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# *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

Yahaya Gavamukulya[1,2✉], Fred Wamunyokoli[1,3], Hany A. El-Shemy[1,4]

[1]*Department of Molecular Biology and Biotechnology, Pan African University Institute for Basic Sciences, Technology and Innovation (PAUSTI), P.O. Box, 62000-00200 Nairobi, Kenya*

[2]*Department of Biochemistry and Molecular Biology, Busitema University Faculty of Health Sciences, P.O. Box, 1460 Mbale, Uganda*

[3]*Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, P.O. Box, 62000-00200 Nairobi, Kenya*

[4]*Department of Biochemistry, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt*

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ABSTRACT

*Annona muricata* (*A. muricata*) is a tropical plant species belonging to family Annonaceae and known for its many medicinal uses. This review focuses on the research history of its traditional uses, phytochemicals, pharmacological activities, toxicological aspects of the extracts and isolated compounds, as well as the *in vitro* propagation studies with the objective of stimulating further studies on this plant for human consumption and treatment. *A. muricata* extracts have been identified in tropical regions to traditionally treat diverse conditions ranging from fever to diabetes and cancer. More than 200 chemical compounds have been identified and isolated from this plant, the most important being alkaloids, phenols and acetogenins. Using *in vitro* studies, its extracts and phytochemicals have been characterized as antioxidant, anti-microbial, anti-inflammatory, insecticidal, larvicidal, and cytotoxic to cancer cells. *In vivo* studies have revealed anxiolytic, anti-stress, anti-inflammatory, immunomodulatory, antimalarial, antidepressant, gastro protective, wound healing, hepato-protective, hypoglycemic, anticancer and anti-tumoral activities. *In silico* studies have also been reported. In addition, clinical studies support the hypoglycemic as well as some anticancer activities. Mechanisms of action of some pharmacological activities have been elucidated. However, some phytochemical compounds isolated from *A. muricata* have shown a neurotoxic effect *in vitro* and *in vivo*, and therefore, these crude extracts and isolated compounds need to be further investigated to define the magnitude of the effects, optimal dosage, and mechanisms of action, long-term safety, and potential side effects. Additionally, more clinical studies are necessary to support the therapeutic potential of this plant. Some studies were also found to have successfully regenerated the plant *in vitro*, but with limited success. The reported toxicity notwithstanding, *A. muricata* extracts seem to be some of the safest and promising therapeutic agents of the 21st century and beyond that need to be studied further for better medicinal formulations and diseases management.

## 1. Introduction

*Annona muricata* L. (*A. muricata*) is a species of the Annonaceae family that has been widely studied in the last decades due to its therapeutic potential. The medicinal uses of the Annonaceae family were reported long time ago and since then, this species has attracted the attention due to its bioactivity and traditional uses [1–7]. Medicinal plants are considered as the basis for health preservation and care worldwide. Chronic degenerative diseases have reached epidemic proportions and are considered as a serious health problem; therefore, the treatments of these diseases are of clinical importance [8].

Ethnobotanical studies have indicated that *A. muricata* has been used as insecticide [2] and parasiticide [3]. Fruit juice and infusions of leaves or branches have been used to treat fever [4,9], sedative

✉First and corresponding author: Yahaya Gavamukulya, Department of Molecular Biology and Biotechnology, Pan African University Institute for Basic Sciences, Technology and Innovation (PAUSTI), P.O. Box, 62000-00200 Nairobi, Kenya.
Tel: +256 775 869 783.
E-mail: gavayahya@yahoo.com
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[5,10], respiratory illness [11–13], malaria [14,15], gastrointestinal problems [6,9,16], liver, heart and kidney affections [7,17]. In recent years it has become widely used for hypoglycemic [18], hypotensive [16,18,19] and cancer treatments [20].

A number of publications and reviews about *A. muricata* have been conducted to integrate the available scientific studies on this plant with special interest on acetogenins as principal bioactive compounds [1,7,21,22]. Other bioactive compounds have been identified; more bioactivities have been evaluated; and medicinal uses have been extended, as well as a few reported toxicities. The aim of this systematic review was to integrate the scientific studies reported until February 2017 that describe past research history on the traditional medicinal uses, phytochemical contents of *A. muricata*, and relate them with the pharmacological activities, studied mechanisms of action, toxicological evaluation, as well as methods of propagation for sustainable use and give an expert opinion on its future prospects. The bioactivity tested can be the base for therapeutic utilization, but the toxicological research results are important to consider the therapeutic uses of this plant versus its toxicity, and the potential harmful effects of products prepared from this plant. Over 150 papers have been reviewed for this manuscript.

## 2. Taxonomy, ecology and physiology

### 2.1. Taxonomy

*A. muricata* is known as Soursop (English), Graviola (Portuguese), Guanábana (Latin American Spanish), Omusitafeli/Ekitafeli (Uganda), and other local indigenous names as has been enlisted [21]. This plant is a species of the genus *Annona* with the following taxonomic classification. Kingdom: Plantae, Division: Angiosperms (Magnoliophyta), Class: Magnolids, Order: Magnoliales, Family: Annonaceae, Genus: *Annona*, Species: *A. muricata* L. [1]. The genus *Annona* comprises over 70 species among which *A. muricata* is the most widely grown.

### 2.2. Ecology and physiology

The *A. muricata* tree is about 5–10 m tall and 15–83 cm in diameter with low branches [23–25]. It tends to bloom and fruit most of the year, but there are more defined seasons depending on the altitude [1]. It is widely distributed in the tropical regions of Central and South America, Western Africa, Central and Eastern Africa and Southeast Asia [1,26] at altitudes below 1 200 m above sea level, with temperatures between 25 °C and 28 °C, relative humidity between 60% and 80%, and annual rainfall above 1 500 mm. The fruit is an edible collective ovoid berry, dark green in color. Its average weight is 4 kg in some countries [1], but in Mexico [24], Venezuela [27] and Nicaragua [23], it ranges between 0.4 kg and 1.0 kg. Each fruit may contain 55–170 black seeds [28] when fresh and they turn light brown when dry. The flesh is white and creamy with a characteristic aroma and flavor [1].

## 3. Traditional medicinal uses

A number of medicinal uses have been reported across the globe ranging from the use of leaves, bark, roots, fruits to seeds of *A. muricata* [7]. The most widely used preparation in traditional medicine is the decoction of bark, root, seed or leaf but applications are varied. In a number of tropical sub-Saharan countries such as Uganda, all parts are used to treat malaria, stomachache, parasitic infections, diabetes [29], and cancer [30]. The use of leaves to treat malaria is very important in tropical countries such as Cameroon, Togo, and Vietnam [14,15,31,32]. In Ghana, *A. muricata* and some other plants are decocted into a mixture and used in bath for pregnant mothers prior to birth [33]. In Indonesia, the Caribbean islands [34] and South Pacific countries, the leaves are used in bath to treat skin ailments; while in Mauritius [35], New Guinea [36] and Ecuador [37], the application of leaves is done locally on the pain site. The ingestion of leaves decoction is used as analgesic in Brazil [32], Martinique [38], Mexico and Nicaragua [32] while in several countries such as Benin [12], the Caribbean [10], and Cuba [11], it is used to treat discomfort associated with colds, flu and asthma. Natives of Malaysia used *A. muricata* leaves to treat cutaneous (external) and internal parasites [7].

