Scientific Journals
of the Maritime University of Szczecin

Zeszyty Naukowe
Akademii Morskiej w Szczecinie

2019, 60 (132), 203–209
ISSN 1733-8670 (Printed)
ISSN 2392-0378 (Online)
DOI: 10.17402/390

Received: 19.04.2019
Accepted: 27.10.2019
Published: 18.12.2019

Phytochemical screening and *in vitro* antimicrobial properties of *Annona muricata* extracts against certain human pathogens

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Key words: *Annona muricata*, pathogens, bacteria, fungi, antibiotics, medicinal plants

Abstract

All over the world, the use of medicinal plants is gaining more acceptability due to the possibility of discovering novel drugs from them and solving the problem of antimicrobial resistance associated with conventional antibiotics. The phytochemical composition and antimicrobial properties of crude extracts of the leaves, stems, and bark of *Annona muricata* were evaluated on *Escherichia coli, Staphylococcus aureus,* and *Salmonella typimurium*, while the antifungal properties were evaluated against *Candida albicans* and *Candida tropicalis*. The Agar well method was used for the study. At concentrations of 150 mg/ml and 300 mg/ml, inhibitory effects were observed on *E. coli* and *S. aureus,* with a visible zone of inhibition ranging from 15 mm to 21 mm respectively, and with respect to N- hexane, an antimicrobial activity range of 5 mm to 20 mm, for the leaf extract, which shows effective antimicrobial action against *E. coli* and *S. aureus*. Hot water extracts were observed to possess more bioactive compounds compared to organic solvent extracts, and exhibit higher ranges of activity against the tested bacterial species. All extracts exhibited low anti-fungal activity in the range of 8 mm to 15 mm. The phytochemical screening of the extracts of different parts of *A. muricata* revealed the presence of secondary metabolites such as tannins, alkaloids, saponins, flavonoids, steroids, and cardiac glycosides. The antimicrobial activity of the extracts was compared with a standard antibiotic, ketoconazole, and with ampicillin, which served as the controls). The results showed that *A. muricata* can be used as an anti-bacterial substance, since it shows broad spectrum activity against a range of bacteria responsible for the most common bacterial illnesses. Further research will be necessary to ascertain its full spectrum of efficacy.

Introduction

All over the world, herbal medicine has served as perhaps the most valuable and popular field of traditional medicine. Medicinal plants have been used to treat illnesses since before recorded history (Gajalakshmi, Vijayalakshmi & Rajeswari, 2012). The study of medicinal plants is essential to promoting proper use of herbal medicines and in order to identify potential sources of new drugs (Parekh & Chanda, 2007). According to a World Health Organization (WHO) report, greater than 80% of the world's population depends on traditional medicine to satisfy their primary health care needs (Vashist & Jindal, 2012). Finding new naturally active components of plants and plant-based products has interested many scientific researchers. In this regard, the antimicrobial properties of botanicals have attracted a great deal of attention as a promising potential source of novel pharmaceutical drugs.

Soursop is one of the medicinal plants reported to have properties beneficial to health. Its scientific name is *Annona muricata* (Sarah, Mustafa & Rehab, 2015). *Annona muricata,* commonly known as graviola or soursop, belongs to the family of *Annonaceae*. It is a typical tropical tree, with heart-shaped edible fruits and widely distributed in most tropical countries (Foong & Hamid, 2012). It is a small tree, native to and widespread in Central America and the Caribbean, but now also widely

cultivated, and in some areas becoming invasive, in tropical locales throughout the world (Le Ven et al., 2011; Moghadamtousi et al., 2015). It has become an important crop because of its tasty flavor, high pulp content, nutritional value, and antioxidant properties (Moghadamtousi et al., 2015). The plant has various native names, depending on the country where it is found. It is called Guanabana in China and, in Nigeria, it is proudly called Shawahopu in the Igbo language (Le Ven et al., 2011; Moghadamtousi et al., 2015).

