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RESEARCH ARTICLE

A PRELIMINARY SCREENING OF THE ANTIMICROBIAL ACTIVITY OF THE RIND OF *ANNONA SQUAMOSA* L. ON PATHOGENIC BACTERIA

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ABSTRACT

Annona squamosa L. is a multipurpose plant in which the fruits, leaves, bark, roots and the cotyledons are reported to have medicinal properties. Folklore has it that this plant is endowed with numerous properties namely anti-tumor, anti-diabetic, anti-oxidant, anti-lipidimic, anti-inflammatory and insecticidal. Numerous reports are available on the phytochemical and antimicrobial and therapeutic properties on the different parts of this plant using different solvents. In this preliminary report, the phytochemical properties of the rind of custard apple was studied which confirmed the presence of tannins, saponins, flavonoids in different concentrations. The antimicrobial activity against different pathogens showed good activity as seen by the zone of inhibition to the different extracts.

INTRODUCTION

Traditional medicines have been used worldwide since time immemorial. The term, traditional medicine is interchangeably used with herbal medicine and natural medicine. Since antiquity, man has used plants to treat common infectious diseases and even long before mankind discovered the existence of microbes; the idea that certain plants had healing potential was well accepted (Rios and Recio, 2005) A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Doherty *et al.,* 2010). Plants been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics. Natural products and their derivatives have historically been invaluable as a source of therapeutic agents. However, in the past decade, research into natural products in the pharmaceutical industry has declined, owing to issues such as the lack of compatibility of traditional natural-product extract libraries with high-throughput screening (Koehn and Carter, 2005). Nearly all cultures from ancient times to the present day have used plants as a source of medicines as a result different remedies tended to develop in different parts of the world. Current strategies to overcome the global problem of antimicrobial resistance include research in finding new and innovative antimicrobials from plants (Lino and Deogracious, 2006). The custard-apple, also called bullock's heart or bull's heart, is the fruit of the tree *Annona squamosa*. This tree is a small deciduous or semi-evergreen tree sometimes reaching 10 meters (33 ft.) tall and a native of the tropical New World that prefers low elevations, and a warm, humid climate. It is cultivated in many tropical countries, and also occurs as feral populations in many parts of the world including Southeast Asia, Taiwan, India, Australia, and Africa.

Annona squamosa is a multipurpose indigenous plant belonging to the family *Annona*ceae. It has been used for centuries as folk remedy in India to treat different disease such as antidiuretic, antioxidant, anti-inflammatory and neurological disorders such as sedative hypnotics, ovary complication. It contain several constituent belonging to category alkaloids, Glycoside, flavonoids, terpenoids, steroids and fruit pulp contain important nutrients (Gajalakshmi *et al.,* 2011). Numerous reports are available on the phytochemical, antimicrobial, and therapeutic properties of A. *squamosa* L. with respect to roots (Mariod, 2012), leaves (Gupta *et al.,* 2005), rhizome (Gnanamani and Priya, 2003), bark (Craig Hopp *et al.,* 1998; Shirwaikar *et al.,* 2004), seeds (Morita *et al.,* 1999; Panda and Kar, 2007; Rahman *et al.,* 2005). Phytochemical analysis carried out on *Annona squamosa* revealed the presence of alkaloids, tannins, saponin, steroids, cardiac glycoside, flavonoid, terpenoids and phenol. The presence of these phytochemicals support the use of this plant as antimicrobial agent (Anyanwu and Nwosu, 2014). However, there has been no report on the phytochemistry and

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antimicrobial activity of the rind of custard apple. The present work was a preliminary screening of this objective.

MATERIALS AND METHODS

Healthy and good looking custard apples were procured from the local market. They were washed thoroughly to remove any grime and dust. The rind of the fruit was carefully peeled off. 400 g of the sample were dried at room temperature for one week. After drying, 350 g were obtained. The sample was then powdered using mixer and grinder.

Extraction

The powdered sample was subjected to extraction in a soxhlet apparatus using different solvents namely benzene, chloroform, acetone, methanol, ethyl acetate and water, each for a period of 48 h.