In addition to being used as a food, the fruit juice is used to treat diarrhea, heart and liver diseases [7,19], and against intestinal parasites in South America [7]. Lately, the medicinal uses of *A. muricata* leaves included treatments for hypertension [7,19,39,40], diabetes [7,18,39] and cancer [6,20,30,41]. Furthermore, some patients use decoctions or capsules of *A. muricata* for cancer and pharmacological treatments.

Unripe fruit, seeds, leaves and roots are also used as bio-pesticides, bioinsecticides and topical insect repellents. The importance of this species in pest control was indicated in the edition of 'Pesticide action and alternatives for Latin America', which recommended the use of aqueous extract of *A. muricata* to control lepidopteran larvae, aphids and thrips, among others [2,42]. These are just some of the reported traditional uses, but there are very many as has been enumerated by previous reviews [1,7,21,22], including very many that have not yet been documented.

## 4. Phytochemicals

As of February 2017, two hundred and twelve bioactive compounds had been reported to be found in *A. muricata*. These 212 bioactive compounds, their structures and corresponding biological activities have been enlisted in the review [21]. The predominant compounds are acetogenins followed by alkaloids, phenols and other compounds. Leaves and seeds were the main plant studied organs, probably because they are the most traditionally used. A brief description of some of the major phytochemicals is, however, given below.

### 4.1. Acetogenins

More than 120 acetogenins have been identified in ethanolic, methanolic or other organic extracts of different organs and tissues of *A. muricata* such as leaves, stems, bark, seeds [43–46], pulp [47], and fruit peel [21,48]. Acetogenins are characterized by a long aliphatic chain of 35–38 carbons bonded to a γ-lactone α ring, terminally substituted by β-unsaturated methyl (ketolactone), with one or two tetrahydrofurans (THF) located along the hydrocarbon chain and a determined number of oxygen groups (hydroxyl, acetoxyls, ketones, epoxy). Most of the acetogenins found in *A. muricata* contain a THF ring,

although acetogenins have also been reported with two adjacent or nonadjacent THF rings. Acetogenins are linear and may have one or two epoxy groups. Some studies suggested that its bioactivity depends on its structure [49]. Annonacin was the most abundant acetogenin reported in both leaves [45] and fruit [50,51] of *A. muricata*, but has also been reported in seeds [52], peel [48] and roots [53]. The contents of acetogenins in leave extracts range from 3.38 to 15.05 mg/g measured by A $^{1}$H NMR, while HPLC-MALDI quantified 0.299 mg/g [54]. Acetogenins are considered the main bioactive compounds of the Annonaceae family [43]. Some studies have shown that acetogenins are more cytotoxic than alkaloids and rotenone, a synthetic cytotoxic compound. Acetogenins and alkaloids are widely studied in a controversial form, due to their therapeutic potential versus neurotoxic activity, as will be discussed later on in the review.

### 4.2. Alkaloids

Alkaloids are naturally occurring compounds containing basic nitrogen atoms. The most abundant in *A. muricata* are reticuline and coreximine [55], and leaves contain the higher alkaloid concentration [56–59], although they have also been found in roots, stems [55] and fruit [60,61]. The alkaloids reported in *A. muricata* are mainly of the isoquinoline, aporphine and protoberberine type [62]. Previous studies have shown that alkaloids isolated from *Annona* species possess an affinity for the 5-HT1A receptors *in vitro* and participate in dopamine biosynthesis [60,61]. Thus, it has been proposed that alkaloids derived from the *Annona* could induce antidepressant-like effects [60,61], and cytotoxic activity [57]. Neurotoxic effects have also been reported for some alkaloids, and suggested that neuronal death occurred by apoptosis [63].

### 4.3. Phenolic compounds

Thirty seven phenolic compounds have been reported to be present in *A. muricata*. The important phenolic compounds found in *A. muricata* leaves include quercetin [64] and gallic acid [65]. The presence of flavonoids and lipophilic antioxidant compounds such as tocopherols and tocotrienols has been reported to be present in the pulp [65]. In different studies, when organic or aqueous extracts have been used, the quantity of extractable total phenols is considerably different. This is important to mention because the most common medicinal use is aqueous infusion and the majority of phenols are soluble in water. Phenolic compounds are considered as the major phytochemicals responsible for the antioxidant activity [30,66].

### 4.4. Other compounds

Other compounds such as vitamins, carotenoids, amides, and cyclopeptides have also been identified in *A. muricata*. Vitamins and carotenoids have been found in leaves, seeds and fruit pulp [65,67]. The presence of the amide N-p-coumaroyl tyramine [68] and cyclopeptides [69,70] have been reported in the seeds and showed to have anti-inflammatory and anti-tumor effects. On the other hand, 37 volatile compounds have been identified in the fruit pulp of *A. muricata*, and most of these compounds are aromatic and aliphatic esters [71]. In addition, 80 essential oils, mainly sesquiterpenes derivates [12,72], have been identified in the leaf [73]. The study of volatiles of *A. muricata* is promising because of their bioactivity.

## 5. Pharmacological activities

From the 101 papers of pharmacological studies that we have reviewed for this manuscript, around 60% corresponded to *in vitro* studies, 36% to *in vivo* studies in murine models, 2% to *in silico* modeling, and 2% to clinical studies. Regarding the type of extracts used, about 85% corresponded to maceration of any part of the plant in organic solvents and 15% corresponded to aqueous preparations.

### 5.1. In vitro studies

The *in vitro* studies reviewed include cytotoxic activity, antioxidant activity, antiprotozoal activity, insecticidal, larvicidal and repellent activity, anti-inflammatory activities, immunomodulatory activity, antibacterial activities, antiviral activity, and antipediculicidal activities among others.

#### 5.1.1. Cytotoxic activity

The most studied cancer cell lines using *A. muricata* extracts *in vitro* include: ECV304, human leukemia carcinoma cells; FG/COLO357 and CD18/HPAF, pancreatic cancer cells; U937, histiocytic lymphoma cell line; HeLa, uterine cervical cancer cell line; MDA-MB-435S, breast carcinoma cells; HaCat, immortalized human keratinocytes; WRL-68, normal human liver cells; MBDK, bovine cell line; MCF-7, human breast carcinoma; K562, human bladder carcinoma cells; H-460, human large lung cell carcinoma; S-F-268, glioma; CCD841, normal human colon epithelial cells; HT-29 and HCT-116, colon cancer cell; VERO, kidney epithelial cells; C-678, stomach cancer cells; EACC: Ehrlich ascites carcinoma cells; SKBR3: breast adenocarcinoma cell line; T47D, breast cancer cells; HL-60, human promyelocytic leukemia; Capan-1, pancreatic cancer cells; BPH-I, human benign prostate cells; and Raji cell lines.