Gajalakshimi et al. (2012) reports that *A. muricata* is a traditional medicinal plant with phytochemical constituents and bioactive compounds possessing diverse medicinal properties. Intensive chemical investigation of the leaves and seeds of the species have resulted in the isolation of a great number of acetogenins (Moghadamtousi et al., 2015). The isolated compounds display some desired biological and pharmacological effects such as anti-tumoral properties, cytoxicity, and pesticidal properties (Moghadamtousi et al., 2015). These conclusions are supported by the use in traditional medicine of the roots of the species for their anti-parasitical and anti-pesticidal properties (Moghadamtousi et al., 2015; Sarah, Mustafa & Rehab, 2015). *A. muricata* has been traditionally used to treat headaches, hypertension, cough, and asthma and used as an antispasmodic, sedative, and nervine for heart conditions (Sarah, Mustafa & Rehab, 2015).

Soursop leaves contain flavonoids, tannins, alkaloids, saponins, calcium, phosphorus, carbohydrates, vitamins A, B, and C, phytosterol, and calcium oxalate (Edeoga, Okwu & Mbaebie, 2005; Abdul Wahab et al., 2018). The leaves are traditionally used to prevent and treat asthma, bronchitis, biliary disorder, diabetes, heart diseases, hypertension, worm disease, liver disorder, malaria, rheumatism, arthritis, other sources of joint pain, tumors, and cancer (Padma et al., 2001; Wicaksono et al., 2011). The leaves are also used to treat several types of bacterial disease, such as pneumonia, diarrhea, urinary tract infection, and various skin diseases (Gajalakshmi, Vijayalakshmi & Rajeswari, 2012). Additionally, the leaves, roots, and seeds of soursop have been reported to demonstrate significant insecticidal properties (Tattersfield, 1940). It has also been documented to possess both hypoglycemic and antioxidant properties without any adverse effects (Lenk et al., 1992). The leaves act also as molluscicidal and anti-parasitical agents (De S. Luna et al., 2005). Extracts from the roots, leaves, and stem are used to make tea and other solutions for patients (Padma, Chansouria & Khosa, 2009). The leaves can be crushed along with raw fruit from the plant and mixed with olive oil to treat various skin disorders, such as rashes, boils, and sores (Padma et al., 2001; Vijayameena et al., 2013). The plant has also been reported to exhibit anti-inflammatory and analgesic effects (Lans, 2006; Roslida et al., 2010; Sarah, Mustafa & Rehab, 2015).

Considering the widespread traditional use of this plant among local communities in Nigeria, it is pertinent to provide scientific support for its application. There is a dearth of such information in some communities, particularly in Ekiti State, where the plants is voraciously exploited for herbal medicinal purposes. Hence, this study was carried out to add to the existing lean body of knowledge on the phytochemical composition as well as antimicrobial properties of the leaves, stem, and bark of *A. muricata* plant on some human pathogens of public health concern.

Materials and methods

Sources of plants for extraction

The fresh leaves, stems, and bark of soursop (*Annona muricata*) (Figure 1) were acquired from a market in Ado-Ekiti, Ekiti State, Nigeria, and transported to the laboratory. These parts of the plant were identified at the herbarium unit of the Plant Science Department of Ekiti State University, in Ado-Ekiti.

Figure 1. *Annona muricata* plant (Moghadamtousi et al., 2015)

Source of test microorganisms

The bacteria and fungi used in this study were clinical isolates from the Department of Medical Microbiology, Federal Teaching Hospital, Ado-Ekiti. They included *Staphylococcus aureus*, *Escherchia coli*, *Salmonella typhi*, *Candida albcans*, and *Candida tropicalis.* The bacteria were maintained on nutrient agar slant at 4°C, while the fungi were maintained on the Potato Dextrose agar slant until needed for assay.