Phytochemical analysis

The dry powder or aqueous preparations of the powder were used for testing for tannins, phlobatannins, saponins, phenols, alkaloids, flavonoids and cardiac glycosides using standard protocols.

Thin - layer chromatography

TLC was perfomed to detect the different compounds. The extracts extracted with different solvents were submitted to thin-layer chromatography (TLC). Plates 250 mm thick were developed with $CHCl_3$: CH_3OH : H_2O (80 : 18 : 2), which separate components into a wide range of Rf values. The components were visualized under visible and UV light (254 and 366 nm) and sprayed with the following reagents in order to reveal spots of different groups: modified Dragendorf's reagent for alkaloids, methanolic potassium hydroxide for coumarins, aluminium chloride for flavonoids and anisaldehyde/sulphuric acid for steroids and terpenes.

Antimicrobial assay

Antimicrobial assay was done using the different solvent extracts on pathogenic strains of *Escherichia coli, Klebsiella sp., Shigella sp., Staphylococcus aureus* and *Serratia sp.*

Agar diffusion method

Nutrient agar was poured in sterile petri dishes and was allowed to solidify. 1ml of the test culture was dropped on the solidified agar and the organism was spread all over the surface of the agar using a spreader. Wells of approximately 5mm in diameter were made on the surface of the agar medium using a sterile cork borer. The plates were turned upside down and the wells labeled with a marker. Each well was filled with 0.2ml of the extract. Streptomycin disc was used as control for the cultures. The plates were incubated aerobically at 37°C for 24 hours. Sensitivity of the organisms to the extract was recorded as the diameter of the zone of inhibition.

RESULTS AND DISCUSSION

The phytochemical analysis of the rind confirmed the presence and the percentage of each compound present in the rind of A. *squamosa* as seen in Table 1.

Table 1. Presence and percentage composition of compounds in the rind of A. *squamosa*

Constituents	Results (%)
Phenol	0.81
Alkalcids	0.86
Tannins	12.21
Flavonoids	0.51
Saponin	0.92

The antimicrobial activity of the extracts using different solvents was studied and the results tabulated as follows.

Table 2. Zones of inhibition (cm) of ethyl acetate extracts of the rind of *Annona squamosa*

Isolates	Ethyl acetate (100µl)
Escherichia coli	3.4
Staphylococcus aureus	3.2
Klebsiella sp	4.2
Shigella sp	2.1
Serratia sp	2.2

Table 3. Zones of inhibition (cm) of aqueous extracts of the rind of *Annonasquamosa*

Isolates	Aqueous extract(100µl)
Escherichia coli	1.3
Staphylococcus aureus	4.0
Klebsiella sp	4.5
Shigella sp	2.6
Serratia sp	4.1

Table 4. Zones of inhibition (cm) of methanol extracts of the rind of *Annonasquamosa*

Isolates	Methanol extract(100µl)
Escherichia coli	4.2
Staphylococcus aureus	4.0
Klebsiella sp	4.6
Shigella sp	2.8
Serratia sp	3.4

Table 5. Zones of inhibition (cm) of acetone extracts of the rind of *Annonasquamosa*

Isolates	Acetone Extract (100µl)
Escherichia coli	4.5
Staphylococcus aureus	3.4
Klebsiella sp	3.6
Shigella sp	4.0
Serratia sp	3.2

Table 6. Zones of inhibition (cm) of benzene extracts of the rind of *Annonasquamosa*

Isolates	Benzene extract(100µl)
Escherichia coli	4.1
Staphylococcus aureus	3.4
Klebsiella sp	4.2
Shigella sp	2.9
Serratia sp	2.4

Table 7. Zones of inhibition (cm) of chloroform extracts of the rind of *Annonasquamosa*

Isolates	Chloroform extract(100µl)
Escherichia coli	4.2
Staphylococcus aureus	4.0
Klebsiella sp	4.3
Shigella sp	2.8
Serratia sp	3.6