The increasingly popular use of *A. muricata* as an anticancer treatment reported ethnobotanically may be related to reports of its selective cytotoxic activity [74]. This bioactivity is considered selective as some of the extracts studied *in vitro* were shown to be more toxic to cancer cell lines than to normal cells – actually, with most of them having no cytotoxic effects on the normal human cells [4,30,74–76]. It was reported that 1.6 μg/mL and 50 μg/mL from hydroalcoholic extract of *A. muricata* leaves increased the viability of non-cancerous cells while 100 μg/mL did not alter their viability [64]. This selective activity has been reported to induce healing, with minimum effects.

While studying bioactivities of other compounds, the type of extract is decisive in the results obtained. Organic solvents, pentanoic and ethanolic, were the most active *A. muricata* extracts against cancer cells grown *in vitro*. For these extracts, activity has been reported to be 10 and 4.5 times higher, respectively, than the activity of the aqueous extract in the A375 cell culture [77]. According to Osorio *et al.*, 2007 [78], extracts with $LC_{50}$ < 10 μg/mL can be classified as highly cytotoxic while the National Cancer Institute [31] suggested that plant extracts with $LC_{50}$ values ⩽20 μg/mL are suitable for cancer drugs from plants. Ethyl acetate *A. muricata* leaf extract showed inhibition of the U-937 cell line with

7.8 μg/mL [78]. Although *A. muricata* extracts exhibit good cytotoxicity, there are plants with more cytotoxic effect, like *Thevetia ahouai* with $LC_{50}$ < 1 μg/mL. Both plant species are used in Latin American countries to treat cancer [79]. The hexane extract of leaves had the highest content of flavonoids and the most effective inhibition of cell proliferation than the methanol or chloroform extracts [80]. In another study, it was demonstrated that the chloroform fraction from *A. muricata* leaves exert potent cytotoxic effect on Raji and Hela cells [81].

It has been proposed that the mechanism of action of the extract implies the disruption of mitochondrial membrane to arrest cells in $G_0/G_1$ phase, and the induction of apoptosis suppressing the migration and invasion of cancer cells [31,82]. Furthermore, according to Pieme *et al.*, 2014 [31] *A. muricata* extracts induce apoptosis by reactive oxygen species (ROS), and downregulates Bcl-2 proteins. Bax protein Bcl-2 are anti-apoptotic proteins that suppress the function of apoptosis, while Bax are proteins that mediate the leakage of pro-apoptotic factors, including cytochrome *c*, $Ca^{2+}$ and the mitochondrial protein Smac/DIABLO into the cytosol through dimerization and translocation to the outer mitochondrial membrane; a property that was also observed for acetogenins [83]. The detailed mechanisms of action are, however, yet to be completely elucidated.

The acetogenins with anti-tumor and anticancer activity have also been studied using *in vitro* assays, and cytotoxic effects against more than 15 cancer cell lines as outlined earlier has been documented [41,45,84–88]. Isolated acetogenins have demonstrated selective cytotoxic effects [82]. Acetogenins bioactivity has been related to their molecular structure [49,89]. It is agreed that the two adjacent THF rings acetogenins are the most [89–91], especially bullatacin and squamocin, which have been reported mainly in the seeds [49,89]. The mechanism of the acetogenins cytotoxic action is the inhibition of the mitochondrial complex I [92], and the inhibition of ubiquinone-linked NADH oxidase in the plasma membranes of cancerous cells causing apoptosis [43]. It was demonstrated that *A. muricata* extracts suppressed phosphorylation of the key molecules involved in the extracellular signal-regulated kinase (ERK) and the phosphatidylinositol 3′ kinase (PI3 K/Akt) pathway which play a crucial role in the proliferation and survival of pancreatic cancer cells [88]. In addition to the above, plant extract inhibited the expression of glucose transporter and glycolytic enzymes, all of which lead to the reduction of glucose uptake and ATP production by PC cells [88].

Biochemical apoptosis implied a transverse redistribution of phosphatidylserine (PS) on the outer plasma membrane arises during early apoptosis [82]. Other events in apoptosis are the complex cascade of caspases. It was reported that Annomuricin E caused depletion of mitochondrial membrane potential (MMP) leading to opening of mitochondrial permeability transition pores and further release of pro-apoptotic proteins, such as cytochrome *c* from the mitochondria to the cytosol, resulting in the formation of the apoptosome and the activation of caspase 9 and caspase 3/7, which have been linked to the mitochondrial death pathway. Isolated Annomuricin E was found to down regulate Bcl-2 proteins and up regulate Bax protein. This finding confirms that annonacin E-induced apoptosis was through the mitochondrial-mediated pathway [82]. Finally, it was suggested that selective cytotoxicity of *A. muricata* is due to the enhanced ATP demand of cancer cells with respect to normal cells [93].

### 5.1.2. Antioxidant activity

Several antioxidant screenings have been conducted on *A. muricata*. Natural antioxidants from plant species have gained interest due to their protective effect against oxygen-derived from free radicals involved in the development of many diseases such as cancer, cardiovascular affections, arthritis, as well as degenerative illness such as Parkinson and Alzheimer [94]. A compilation of studies on the antioxidant activity of *A. muricata* considering different assays, the different plant parts, and the different solvents used has been done [65]. Some of the methods used for determining the total antioxidant capacity included the free radical scavenging capacities using DPPH and the $ABTS_+$ assays, determination of oxygen radicals by the ORAC assay, reduction power by the FRAP assay and β-carotene bleaching [30,95].

The antioxidant activity has been evaluated in fresh and frozen pulp, juice, and fresh or dried leaves. The pulp antioxidant activity measured by ABTS, FRAP and ORAC suggested that the antioxidant compounds from *A. muricata* are mainly lipophilic, and the mechanism of action is by hydrogen donation [65].

The composition of the extract varies depending on the solvent used. For example, methanolic, ethanolic, *n*-butanolic and aqueous leaf extracts showed different antioxidant activity measured by DPPH. For example, the aqueous extract of fresh leaves of *A. muricata* was 1 000 times less active than the commercial antioxidant butylated hydroxytoluene [96]. A positive correlation between antioxidant activity and the total polyphenol content was reported [30,74].

### 5.1.3. Anti-protozoal activity

*A. muricata* extracts and some of their isolated compounds have shown effective activity against protozoans responsible for some human diseases, as is the case of the genera *Plasmodium* [14], *Leishmania* [78], *Biomphalaria* [97], *Trypanosoma*, and *Entamoeba* [32], responsible for malaria, leishmaniasis, schistosomiasis, chagas, and amebiasis diseases, respectively. The anti-plasmodic effect has particular interest due to the necessity for antimalarial drugs in tropical areas. Methanol extract of this species has shown inhibition of this parasite *in vitro* but with less activity compared to the commercial drugs chloroquine and artemisinin [14], though more studies on this aspect are yet to be conducted. The highest effectiveness was found in seed extracts [14]. It has further been reported that alkaloids [56], acetogenin, anonaine, and gallic acid [98] isolated from *A. muricata* had anti-plasmodial activity. It has been demonstrated that phenolic compounds inhibit the activity of β-ketoacyl-ACP-reductase (FabG), β-hydroxyacyl-ACP-dehydratase (FabZ) and enoyl acyl-ACP reductase (FaBI), important enzymes for fatty acid biosynthesis in *Plasmodium falciparum* that compromises its growth [99]. In the case of FabG, phenols like luteolin act as noncompetitive inhibitor of FabG with respect to acetoacetyl-CoA as well as NADPH, while in FabZ, luteolin acts as competitive inhibitor of the substrate crotonyl-CoA [99].