Preparation of various solvent extracts

The extraction of the leaves, stem, and bark of *Annona muricata* was carried out by the maceration method, using the solvent polarity of, in order, ethyl acetate, n-hexane, and hot water (100°C). The maceration method used was that described by Ginda et al. (Ginda, Niky & Erly, 2014) and Rarassari and Maftuch (Rarassari & Maftuch, 2016). The soursop leaves, stems, and bark were macerated separately with disinfected mortars and pestles. Exactly 100 g each of the coarsely powdered plant parts were placed in stoppered containers containing 250 ml of solvent (ethyl acetate, n-hexane, or water). They were each labeled appropriately and allowed to stand at room temperature for three days with frequent agitation, until the soluble matter was dissolved. The mixture was strained, the "marc" (the damp solid materials) pressed, and the liquids clarified by filtration after standing (Sukhdev et al., 2008; Sasidharan et al., 2011). These extracts were then concentrated using a rotary evaporator with the temperature not exceeding 40°C until the concentrated extracts were obtained (Rarassari & Maftuch, 2016).

Phytochemical screening

Qualitative tests were carried out on the crude solvent extracts for alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, terpenoids, proteins, and anthraquinone (Harborne, 1973). These tests were carried out at the Federal University of Technology, Akure, Ondo State, Nigeria, as described below:

Test for alkaloids

Exactly 5 ml of the extract was diluted with sulphuric acid to make it acidic. Mayer's regent was added to the acidic extract, a white precipitate indicating the presence of alkaloids, as a positive result.

Test for saponins

Exactly 20 ml of the extract was evaporated to dryness and the extract dissolved in 3 ml of chloroform, the filtrate treated with 3 drops of a mixture of concentrated sulphuric acid and acetic anhydride, and a colour of different shade was observed, indicating a positive test for saponins.

Test for steroids and terpenes

Five milliliters (5 ml) of the extract was divided into 2 equal parts and evaporated to dryness and the extract dissolved in 3 ml of chloroform. The filtrate was then treated with 3 drops of a mixture of concentrated sulphuric acidic and acetic anhydride. Colors of different shades were observed indicating a positive test. The second portion of the extract was heated with hot acetic anhydride, allowed to cool and six drops of concentrated sulphuric acid added, and a blue-green color was observed, indicating terpenes.

Test for tannins and phenols

Exactly 3 ml of extract was treated with 5% ferric chloride solution; a green to blue color was observed indicating a positive test for tannins. Similarly, 3 ml of extract was added to 3 ml of lead acetate solution and a white precipitate occurred, indicating tannins and phenols.

Test for proteins

Exactly 1 ml of 4% sodium hydroxide and 1% dilute copper sulphate was added to 5 ml of the extract, and a red solution confirmed proteins. Additionally, a xanthoprotein test was also done by adding 3 ml of extract to 1 ml of concentrated sulphuric acid. The presence of white precipitate which turned to yellow on boiling, and orange on addition of 1 ml ammonium hydroxide, indicated the presence of proteins.

Test for carbohydrates

To 2 ml of the extract, 2–3 drops of alpha naphthalene solution in alcohol were added, the solution shaken for 2 minutes, and 1 ml of concentrated sulphuric acid added slowly from the side of the test tube, until it gave a deep purple color at the junction of two layers, indicating the presence of carbohydrate. Adding Benedict's reagent to the extract, it

yielded a yellow to brown precipitate after boiling in a water bath.

Test for glycosides

Into 2 ml of extract, 1 ml of pyridine and 1 ml of sodium nitro-prusside were added. A red color indicated the presence of cardiac glycosides.

Keller-killiani test

To a test tube containing 2 ml of extract, 1 ml of glacial acetic acid was added with 3 drops of 5% ferric chloride and concentrated sulphuric acid, and the disappearance of the reddish brown color at the junction of the two layers and bluish green in upper layer indicated the presence of cardiac glycosides.

Test for flavonoids

Into 2 g of dry extract, 5 ml of ethanol, 5 drops of hydrochloric acid, and 0.5 g of magnesium were added; a pink color indicated the presence of flavonoids.

The preparation of extract concentration for antibacterial application

About 3 g each of the concentrated aqueous, ethyl acetate, and n-hexane extracts were dissolved separately in dimethyl sulfoxide (DMSO) until 10 ml of volume was obtained of concentrate from the extract of 300 mg/ml. The dilution was made in order to obtain extracts with concentrations of 5 mg/ml, 10 mg/ml, 50 mg/ml, 150 mg/ml, and 300 mg/ml.

