and preservation of male features namely the development of the vocal cords and body hair (Sultan and Raza, 2015), excite the growth of bones (Haines, 2001), affect need to eat, encourage manly puberty and cure lingering degenerative settings, cancer and AIDS (Pinna et al., 2006).

Proteins obtained from several medicinal plants show different potentials in the inhibition of microbial growth and also function as anti-microbes, anti-oxidants, anti-HIV infection, anticarcinogenic agent and also help to inactivate ribosomes and neuro-modulation in animals (Wani et al., 2020).

Coumarins, with the IUPAC name; 1-benzopyran-2-one belong to a class of chemical complexes in the benzopyrone group of organic compounds that are present in several plants. The health benefits of Coumarins and anti-microbial behaviour include anti-bacterial, anti-viral, anti-inflammatory, antidiabetic, antioxidant, and enzyme inhibitory activity (Venugopala et al., 2015).

The preliminary results of the inhibition of the various solvent extracts of the plants (*A. muricata* and *C. maxima*) on the bacterial isolates (*S. aureus*, *V. cholera* and *E. coli*) are shown in Table 3. The responses of the bacterial strains to the different extracts showed that *S.*

aureus was inhibited in chloroform and hexane extracts of *A. muricata* and hexane and methanol extracts of *C. maxima*, but resisted the effects of chloroform extract of *C. maxima* and methanol extract of *A. muricata*. The growth of *V. cholera* was inhibited in all the solvent extracts from the solvents. The *A. muricata* and *C. maxima* extracts in chloroform and methanol did not inhibit the growth of *E. coli*, while hexane extracts of both plants inhibited the growth of *E. coli*.

The inhibition of the growth of the different bacterial isolates in the solvent extracts is due to the presence of the different phytochemicals present (Edori and Ekpete, 2015; Marcus et al., 2019). The exhibition of antimicrobial activity by the various plant extracts is in agreement with the observations of different researchers in similar studies (Donkor et al., 2019; Larayetan et al., 2019). Some of the phytoconstituents (flavonoids, triterpenoids and alkaloids) observed in the present study have been identified as potential inhibitors of microbial growth and replication (Romero et al., 2011). The effectiveness of any extract in exhibiting biological activity is dependent on the concentration of the extract. Therefore, the potency of the phytoconstituents is a function of the amount of the potent type of the specific functional inhibiting phytochemicals present in the final composition of the extract (Suffredini et al., 2004; Edori and Marcus, 2017). Therefore, the higher the concentrations, the more the antimicrobial activity, and the lesser the concentrations of the phytochemical, the more the observed decrease in bioactivity (FAO/WHO, 1984).

Conclusion

The extracts from the plant contained different phytoconstituents and exhibited some degree of antibacterial properties against the tested microbes. Hence, they are good sources of herbal treatment of diseases associated with the tested microorganisms. Therefore, they can be used in traditional medicine and serve as potential sources of science-based drugs.

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