Ethyl acetate and methanolic extracts of *A. muricata* peel showed higher anti-leishmanial activity than the commercial compound Glucantime® used to treat diseases caused by different strains of protozoa [48]. It has further been reported by Tempone *et al.* (2005) [100] that isoquinoline alkaloids are strongly implicated in the inhibition of an essential antioxidant enzyme of *Leishmania* and *Trypanosoma*, trypanothione

reductase, and enzyme that protects the parasites from ROS generated by the host defense cells.

Extracts of *A. muricata* also have anti-parasitic activity against the metazoan or helminth *Haemonchus contortus*, a gastrointestinal parasite of sheep [101]. The extracts of *A. muricata* were active against eggs, infective larvae and adult forms of the parasite, and the effect was comparable to that obtained with using the anthelmintic drug, levamisole [101]. The trypanocidal activity of *A. muricata* was found in extracts from different plant parts and in different solvents. However, its effectiveness was 100 times lower than the commercial trypanocide benznidazole [76,78].

### 5.1.4. Insecticidal, larvicidal and repellent activity

*A. muricata* has shown insecticidal activity from all its parts including the seeds, leaves, barks, stems, roots and flowers [2,102,103]. Ethanolic extracts inhibited insect larvae of *Aedes aegypti* (*Ae. Aegypti*) [102,104,105], *Anopheles albimanus* [104], and insects that affect plants such as *Spodoptera litura* [2], *Callosobruchus maculatus* and *Plutella xylostella* [103]. *A. muricata* seed extracts have shown the most active insecticidal activity [102,104,105], this probably due to its content of chemical compounds such as alkaloids, fatty acids and acetogenins, though the insecticidal action of *A. muricata* alkaloids has not been fully studied. Fatty acids for example are toxic to insects in different manners: by inhalation of volatile compounds, by contact with film at the surface of water, and by penetration due to the amphibolic property of some compounds [106]. New technologies, such as nano science, are exploring the development of environmentally friendly, effective, inexpensive and easy to apply mosquito control products. For this effect, green silver nanoparticles synthesized using aqueous crude extract of *A. muricata* showed larvae toxicity of *Ae. aegypti* [107].

Acetogenins have *in vitro* activity on larvae of *Myzus persicae*, *Leptinotarsa decemlineata*, *Blattella germanica*, *Ae. aegypti*, *Rhodnius prolixus*, and *Rhodnius pallescens* [90,108]. Studies that evaluated the insecticidal activity of 44 acetogenins isolated from different species of *Annona* showed that there was a relationship between the acetogenin structure and their toxicity to mosquito larvae. Similarly, compounds with adjacent bis-tetrahydrofuran rings and three hydroxyls were more active than compounds with a mono-tetrahydrofuran ring.

The majority of the active acetogenins evaluated in a study by Isman and Akhtar (2007) [42] were equitoxic to the commercial compound rotenone ($LC_{50}$ = 1.2 ppm). It has been suggested that the insecticidal mechanisms of acetogenins are due to THF ring having strong interaction with the interface of lipid bilayers, and alkyl spacer between the γ-lactone and hydroxylated THF ring moieties elicited potent inhibitory activities on the NADH oxidase, resulting in the inhibition of mitochondrial complex I [42,108] and thus damaging the respiration chain and the integrity and function of the cell. Using the insecticidal activity of isolated acetogenins as a base, commercial products were developed but failed mainly because their mechanism of action involves inhibition of mitochondrial electron transport with a specific action at complex I, thus becoming detrimental to other organisms. In the case of other plants, using crude extracts can be more promising than the development of products using individually isolated compounds as active ingredient [42]–this may majorly be related to their synergistic nature.

### 5.1.5. Anti-inflammatory activities

A study aimed to evaluate anti-inflammatory potential of *A. muricata* extract (AME) on LPS-stimulated murine macrophage cell line (RAW264.7) was reported [109]. Cell viability assay to evaluate nontoxic concentration in cell line was performed with MTS assay. The parameters used to determine anti-inflammatory activity between treatment group and non-treated cells, were IL-1β, TNF-α, and IL-6 which was measured with ELISA, and NO level which was measured with nitrate/nitrite colorimetric assay. The AME of 50 and 10 μg/mL showed high viability (>90%) and it was not significantly different compared to control making it suitable for treatment. The AME of 50 μg/mL resulted low TNF-α level in RAW264.7 (264.69 pg/mL), as well as IL-1β level (905.00 pg/mL) and IL-6 (219.13 pg/mL). Also, it was reported that AME of 75 μg/mL showed lower NO level (9.79 μm) compared to untreated cells [109]. The research revealed that AME possess the anti-inflammatory potential indicated by inhibition of inflammatory mediators including TNF-α, IL-1β, IL-6 and NO.

### 5.1.6. Immunomodulatory activity

The immune-enhancing activity of Graviola leaf extracts (GE) in RAW 264.7 macrophage cells have been examined [110]. In one study, both steam and ethanol GE induced the transcriptional expression of cytokines, including TNF-α and interleukin-1α, but only the steam extract up regulated inducible nitric oxide synthase (iNOS). In consistence with mRNA expression, the production of TNF-α and nitrite was elevated by both steam and ethanol extracts of *A. muricata* leaves. It was reported that this was mainly due to activation of mitogen-activated protein (MAP) kinase signaling pathways. These results suggest that *A. muricata* leaves enhance immunity by activation of the MAP kinase pathways [110]. These bioactive properties indicate that GE has immune stimulatory potential and can be applied to boost the innate immune system in immuno-compromised patients. It should further be noted that in addition to its high contents of bioactive components and ROS-scavenging capacity, the immune-boosting ability of *A. muricata* can be applied for the development of health-promoting functional foods.

### 5.1.7. Antibacterial activities

Comparable with the standard antibiotic streptomycin, *A. muricata* extracts showed antibacterial activity against gram-positive and gram-negative bacteria. Its bioactivity efficacy depends on the kind of solvent used in the extraction. For instance, ethanolic and methanolic extracts of *A. muricata* showed antibacterial activity against *Staphylococus aureus*, while the peel aqueous extract did not show such activity. In addition to the direct antimicrobial activity, a modulatory activity has also been reported. The combination of ethanolic extract and antibiotic treatment increased the potentiation of the antibiotic against multidrug-resistant strains of *Escherichia coli* and *Staphylococcus aureus* [59,111–113].

Antimicrobial bioactivity of *A. muricata* extracts is attributed to flavonoids, steroids and alkaloids present in the plant extracts [114]. The mechanism of action is probably due to a synergism of these compounds. It has been reported that some alkaloids have

the ability to bind with DNA of microorganisms and inhibit RNA synthesis [115], and have shown antimicrobial activity by glycosidase inhibition [62]. It has also been reported that flavonoids act by inhibiting both cytoplasmic membrane function and DNA synthesis, such as quercetin that binds to GyrB subunit of *Escherichia coli* DNA gyrase and inhibits the enzyme ATPase activity. Phenylphenol was reported to bind to membrane protein or hydrogen with vital proteins such as microbial enzymes and inhibit and change their functions [114].