Antimicrobial activity assay

The test bacterial innocula (*S. aureus*, *E. coli,* and *S. typhi*) were prepared from an overnight culture of nutrient agar slant. The bacterial cultures were directly suspended in sterile Mueller Hinton broth (oxoid) and the suspension adjusted to the 0.5 Macfarland turbidity standard (10^5 cells/ml) needed for the experiment. The fungi innocula were prepared directly using Sabouraud dextrose broth. The already dried Mueller Hinton plate was inoculated with test bacteria using sterile swabs by rotating the plate 3 times between each smear and leaving to dry for 10 minutes at ambient temperature before wells were made. This was repeated for the fungi using a Sabouraud dextrose plate. Exactly 20 µl of each extract concentration (300 mg/ml, 150 mg/ml, 50 mg/ml, 10 mg/ml, and 5 mg/ml (w/v)) was introduced into the wells on already inoculated culture plates with the test bacterial and fungal isolates. These were incubated at 37°C for 24 hours for the bacteria and 48 hours for the fungi. After incubation, each extract's zone of inhibition was noted for each isolate. All tests were done in triplicate. The reference antimicrobial agent, ampicillin, was used as a positive control, while DMSO was used as a negative control. The diameters of zones of inhibition were measured in mm using a Vernier caliper (Vijayameena et al., 2013).

Results

The phytochemical compositions of extracts of *A. muricata* showed that the leaf, stem, and fruit possess mainly tannins, flavonoids, saponins, reducing sugars, carbohydrates, alkaloids, steroids, proteins, nitrate ions, and starch (Table 1). They also show that only the leaves of *Annona muricata* possess glycosides and there are no phlobatannins and anthraquinone in the leaves, stems, or fruits.

The antimicrobial activities of n-hexane leaf extract of *Annona muricata* on the selected clinical isolates are presented in Table 2. The n-hexane leaf extract showed zone of inhibition diameters of 21 mm against *S. aureus* at 300 mg/ml and 18 mm against *E. coli* at 150 mg/ml. Similarly, the same extract showed 15 mm and 8 mm against *C. tropicalis* at concentrations of 300 mg/ml and 150 mg/ml respectively. Similarly, Tables 3 and 4 show that n-hexane stem extract showed 22 mm and 15 mm against *S. aureus* and *E. coli* respectively.

The ethyl leaf extract at concentrations of 300 mg/ml showed zones of inhibition of 12 mm and 13 mm against *E. coli* and *S. aureus* respectively, while at concentrations of 150 mg/ml, 8 mm was recorded against *E. coli* and *S. aureus* (Table 5). *E. coli* and *S. aureus* showed zones of inhibition of 14 mm and 20 mm respectively at 300 mg/ml (Table 6), and 15 mm and 8 mm respectively at 300 mg/ml (Table 7).

The hot water extracts of *A. muricata* also exhibited antimicrobial effects recorded in Tables 8, 9, and 10. They show diameters of inhibition of 10 mm at 150 mg/ml and 18 mm at 300 mg/ml against *E. coli*. *Candida albicans* and *Candida tropicalis* displayed 5 mm and 8 mm respectively at 300 mg/ml. (Table 8). *E. coli* and *S. aureus* showed zones of inhibition of 10 mm and 22 mm respectively at 300 mg/ml (Table 9), and 2 mm each at 300 mg/ml (Table 10).

Table 1. Phytochemical constituents of leaf, stem, and fruit extracts of soursop

Phytochemical constituents	Leaf	Stem	Fruit
Tannins	+	+	+
Flavonoids	+	+	+
Glycosides	+	–	–
Saponins	+	+	+
Phlobatannins	–	–	–
Reducing sugar	+	+	+
Carbohydrate	+	+	+
Alkaloids	+	+	+
Steriods	+	+	+
Protein	+	+	+
Nitrate ion	+	+	+
Starch	+	+	+
Anthraquinone	–	–	–

+ present; – absent

Table 2. Antimicrobial activities of n-hexane leaf extract of *Annona muricata* (mm)