The result of this work showed that the rind extract of *Annona squamosa* inhibited the growth of all the bacteria tested. This suggests that the plant extract is broad spectrum in activity. Higher antimicrobial activity of the extracts was observed on *E.coli* and *Klebsiella* sp. The antimicrobial effect of *Annona squamosa* is due to the phytochemical constituents present in it. It is rich in phytonutrient such as flavonoids, phenolic compound tannins, saponin, terpenoids, cardiac glycosides and alkaloids. The plant *Annona squamosa* can be of immense use in phytomedicine and can be included in health care system. The phytochemicals obtained from the rind of *Annona squamosa* can be used in the treatment of infectious diseases. Extract from *Annona squamosa* contain phytochemicals which offer S an enormous potential for biocontrol of these pathogens and source of antimicrobial agents of therapeutic importance.

REFERENCES

Anyanwu C.U. and Nwosu G.C. 2014. Assessment of the antimicrobial activity of aqueous and ethanolic extracts of *Piperguineense* leaves. *J Med Plant Res.,* Vol 8(10); 436-440.

Craig Hopp D., Feras Q. Alali, Zhe-mingGu and Jerry L. McLaughlin, 1998. Three New Bioactive Bis-adjacent THF-ring Acetogenins from the Bark of *Annona squamosa*. *Bioorganic and Medicinal Chemistry,* 6: 569-575.

Doherty V.F., Olaniran O.O. and Kanife U.C. 2010. Antimicrobial activities of *Aframomum melegueta* (Alligator Pepper). *Int J Biol.,*Vol 2(2): 126-131.

Gajalakshmi S., Divya R., DivyaDeepika V., Mythili S. and Sathiavelu A. 2011. Pharmocological activities of *Annona squamosa*: A Review. *Int J Pharm Sci Rev Res.,*Vol 10 (2); 24-29.

Gnanamani A. and Priya K.S. 2003. Antibacterial activity of two plant extracts on eight burn pathogens, *J.Ethnopharmacol.,* 86: 59-61.

Gupta R.K., Achyut Narayan Kesari, Murthy P.S., Chandra R., Tandon V. and GeetaWatal 2005. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa L.* in experimental animals. *J Ethnopharmacol.,* 99 :2005,75–81.

Koehn F.E. and Carter G.T. 2005. The evolving role of natural products in drug discovery. *Nature,*Vol 4: 206- 220.

Lino, A. and Deogracious, O. 2006. The invitroantibacterial activity of *Annonasenegalensis*, *Sacuridecaelongipendiculata*and *Steganotaemaaraliacea*. Uganda medicinal plants. *J Afr Health Sci.,* 6 (1):31-35.

Mariod AA., Abdlwahab SI., Elkheir S., Ahmed YM., Fauzi PN. and Chuen CS. 2012. Antioxidant activity of different parts from *Annona squamosa* and *Catunaregamnilotica*methanolicextract. *Acta Sci Pol Technol Ailment.* Vol 11(3): 249-58.

Morita H., Yumiko Sato, and JunichiKobayashi, 1999. Cyclosquamosins A - G, Cyclic Peptides from the Seeds of *Annona squamosa*. *Tetrahedron,* 55:7509-7518.

Panda S. and Kar A. 2007. *Annona squamosa* seed extract in the regulation of hyperthyroidism and lipid-peroxidation in mice: Possible involvement of quercetin.*Phytomedicine,*14:799–805.

Rahman MM., ShahnajParvin, EkramulHaque M., Ekramul Islam M., Mohammad A. Mosaddik 2005. Antimicrobial and cytotoxic constituents from the seeds of *Annona squamosa*.*Fitoterapia,* Vol. 76: 484–489

Rios J.L. and Recio M.C. 2005. Medicinal plants and antimicrobial activity. *J Ethnopharmcol.* Vol100 (1-2): 80-4

Shirwaikar A., Rajendran K., Dinesh Kumar C. and RamgopalBodla, 2004. Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin–nicotinamide type 2 diabetic rats. *JEthnopharmacol.,*91 :171–175.

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