#### 5.1.8. Antiviral activity

With respect to antiviral bioactivity, it is known that plant extracts interfere with HIV-I replication at an early step of the virus. In the first step, plant extracts interfere with virus entry into the host cell by reduction of input viral RNA and by interfering with the function of the envelope proteins that diminish the infectivity of viral particles. This indicates that plant extracts have virucidal activity and act before the interaction with the host cell. Also, plant extracts inhibit attachment of virus to the host cell. It is demonstrated that antiviral activity of plant extracts is mediated by polyphenol compounds [116]. In one of the studies, ethanolic extracts from stem and bark of *A. muricata* showed antiviral activity *in vitro* against the herpes simplex virus [117].

#### 5.1.9. Antipediculicidal activity

A study was carried out to investigate the *in vitro* antipediculicidal activity of *A. muricata* seeds against lice infestation in backyard poultry [118]. The dried powdered seeds of *A. muricata* were extracted, using petroleum ether, ethyl acetate and methanol by a Soxhlet extractor. *In vitro* antipediculicidal activity of petroleum ether, ethyl acetate and methanolic seed extracts of various concentrations (12.5 mg/mL, 25.0 mg/mL, 50.0 mg/mL and 100.0 mg/mL) were then used and analyzed for average percentage mortality. Results showed that ethyl acetate had highest average mortality percentage rather than petroleum ether and methanolic extracts. Highest concentration of drugs in all extracts performed better than lowest concentration. It was concluded that the seed extracts of *A. muricata* exhibited strong antipediculicidal activity and, thus, could be a good source of acaricidal activity [118].

### 5.2. In vivo studies

The pharmacological activities of *A. muricata* extracts evaluated included anticancer and anti-tumorigenic, hypoglycemic, hypotensive, anti-inflammatory and anti-nociceptive, immunomodulatory, antimalarial, hepato-protective, gastro protective, anxiolytic and anti-stress, antidepressant, wound healing and antipediculicidal activities.

#### 5.2.1. Anticancer and anti-tumorigenic activity

Ethyl acetate extract of *A. muricata* leaves showed chemopreventive properties on azoxymethane-induced colonic aberrant crypt foci in rats [82]. As acetogenins, the extract down regulates PCNA and Bcl-2 proteins, upregulates Bax protein and restores the levels of the antioxidant enzymes. An excessive ROS generation results in the production of lipid radicals such as malondialdehyde (MDA), and an elevated concentration of MDA was observed in patients suffering from colorectal cancer [82]. *A. muricata* extract treatment reduced MDA formation in colon tissue, confirming its protective effect against oxidative stress.

Anti-tumoral activity has been reported for extracts and some isolated acetogenins of *A. muricata*. It was reported by reported Hamizah *et al.* (2012) [119] that the ethanolic extract of *A. muricata* leaves showed greater anti-tumor activity in murine models than curcumin, a known natural chemopreventive. This extract has shown protective effect in biochemical events and in morphological changes in induced colorectal carcinogenesis. Breast tumor in rats was reduced by treatment for 5 weeks with *A. muricata* fruit extract [75]. Aqueous extract of commercial powder capsules containing leaf and stem of *A. muricata* also showed anti-tumorigenic and anti-metastatic activities on pancreatic tumors in murine models [88]. In another study, the therapeutic effects of the B1 AMCE treatment in mice bearing the 4 T1-induced tumors were assessed and found that the mean tumor volume of the group treated with B1 AMCE was smaller than the untreated group [120].

The mechanism of action suggests the inhibition of multiple signaling pathways that regulated metabolism, metastasis [120], induction of necrosis and cell cycle arrest [75,88], has been shown in cytotoxic mechanism. Anti-tumor activity was also reported for two acetogenin isolates of *A. muricata* [121,122]. It was reported by Ko *et al.* (2011) [121] that bullatacin at doses of 400 mg/kg was able to reduce a tumor induced in rodents 300 times better than the commercial drug Taxol (paclitaxel). Meanwhile, annonacin at doses of 10 mg/kg reduced tumor size induced in murine models comparable to the commercial drugs cisplatin and adriamycin [122]. Another study by Yang *et al.* (2015) [123] demonstrated that crude leaf extract showed more *in vivo* inhibition of prostate cancer proliferation and more effect on tumor growth-inhibition than flavonoid-enriched extract. This report suggests that the effectivity of crude extract is probably due to a synergistic interaction between flavonoids and acetogenins.

#### 5.2.2. Hypoglycemic activity

*A. muricata* leaf extracts showed hypoglycemic activity in murine models [124]. In these studies, the effect of aqueous and methanolic extracts of *A. muricata* leaves on reducing the concentration of blood glucose in rats with diabetes induced with streptozotocin (STZ) was evaluated, and the histology and biochemistry of the pancreas were observed. Pancreatic β-cells in rats that were administered with extracts of *A. muricata* did not show the alterations that are normally found in diabetic rats. An increase in the antioxidant enzymatic activity and insulin content in pancreatic serum was reported. Near normal blood glucose levels, body weight, food and water intake, lipid profile and oxidative defense were achieved after a month of daily treatment with *A. muricata* extract, which could prevent the deleterious effect of STZ by its antioxidant and protective effect of pancreatic β-cells [125]. It has also been reported that there is a positive correlation between tannins, flavonoids and triterpenoids content and the inhibition of α-glucosidase. Flavonoids inhibit α-glucosidase through hydroxylation bonding and substitution at β ring [126]. This inhibition decreases carbohydrate hydrolysis and glucose absorption, and inhibits carbohydrates metabolism into glucose [126].

Additionally, glycemic index (GI) and glycemic load (GL) have been reported for *A. muricata* fruit. GI indicates the effect

of the content and type of carbohydrates of a food on blood glucose content, while GL estimates how much the food will raise blood glucose level after eating it. GI and GL are considered low for *A. muricata*, which agrees with its hypoglycemic potential [127].

### 5.2.3. Hypotensive activity

Leaf extract of *A. muricata* caused a dose-dependent reduction in MAP in normotensive rats [128]. The suggested hypotensive mechanism of action of aqueous extract of *A. muricata* did not involve the endothelial or nitric oxide-dependent pathways. Studies by Nwokocha *et al.* (2012) [128] suggested that plant extracts lower blood pressure through the blockage of calcium ion channel, and this $Ca^{+}$ antagonism is further demonstrated by its ability to relax high $K^{+}$ induced contractions. The hypotensive effect has been attributed to alkaloids such as coreximine, anomurine, and reticulin, and some essential oil components such as β-caryophyllene [128].

### 5.2.4. Anti-inflammatory and anti-nociceptive activities

Anti-inflammatory activity similar to the activity presented by indomethacin, which is a nonsteroidal anti-inflammatory, has been reported [129,130]. The antinociceptive effect of ethanolic and hydroalcoholic extracts of *A. muricata* has been reported using various chemical and thermal nociceptive models. *A. muricata* produced antinociception action of activity in both neurogenic and inflammatory phases [131]. Metabolites of arachidonic acid (called icosanoids) are involved in inflammation process [129]. These metabolites are produced via cyclooxygenase and lipoxygenase when a cell is activated by mechanical trauma, cytokines, growth factors or other stimuli. It has been proposed that the mechanism of antinociception may be by inhibition of cyclooxygenase (COX) and lipoxygenases (LOX) and other inflammatory mediators by flavonoids present in the plant extract [129].