Clinical Isolates	5 mg/ml	10 mg/ml	50 mg/ml	150 mg/ml	300 mg/ml
Escherichia coli	0	0	0	18	20
Staphylococcus aureus	0	0	2	15	21
Salmonella typhi	0	0	0	2	5
Canadia albicans	0	0	0	8	10
Canadia tropicalis	0	0	5	8	15

Table 3. Antimicrobial activities of n-hexane stem extract of *Annona muricata*

Clinical Isolates	5 mg/ml	10 mg/ml	50 mg/ml	150 mg/ml	300 mg/ml
Escherichia coli	0	0	0	10	15
Staphylococcus aureus	0	0	0	6	22
Salmonella typhi	0	0	0	0	4
Canadida albicans	0	0	0	0	0
Canadida tropicalis	0	0	0	0	0

Table 4. Antimicrobial activities of n-hexane fruit extract of *Annona muricata*

Microorganism	5 mg/ml	10 mg/ml	50 mg/ml	150 mg/ml	300 mg/ml
Escherichia coli	0	0	0	10	15
Staphylococcus aureus	0	0	0	6	22
Salmonella typhi	0	0	0	0	4
Canadida albicans	0	0	0	0	0
Candida tropicalis	0	0	0	0	0

Table 5. Antimicrobial activities of Ethyl acetate leaf extract of *Annona muricata*

Microorganism	5 mg/ml	10 mg/ml	50 mg/ml	150 mg/ml	300 mg/ml
Escherichia coli	0	0	0	8	12
Staphylococcus aureus	0	0	4	8	13
Salmonella typhi	0	0	0	0	5
Candida albicans	0	0	0	4	8
Candida tropicalis	0	0	3	6	10

Table 6. Antimicrobial activities of Ethyl acetate extract stem extract of *Annona muricata*

Clinical Isolate	5 mg/ml	10 mg/ml	50 mg/ml	150 mg/ml	300 mg/ml
Escherichia coli	0	0	5	8	14
Staphylococcus aureus	0	2	8	15	20
Salmonella typhi	0	0	0	0	0
Candida albicans	0	0	0	0	4
Candida tropicalis	0	0	0	0	5

Table 7. Antimicrobial activities of Ethyl acetate extract fruit extract of *Annona muricata*

Clinical Isolate	5 mg/ml	10 mg/ml	50 mg/ml	150 mg/ml	300 mg/ml
Escherichia coli	0	0	0	10	15
Staphylococcus aureus	0	0	0	0	8
Salmonella typhi	0	0	0	0	0
Candida albicans	0	0	0	0	0
Candida tropicalis	0	0	0	0	0

Table 8. Antimicrobial activities of hot water leaf extract of *Annona muricata*

Clinical Isolates	5 mg/ml	10 mg/ml	50 mg/ml	150 mg/ml	300 mg/ml
Escherichia coli	0	0	0	10	18
Staphylococcus aureus	0	0	0	5	20
Salmonella typhi	0	0	0	2	4
Candida albicans	0	0	0	0	5
Candida tropicalis	0	0	0	4	8

Table 9. Antimicrobial activities of hot water stem extract of *Annona muricata*

Clinical Isolates	5 mg/ml	10 mg/ml	50 mg/ml	150 mg/ml	300 mg/ml
Escherichia coli	0	0	0	0	10
Staphylococcus aureus	0	0	0	6	22
Salmonella typhi	0	0	0	0	0
Candida albicans	0	0	0	0	0
Candida tropicalis	0	0	0	0	0

Table 10. Antimicrobial activities of hot water fruit extract of *Annona muricata*

Clinical Isolates	5 mg/ml	10 mg/ml	50 mg/ml	150 mg/ml	300 mg/ml
Escherichia coli	0	0	0	0	2
Staphylococcus aureus	0	0	0	0	2
Salmonella typhi	0	0	0	0	0
Candida albicans	0	0	0	0	0
Candida tropicalis	0	0	0	0	0

Discussion

The phytochemical constituents of leaf, stem, and fruit extracts of *Annona muricata* revealed tannins, flavonoids, saponins, reducing sugars, carbohydrates, alkaloids, steroids, proteins, and starch. Previous studies have shown that the antimicrobial

properties of plants are due to tannins, alkaloids, saponins, flavonoids, sterol, triterpenes, and reducing sugars (Pathak et al., 2010; Ogu & Amiebenomo, 2012; Ogu et al., 2012; Ogu, Ezeadila & Ehiobu, 2013). Thus, the antibacterial activities of the leaf, stem, and bark extracts observed in this study are most likely due to the presence of one or more of these bioactive principles in the extract. The leaves possess more phytochemical components than the stem or fruits. This suggests that that the leaves may be utilized for the strongest beneficial effects on human health. This finding agrees with studies conducted by Edeoga et al. (Edeoga, Okwu & Mbaebie, 2005) and Usunobum and Paulinus (Usunobum & Paulinus, 2015), who report that *Annona muricata* are reservoirs of free radical scavenging molecules, rich in antioxidant activity.

This study tested for and observed antimicrobial activities of n-hexane, ethyl acetate, and aqueous extracts of the leaves, stems, and fruits of soursop on selected clinical isolates. It showed that n-hexane, ethyl acetate, and aqueous extracts of soursop leaves inhibited *E. coli*, *S. aureus*, *S. typhi*, *C. albicans* and *C. tropicalis* at concentrations of 300 mg/ml and 150 mg/ml. There was no sensitivity recorded at lower concentrations against the tested isolates except at the concentration of 50 mg against *S. aureus* and *C. tropicalis.* This corroborates the earlier report of Sarah et al. (Sarah, Mustafa, & Rehab, 2015), of the antibacterial effect of methanolic and aqueous extracts of the leaves of *Annona muricata* against various bacterial strains: *Staphylococcus aureus* ATCC29213, *Escherichia coli* ATCC8739, *Proteus vulgaris* ATCC13315, *Streptococcus pyogenes* ATCC8668, *Bacillus subtilis* ATCC12432, *Salmonella typhi* ATCC23564, and *Klebsiella pneumonia.* This is supported by the previous reports of Lans (2006) who demonstrated that the leaf, bark, root, stem, fruit and seed extracts of *Annona muricata* possess anti-bacterial, antifungal, and anti-malarial properties. Similarly, the n-hexane, ethyl acetate, and aqueous extracts of soursop stems in this study demonstrated antibacterial and antifungal effects against *E. coli*, *S. aureus*, *S. typhi*, *C. albicans*, and *C. tropicalis* at concentrations of 300 mg/ml and 150 mg/ml. Thus, *Annona muricata* extract contains a wide spectrum of activity against a group of bacteria responsible for the most common bacterial diseases. Pathak et al. (2010) also reported that leaf extract of *Annona muricata* is used in the treatment of various bacterial infectious diseases. Thus, the plant possesses an abundance of antibacterial compounds as reported earlier (Moghadamtousi et al., 2015).

Nevertheless, in this study, although the n-hexane extract of the fruit showed antibacterial effects on all the selected bacteria at 300 mg/ml and 150 mg/ml, the *Candida* species were resistant at the same concentrations. This suggests that more of the antibacterial bioactive ingredients were soluble in n-hexane than the antifungal components. The slightly greater antimicrobial activities recorded in this study for leaf extract over stem or bark extracts, suggests that more of the bioactive ingredients are lodged in the leaves, as reported by Ogu et al. (Ogu et al., 2012). This is probably one of the reasons herbal practitioners have almost always recommended using leaf extracts over those of stems or barks in native herbal medicine. This submission is in consonance with the submissions of previous studies (Adeshina, Onujagbe & Onaolapo, 2010; Ogu et al., 2012). The aqueous extract of the stem and leaves showed that hot water leaf extraction resulted in better antibacterial effects than cold extraction, indicating that most of the active agents were expressed by hot rather than cold maceration.

Studies in the past have reported similar finding (Matsushige, Kotake & Takeda, 2012). The findings in this study further support earlier claims that medicinal plants can be used for effective treatments of infectious diseases caused by a variety of microorganisms, and thus should be exploited.