### 5.2.5. Immunomodulatory activity

One of the studies showed that in mice treated with *A. muricata* crude extracts (AMCE) there was increased levels of white blood cell, T-cell, and natural killer cell population [120]. It was further observed that AMCE sample regulated several immune systems markers *in vivo* and increased the level of white blood cells. In order to gain the knowledge on the effect of B1 AMCE in modulating several important immune markers, immunophenotyping of the splenocyte cell population was carried out. The results showed that whereas the splenocyte population of CD4/CD3-T cell was decreased in the normal and control/untreated groups there was a significant increase observed in the AMCE-treated group. A similar trend was also observed in CD8/CD3-Tcell population. In addition, the population of natural killer (NK) 1.1/$CD3^{+}$ cell was increased in the AMCE-treated group compared to the control/untreated group. Finally, the total white blood cell count observed was in the AMCE-treated mice group was higher than in the control group [120]. This shows how effective the AMCE are in immunomoduration *in vivo*.

### 5.2.6. Antimalarial activity

A study to evaluate the antimalarial activity of the *A. muricata* aqueous leaf extract in *Plasmodium berghei* infected mice was reported [132]. Aqueous leaf extract of *A. muricata* was prepared and tested for acute toxicity in mice and for efficacy test *in vivo*, standard 4-day suppressive test was carried out. ICR mice were inoculated with $10^{7}$ parasitized erythrocytes of *Plasmodium berghei* ANKA by intraperitoneal injection. The extracts at varying dosages were then given orally by gavage once a day for 4 consecutive days, and then parasitemia, percentage of inhibition, and packed cell volume were subsequently calculated. In the study, Chloroquine (10 mg/kg) was given to infected mice as positive control while untreated control was given only distilled water. It was found that *A. muricata* aqueous leaf extract resulted in dose dependent parasitemia inhibition, prolonged survival time and no mortality to mice was observed with this extract up to a dose of 4000 mg/kg [132]. These results showed the antimalarial potential of *A. muricata* extracts.

### 5.2.7. Hepato protective activities

Studies by Arthur *et al.* 2012 [133] on the hepatoprotective activity of the leaf aqueous extract of *A. muricata* reported that the extract was effective against hyperbilirubinemia or jaundice with similar effect to silymarin (*Silybum marianum*). The extract reduced the harmful effect and preserved the hepatic physiological mechanism of the liver damaged by a hepatotoxin such as paracetamol (Acetaminophen), a drug widely used as antipyretic and analgesic, which can cause liver damage if taken in excessive [133]. This study suggests that *A. muricata* extract reduces bilirubin levels due the glucosides present in the extract, which might be converted into glucuronic acid, conjugating with bilirubin for excretion, or because the extract active regulators increase the activity of enzymes, synthesis of transporter, and steps related to bilirubin clearance pathway [133]. Another study was carried out to evaluate the hepato protective effect of *A. muricata* leaves on extracellular matrix (ECM) accumulation, lysosomal membrane integrity and liver damage in dimethylnitrosamine (DMN) induced fibrotic rats [134]. It was observed that simultaneous treatment with *A. muricata* leaf extract significantly reversed alterations in the indices of liver damage, decreased synthetic ability, lysosomal membrane fragility and altered ECM function [134]. The results suggested that *A. muricata* leaf extracts function as potent fibrosuppressant by suppressing ECM accumulation, enhancing lysosomal membrane stability and liver synthetic ability. A study to investigate the anticholestasis and antisinusoidal congestion properties of aqueous extract of *A. muricata* stem bark following acetaminophen induced toxicity as also conducted. The biochemical analysis and liver microscopy of the study suggested that aqueous stem bark extract of *A. muricata* possess significant hepatoprotective, anticholestasis and antisinusoidal congestion properties [135]. Another study evaluating the effects of aqueous stem bark extract of *A. muricata* on the liver of albino rats was conducted [136]. Blood samples were analyzed using the Reitman and Frankel, cyanmethemoglobin methods. Comparing the control and test groups, results showed significantly increased Hb, PCV, ALP, organ weight and relative weight. The study suggested that *A. muricata* was non-toxic at 25 mg/kg and may possess hematopoietic and hepatoprotective effects [136].

### 5.2.8. Gastro protective activities

Ethyl acetate and ethanol extracts from leaf of *A. muricata* showed protective gastric effect like omeprazole in ethanol-induced ulcerogenesis in rats [131,137]. Antiulcer potential of

*A. muricata* is probably through its antioxidant compounds that increase the mucosal nonprotein sulfhydryl group content [131]. The excessive production of gastric acid in patients with ulcers can reduce the level of gastric wall mucus (GWM). *A. muricata* extract caused attenuation in gastric acidity and retrieved the loss in GWM like proton pump inhibitors drugs as omeprazole but in less proportion. Furthermore, the antioxidant effect of *A. muricata* extract can play an important role in the gastro protection. The ROS produce oxidative damage to the gastric mucosa. *A. muricata* extract restores the activity of enzymes such as glutathione (GHS), catalase (CAT), nitric oxide (NO), superoxide dismutase (SOD), MDA and prostaglandin E2 (PGE-2) that reduces cellular ROS. Histopathological analysis showed that the extract protects the gastric tissue from hemorrhagic lesion associated with attenuation of leukocyte infiltration and submucosal edema [137].

### 5.2.9. Anxiolytic and anti-stress activities

Some of the studies have shown that the anxiolytic and the anti-stress effects were more effective in the alkaloid fraction than in the crude hydroalcoholic extracts [138]. It is possible to attribute this bioactivity to the alkaloid compounds; especially because two of the isolated alkaloids (anonaine and asimilobine) have relaxing activity. These compounds can influence the central nervous system via the $5HT_{1A}$ receptor. The $5HT_{1A}$ receptor binds with the endogenous neurotransmitter serotonin and is involved in the modulation of emotion [60,61]. This bioactivity can validate the reason for the traditional use of *A. muricata* as a sedative.

### 5.2.10. Antidepressant activity

A study to determine the antidepressant and behavioral properties of the Nigerian grown *A. muricata* in Sprague–Dawley rats using the open field test and forced swim test was carried out [139]. In the study, rats were administered *A. muricata* leaf extract (50, 150 and 300 mg/kg) alone, as well as in combination with imipramine or sertraline (10 mg/kg) for 14 d. From the results, the extract was found to reduce the explorative tendencies of the rats in the open field test, whereas in the forced swim it caused a significant reduction in immobility time and increased swimming time. Furthermore, it was observed that the combination of the extracts with imipramine or sertraline further decreased the explorative tendencies at 150 mg/kg concentration and the immobility time at 150 and 300 mg/kg [139]. It was concluded that the results obtained proposed a sedative and antidepressant-like effect of ethanol extract of *A. muricata*, confirming the ethnomedicinal use of the ethanol leaf extract of *A. muricata* for the management of depression.