Conclusions

Many common plant-based foods contain powerful antimicrobial phytochemical substances that can improve human health. The antimicrobial properties demonstrated for different parts and fractions of *Annona muricata* might provide a good alternative to antibiotic drugs in the treatment of some infections. The phytochemicals found in this study could also offer significant protections to consumers against many diet related diseases, including cancer, because of the presence of antioxidants. Therefore this study suggests that every part of the *Annona muricata* can be used for numerous health benefits and should be prepared and consumed in an appropriate manner in order to confer the most health benefits possible.

Annona muricata (soursop) is an essential medicinal plant which has been reported to promote the general health of human beings. Its potential as a source of new drugs cannot be over-emphasized. Therefore, proper and adequate use of the plant will be a welcome development. Also, it would probably be beneficial to incorporate some of the active

substances into foods and drinks, and finally, the molecular study of the plant will provide more vital information about its potential as a good drug alternative.

References

1. Abdul Wahab, S.M., Jantan, I., Haque, M.A. & Arshad, L. (2018) Exploring the Leaves of *Annona muricata* L. as a Source of Potential Anti-inflammatory and Anticancer Agents. *Frontiers in Pharmacology* 9, 661, pp. 1–20.
2. Adeshina, G.O., Onujagbe, O.M. & Onaolapo, J.A. (2009) Comparative Antibacterial Studies on the Root, Stem Bark and Leaf Extracts of *Parkia clappertoniana*. *The Internet Journal of Alternative Medicine* 8, 2.
3. De S. Luna, J., Dos Santos, A.F., De Lima, M.R., De Omena, M.C., De Mendonça, F.A., Bieber, L.W. & Sant'Ana, A.E. (2005) A study of the larvicidal and molluscicidal activities of some medicinal plants from northeast Brazil. *Journal of Ethnopharmacology* 97, 2, pp. 199–206.
4. Edeoga, H.O., Okwu, D.E. & Mbaebie, B.O. (2005) Phytochemical Constituents of some Nigerian Medicinal Plants. *African Journal of Biotechnology* 4, pp. 685–688.
5. Foong, C.P. & Hamid, R.A. (2012) Evaluation of anti-inflammatory activities of Ethanolic extracts of *Annona muricata* leaves. *Brazilian Journal Pharmacology* 22, 6, pp. 1301–1307.
6. Gajalakshmi, S., Vijayalakshmi, S. & Rajeswari, D. (2012) Phytochemical and pharmacological Properties of *Annona muricata:* A review. *International Journal of Pharmacy and Pharmaceutical Sciences* 4, 2, pp. 3–6.
7. Ginda, H., Niky, P.U, & Erly, S. (2014) Study of the antibacterial activities of soursop (*Annona muricata*) Leaves. *International Journal Pharmtechnology Research* 6, 2, pp. 575–581.
8. Harbone, J.B. (1973) *Phytochemical Methods*. London: Chapman and Hall Ltd. pp. 49–188.
9. Lans, C.A. (2006) Ethno-medicinal used in Trinidad and Tobago for urinary Problems and diabetes mellintus. *Journal of Ethnobiology* 2, pp. 45–55.
10. Le Ven, J., Schmitz-Afonsbo, I., Toubol, D. & Champy, P. (2011) Annonaceace fruits and parkinsonism risk. Metabolisation study of annonacin, a model neurotoxin; evaluation of human exposure. *Toxicology letters* 205, pp. 50–60.
11. Lenk, S.E., Bhat D., Blankency, W. & Andduun, A. (1992) Effects of Streptozotosin Induced diabetes on Rough endoplasmic reticulum and Lysosomes of the rat Liver. *American Physiology* 263, pp. 856–862.
12. Matsushige, A., Kotake, Y. & Takeda, Y. (2012) Annonamine, a new Aporphine alkaloid from leaves of *Annona muricata. Chemical and Pharmacuetical Bulletin* 60, 2, pp. 257–259.
13. Moghadamtousi, S.Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H.M. & Kadir, H.A. (2015) *Annona muricata* (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities. *International Journal of Molecular Sciences* 16, 7, pp. 15625–15658.
14. Ogu, G.I. & Amiebenomo, R. (2012) Phytochemical analysis and in vivo anti-diarrhoeal potentials of *Dialium guineense* Willd stem bark extract. *Journal Intercultural Ethnopharmacology* 1, 2, pp. 105–110.
15. Ogu, G.I., Ezeadila, J. & Ehiobu, J.M. (2013) Antioxidant and antimicrobial activities of Dialium guineense (Willd) leaf extract. *Pharmacy and Pharmacology Research* 1, 1, pp. 1–7.
16. Ogu, G.I., Tanimowo, W.O., Nwachukwu, P.U. & Igere, B.E. (2012) Antimicrobial and phytochemical evaluation of the leaf, stem bark and root extracts of *Cyathula prostrata* (L) Blume against some human pathogens. *Journal of Intercultural Ethnopharmacology* 1, 1, pp. 35–43.
17. Padma, P., Chansauria, J.P.N., Khosa, R.L. & Ray, A.K. (2001) Effect of *Annona muricata* and *Pollyalthia cerasoides* on brain neurotransmitters and enzyme monoamine oxidase following cold immobilization stress. *Journal of Natural Remedies* 1, 2, pp. 144–46.
18. Padma, P., Chansouria, J. & Khosa, R. (2009) Wound healing Activity of *Annona muricata* extract. *Journal of Pharmacy Research* 2, 3, pp. 404–406.
19. Parekh, J. & Chanda, S. (2007) *In vitro* antibacterial activity of the crude Methanol extract of *Woodfordia fruiticosa* Kurz. Flower (lythraceae). *Brazilian Journal of Microbiology* 38, pp. 204–207.
20. Pathak, P., Saraswathy, D.R., Vora, A. & Savai, J. (2010) In vitro antimicrobial Activity and phytochemical analysis of the leaves of *Annona muricata. International Journal of Pharmacology Research and Development* 2, 5, pp. 23–45.
21. Rarassari, M.A & Maftuch, H.N. (2016) Phytochemicals and Antibacterial Activities of Soursop Leaf (*Annona muricata*) against *Edwardsiella tarda* (*In Vitro*). *Journal of Life Science and Biomedicine* 6, 1, pp. 06–09.
22. Roslida, A.H., Tay, C.E., Zuraini, A. & Chan, P.F. (2010) Anti-inflammatory and anti-nociceptive activities of the ethanolic extract of *Annona muricata* leaf. *Journal of National Remedies* 10, 2, pp. 97–104.
23. Sarah, I.A. Mustafa, T.M. & Rehab, A.A. (2015) Study on the trace element and some properties of the fruit juice of soursop and their effect on liver enzymes. *Journal of Pharmaceutical and Chemical Science* 3, 1, pp. 40–45.
24. Sasidharan, S., Chen, Y., Saravanana, D., Sundram K.M. & Yoga Latha, L. (2011) Extraction and Characterization of bioactive compound from plants extracts. *African Journal Traditional Complementary Alternative Medical* 8 (1), pp. 1–10.
25. Sukhdev, S., Suman, P., Gennaro, L. & Dev, D. (2008) Extraction technologies for medicinal nd aromatic plants. *International Centre for Science and High Technology* 23, 6, pp. 34–56.
26. Tattersfield, F. (1940) The Insecidal Properties of certain species of *Annona* and an India strain of *mundulea Sericea. Annals Applied Biology* 27, pp. 262–73.
27. Usonobun, U. & Paulinus, O.N. (2015) Phytochemical Analysis and Mineral composition of *Annona muricata* leaves. *International Journal of Research and Current Development* 1, 1, pp. 38–42.
28. Vashist, H. & Jindal, A. (2012) Antimicrobial Activities of Medicinal Plants –Review. *International Journal of Research in Pharmaceutical Sciences* 3, pp. 222–230.
29. Vijayameena, C., Subhashini, G., Loganayagi, M. & Ramesh, B. (2013) Phytochemical screening and assessment of antibacterial activity for the bioactive compounds in *Annona muricata. Internal Journal Current Microbiology Applied Science* 2, 1, pp. 1–8.
30. Wicaksono, A., Kalahkan, K., Dengan, S. & Media, M. (2011) Pharmacological Properties of *Annona muricata. Journal of Pharmaceutical* 19, 23, pp. 102–233.