### 5.2.11. Wound healing activity

Bark and leaf extracts of *A. muricata* showed elevation in wound contraction compared with wound without treatment [140,141]. Another study using the *A. muricata* crude extracts showed an accelerated wound healing *in vivo* in treated mice groups [120]. Wound healing consists of four complex phases: coagulation, inflammation, proliferation and maturation. *A. muricata* was shown to accelerate some of these phases. In the inflammatory phase the protein expression of heat shock proteins (Hsp70) is important for healing due to their role in cell proliferation. *A. muricata* induced upregulation of Hsp70 in wound tissues. In this phase the inflammatory cells produce cytokines and free radicals that in great quantity can produce lipid peroxidation in wound. Tissues treated with *A. muricata* extracts showed elevated activity of CAT, GPx and SOD that protect tissue against oxidative damage to accelerate the wound healing process. Additionally, *A. muricata* extracts reduce MDA, the biomarker of lipid peroxidation that can cause defect in endothelial cells, fibroblast and collagen metabolism necessary for wound healing. During the maturation phase, the collagen accumulation and fibroblast proliferation occurred. *A. muricata* extracts elevated the deposition of collagen fibers in the wound as observed in histological analysis [82].

### 5.2.12. Antipediculicidal activity

There is report of a study that was carried out to investigate the *in vivo* antipediculicidal activity of *A. muricata* seeds against lice infestation in backyard poultry [118]. The dried powdered seeds of *A. muricata* were extracted, using petroleum ether, ethyl acetate and methanol by a Soxhlet extractor and preliminary phytochemical screening was performed, using standard protocols. *In vivo* antipediculicidal activity was assessed in backyard poultry and premises and had good drug response at higher concentration and the flock recovered dramatically with complete healing. From the results, the ethyl acetate at 100.0 mg/mL had complete recovery of lice infestation in poultry and its premises, indicating that the seed extracts of *A. muricata* is a good source of acaricidal activity [118].

## 5.3. In silico studies

*In silico* studies have been reported [142,143]. In one of the studies, molecular docking and simulations were performed to investigate the inhibitory potential of phytochemicals present in *A. muricata* against antiapoptotic proteins of the B-cell lymphoma 2 (Bcl-2) family including Bcl-2, B-cell lymphoma extra-large (Bcl-Xl), and Mcl-1. Docking results revealed that the acetogenins, such as annomuricin A, annohexocin, muricatocin A, annomuricin-D-one, and muricatetrocin A/B, exhibited strong binding interactions with Bcl-Xl when compared to Bcl-2 and Mcl-1. Binding score and interactions of these acetogenins were notably better than those of currently available synthetic and natural inhibitors [141]. Molecular dynamics simulations of the top-scoring lead molecules established that these molecules could bind strongly and consistently in the active site of Bcl-Xl. These results suggest that acetogenins could be explored as selective natural inhibitors of Bcl-Xl that could assist in promoting the intrinsic pathway of apoptosis [142].

Another study was to evaluate the interaction of *A. muricata* compounds with target proteins (Bcl-2 and survivin) was reported [143]. They used the ArgusLab 4.0.1 docking software using the compounds: annonamuricin A, annonacin, coclaurine, coreximine, corosolone, and synephrine. Among the six compounds identified coclaurine, coreximine and synephrine were found to effectively dock Bcl-2 and survivin. However, coclaurine showed the highest binding affinity −9.95 kcal/mol and −8.23 kcal/mol for Bcl-2 and survivin respectively [143]. It was concluded that the results needed to be validated both by *in vitro* and *in vivo* study to prove proper elucidation of drugs for better treatment.

### 5.4. Clinical studies

Ethanolic extracts of *A. muricata* leaves have been clinically evaluated in relation to their hypoglycemic activity. A randomized, parallel grouped, double blind phase II clinical trial, in patients with type 2 diabetes mellitus has been conducted [144]. Groups of patients were given 1, 2 or 3 capsules of ethanol extract from *A. muricata* leaves (180 mg) plus 5 mg of glibenclamide for 30 d, and another group only received glibenclamide. The results of this study showed a decrease in the blood glucose or glycemia level in patients receiving extract of *A. muricata* compared to patients who did not receive it. Side effects were reported in 11% of patients (five patients) receiving *A. muricata* extract. Two of them mentioned burning pain in epigastrium, one was associated with nausea, and the remaining three reported nausea [144]. Compounds responsible for the hypoglycemic activity found in the *A. muricata* leaf extracts could be flavonoids and alkaloids, which are present in the leaves and the fruit.

Additional to the clinical study described above, two cases of anticancer evaluations have been reported [145,146]. In one of them, tumor markers showed that a breast cancer patient has been stable and had no side effects after therapy for 5 years [145]. Therapy consisted in taking 227 g of leaves decoction of *A. muricata* (10–12 dry leaves in water for 5–7 min) daily and capecitabine (2500 mg PO) 2 weeks on one week off [145]. The other case of study involves the disappearance of the malignancy with substantial regression of colon tumor cells in a patient who combined lifestyle modifications with the intake of some herbal extracts and nutraceuticals. The therapy included the daily ingestion of 5 g of powered leaf and seed of *A. muricata* extract [146]. These form some of the most promising clinical trials for the development and use of *A. muricata* extracts in the management of cancer, diabetes and many other condition that have not yet been studied in clinical trials.

## 6. Toxicological studies

Some information, both formal and informal, is available on the relation of the consumption of *A. muricata* with the appearance of an atypical Parkinson's disease [147,148]. The toxicity reported for the extracts is variable depending on the plant part used, and the solvent employed. A number of studies have been conducted to evaluate the acute toxicity and neurotoxicity associated with the extracts. However, according to studies conducted, the fear was erased in 2010 when it was agreed that the occurrence of the atypical Parkinson's disease is not directly related to consumption of extracts from members of the Annonaceae family, to which *A. muricata* belongs.

### 6.1. Acute toxicity

Aqueous extracts showed a $LD_{50} > 5$ g/kg, while methanolic and ethanolic extracts of leaves, flowers and pulp had a $LD_{50}$ of >2 g/kg [130], which are considered non-toxic according to the guidelines of OECD [149]. The median lethal dose of aqueous extract of leaves is above the expected consumption for a human, which is about 211 mg/kg per day, considering that an average person consumes one cup of tea three times per day [150]. Therefore, for a human to reach the lethal dose of consumption of *A. muricata* leaf infusion would require consuming more than 71 cups of tea a day. For toxicity in organs, it was reported by Arthur *et al.* 2011 [150] that doses greater than 5 g/kg of aqueous extract might cause kidney damage, unlike the 1 g/kg dose that showed hypoglycemic and hyperlipidemia properties. The most toxic extracts that have been reported are methanol extracts of pericarp, fruit pulp or seed [14]. *A. muricata* pulp consumed for 28 d showed no effect in blood hematology and serum biochemistry [151]. A study that evaluated the toxicity of crude leaf extract and its flavonoid and acetogenins enriched extracts shows that acetogenins-enriched extract was more toxic than others [123]. This study suggested that whole extract could pose similar bioactive properties of its fractions or isolated constituents, but without their toxicity.

### 6.2. Neurotoxicity

The association of the consumption of fruit and homemade preparations of *A. muricata* with the appearance of atypical parkinsonism in the Caribbean island of Guadeloupe is based on a case study published in 1999 [147]. This association has also been reported in New Caledonia and Caribbean patients living in London [152]. From these studies, assessment of the neurotoxic effect of the main bioactive compounds of *A. muricata* alkaloids and acetogenins was initiated. It was evident that some of the isolated compounds induce neurotoxicity and neurodegenerative diseases in murine models.

The reticuline and coreximine alkaloids and solamin, annonacinone, isoannonacinone and annonacin acetogenins were shown to be toxic to dopaminergic cells by impairing energy production [50,53,63,148,153]. Annonacin toxicity was greater than the toxicity of the pesticide rotenone, which was used as a positive control. It was reported that in murine models annonacin enters the brain parenchyma, decreases ATP levels and induces neurodegeneration in the basal ganglia [51,148]. According to these authors, this neurodegeneration induced no change in the behavior or locomotor activity in rodents.

Regarding the neurotoxicity, seven acetogenins have been evaluated using mesencephalic dopaminergic neurons, rat striatal neurons cells and laboratory rats. It was also reported that annonacin and reticuline, which are the most abundant acetogenin and alkaloid in *A. muricata*, respectively, are neurotoxic [51]. Annonacin is about 1000 times more toxic for neuronal cell cultures than reticuline, and 100 times more potent than 1-methyl-4-phenylpyridinium (MPP), a known neurotoxin that causes parkinsonism in humans and animal models. This study was conducted by administering isolated annonacin to laboratory rats intravenously. The amount administered to rats was determined by estimating the amount of annonacin a human would consume by ingesting fruit or canned nectar daily for one year. Neurotoxicity studies of annonacin suggest that there is a need for a long exposure to this molecule to observe the effect in murine models, while pharmacokinetic studies estimated low bioavailability of this compound. In this regard, AVIS (l'Agence Francaise de Sécurité des Aliments) in 2010 issued a statement which concluded that on the basis of available experimental data, it is not possible to say that cases of atypical parkinsonian syndromes observed in Guadeloupe are linked to consumption of species belonging to Annonaceae family.

## 7. *In vitro* regeneration

In order to bring about proper conservation of the *A. muricata* tree, *in vitro* regeneration is critical. Earliest studies to propagate *A. muricata* were reported in 1992, however, the explants had low rooting ability or poor performance during acclimatization and therefore, limited success [154]. In 1996, a complete micropropagation system using juvenile or mature explants of *A. muricata* was developed where adventitious bud and shoot proliferation were achieved from hypocotyls of seedlings [155]. This protocol resulted into rooted plantlets being acclimatized successfully [155]. In 1998, adventitious shoot regeneration from internodal explants of mature plants of *A. muricata* was obtained on Nitsch media [156]. In their protocol, meristems were induced with sorbitol as the sole carbon source supplemented with benzylaminopurine and naphthaleneacetic acid. From that study, a hypothesis was proposed for the involvement of sorbitol in the induction and development of de novo shoots from internodal explants of mature trees of *A. muricata* [156]. Research on this process however stagnated for a long time, until 2016 when another study was successfully reported.

In this study, *in vitro* plantlet regeneration through direct shoot regeneration and rooting protocol was reported [157]. They achieved *in vitro* callus production in leaf base explants on MS + BAP 1.5 mg/L + 2,4-D 2.0 mg/L, where callus cell morphogenesis indicated the establishment of proembryo, perfect dichotomy and shoot apices. They also achieved axillary shoot formation from nodal explants on MS + BAP 1.0 mg/L + Kin 0.5 mg/L + IBA 1.5 mg/L and rooting in MS + IBA 2.0 mg/L + NAA 0.5 mg/L + BAP 1.0 mg/L. Finally, they acclimatized and thrived the plantlets in greenhouse and then in natural environmental conditions [157]. From these studies, it is shown that the *in vitro* protocols for direct plantlet regeneration from internodal explants of *A. muricata* will be useful in gaining more information for micropropagation technique of this tree for conservation strategy.

## 8. Conclusion and future prospects

*A. muricata* is widely used in traditional medicine to treat a myriad of conditions including hypertension, diabetes and cancer. Decoctions of all parts are widely used in preparations. *In vitro* and *in vivo* studies support the majority of the traditional uses but lack clinical validation, as most of the trials have not been clinically validated. More than 200 phytochemicals have been identified in this plant, mainly acetogenins, alkaloids and phenols. These phytochemicals have shown pharmacological activities such as antimicrobial, antioxidant, insecticide, larvicidal, selective cytotoxicity to tumoral cells, anxiolytic, anti-stress, anti ulceric, wound healing, anti-jaundice, hepato protective, hypoglycemic, immunomodulatory, and antimalarial among others. Many new phytochemicals are also yet to be identified in *A. muricata*.

Mechanisms of action of the plant extracts and phytochemicals have been proposed or cytotoxicity including disruption of mitochondrial membrane to arrest cells in $G_0/G_1$ phase, the induction of apoptosis, the inhibition of multiple signaling pathways that regulate metabolism, induction of metastasis and necrosis of cancer cells. Mechanism of action of antioxidant activity is by hydrogen donation, while antimicrobial action is because of some phytochemicals having the ability to bind with DNA and inhibiting RNA synthesis and by glycosidase inhibition lacking cytoplasmatic membrane function. Mechanisms of action of antinociception may be by inhibition of cyclooxygenase and lipoxygenase enzymes and other inflammatory mediators. Hypotensive mechanism is thought to be through the blockage of calcium ion channel. Mechanisms of action of other bioactivities have not been completely elucidated, such as anxiolytic, anti-stress and hypoglycemic activities.

Some phytochemicals, such as acetogenins, have shown neurotoxicity *in vitro* and *in vivo* studies. More research is needed to quantify the amount of neurotoxic compounds and to determine the level of human exposure that might cause the toxicity. However, the current intake does not lead to acute toxicity and in 2010 it was agreed that the reported neurotoxicity is not entirely related to consumption of extracts of the Annonaceae family. Metabolic studies are also necessary to determine whether digestive processes decrease or increase bioactivity and/or neurotoxicity of the active compounds. These studies have been extended to the whole extract used in medicinal treatments. Some *in silico* studies have been conducted to model the interaction of the compounds with the target cell. In order to ensure sustainability, especially where the bark and roots might be used, protocols are being developed to support *in vitro* regeneration of the plant. A lot however still remains to be studied about this plant.

Finally, to answer the original question paused in the title, after reviewing all these studies, we can with much certainty affirm that it is true, the natural therapy to most disease conditions including cancer is growing in our back yard, especially in the Tropics. It only requires big steps to be taken for more detailed studies and advocating for pharmaceutical development or local formulations that may not necessarily be a replacement of the current treatment regimens, but where the extracts of *A. muricata* have shown better and promising activity like in cancer and other non-communicable diseases, be used to help save the human race. Using the natural compounds in their proved form, rather than taking years trying to develop a patentable synthetic analog as the human race suffers, may be the best service we might ever give to this world. *A. muricata*, once studied to perfection, is surely here to bail us out.

## Conflict of interest statement

We declare that no conflict of interest exists.

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