Graviola
(*Annona muricata* L.)

Family
Annonaceae, the custard apple tree family comprising approximately 130 genera and 2300 species. The genus *Annona* comprises over 70 species among which *Annona muricata* is the most widely grown.[1,2]

Parts Used
Leaf

Description
Graviola is a fruit tree also known as soursop (English) and guanabana (Latin American Spanish). The fruit is usually called soursop due to its slightly acidic taste when ripe. Graviola (Portuguese) is an evergreen plant that is native to North and South America and the Caribbean. It is now widely distributed throughout tropical and subtropical parts of the world, including Western Africa, Southeast Asia and the Pacific Islands, at altitudes below 1200m and above sea level, with temperatures between 25 and 28°C, relative humidity between 60 and 80% and annual rainfall above 1500mm. Graviola is a terrestrial, erect tree reaching five to 10 metres in height and features an open, roundish canopy with large, glossy, dark green leaves and low branches. The edible fruits of the tree are large, heart-shaped and dark green in colour, and the diameter varies between 15 and 20cm. It tends to bloom and fruit most of the year but there are more defined seasons depending on the altitude. Its average weight is four kilograms in some countries but in México, Venezuela and Nicaragua it ranges between 0.4 and 1kg. Each fruit may contain 55 to 170 black seeds when fresh and they turn light brown when dry. The flesh is white and creamy with a characteristic aroma and flavour.[3,4,5,6]

Traditional and Empirical Use
All parts of the graviola tree including the bark, leaves, root, fruit and seeds are used extensively as traditional medicines against an array of human ailments and diseases, especially cancer and parasitic infections. Research on graviola began in the 1940s. In the 1970s screening of thousands of plants by the National Cancer Institute in the United States began. Initial screening demonstrated cytotoxicity against cancer cells by several *Annona* species. Traditionally the dark leaves are considered sedative and antispasmodic. They are used for headaches, insomnia, cystitis, liver problems, diabetes, hypertension and as an anti-inflammatory and antidysenteric (to prevent dysentery). A decoction of the leaves has parasiticide, antirheumatic and antineuralgic effects when used internally. It is used to kill bed bugs and head lice. For the latter it can have the same effect taken orally or added to bath water. The cooked leaves, applied topically, fight rheumatism and abscesses. The leaves are placed inside a pillow or placed on top of the mattress to induce a good night's sleep. In Indonesia, the Caribbean islands and South Pacific countries, the leaves are used in a bath to treat skin ailments, while in Mauritius, New Guinea and Ecuador the application of leaves is local on the pain site. The ingestion of a leaf decoction is used as an analgesic in Brazil, Martinique, Mexico and Nicaragua, while in several countries including Cuba and Mexico it is used to treat discomfort associated with colds, flu and asthma. Natives of Malaysia used the leaves to treat cutaneous (external) and internal parasites. The use of the leaves to treat malaria is very important in tropical countries such as Cameroon, Togo, and Vietnam. The fruits of graviola are used to prepare syrups, candies, beverages, ice creams and shakes. Generally the fruit and fruit juice are taken internally to eliminate worms and parasites, cool fever, malaria, arthritic pain, neuralgia, increase mother's milk after child birth and as an astringent for diarrhoea and dysentery. The unripe fruit of the plant is astringent and is used in the treatment of intestinal atony (lost muscle strength) and for scurvy. The crushed seeds are believed to have anthelmintic activities against external and internal worms and parasites. In tropical Africa the plant is generally used as an antiparasitic, antispasmodic, astringent, anticancer, antitumour, sedative, hypotensive, insecticide, piscicide (poisonous to fish), vermifuge, and for coughs, fevers, pain and skin diseases. The stem-bark and roots of the plant are commonly used as remedies for diarrhoea, dysentery and intestinal worms. In India, the fruit pods and flower are employed as remedies against catarrh, while the root-bark and leaves are used as anti-inflammatory and anthelmintic agents. In the Peruvian Andes a leaf tea is used for catarrh and the crushed seed is used to kill parasites. In the Peruvian Amazon the bark,

root and leaves are used for diabetes and as a sedative and antispasmodic. Indigenous tribes in Guyana use a leaf and /or bark tea as a sedative and heart tonic. In the Brazilian Amazon a leaf tea is used for liver problems and the oil of the leaves and unripe fruit is mixed with olive oil and used externally for neuralgia, rheumatism and arthritic pain. In Jamaica, Haiti and the West Indies the fruit and/or fruit juice is used for fever, parasites and diarrhoea.[7,8,9,10,11,12]

Constituents

Phytochemical studies reveal that annonaceous acetogenins are the major constituents of graviola. The leaves are also rich in secondary class metabolite compounds such as alkaloids, saponins, terpenoids, flavonoids, coumarins and lactones, anthraquinones, tannins, cardiac glycosides, phenols and phytosterols. More than 100 annonaceous acetogenins have been isolated from the leaves, barks, seeds, roots and fruits of graviola. For example, identified in the leaves were annomuricins A and B, gigantetrocin A, annonacin-10-one, muricatetrocins A and B, annonacin, goniothalamicin, muricatocins A and B, annonacin A, (2,4-trans)-isoannonacin, (2,4-cis)-isoannonacin, annomuricin C, muricatocin C, gigantetronenin, annomutacin, (2,4-trans)-10R-annonacin-A-one, (2,4-cis)-10R-annonacin-A-one, annopentocins A, B and C, cis- and trans-annomuricin-D-ones, annomuricine, muricapentocin, muricoreacin and murihexocin C and annocatacin A and B. These acetogenins have cytotoxic properties against tumour cell lines and molluscicidal (fatal to molluscs) activity. They are derivatives of long chain (C35 or C37) fatty acids derived from the polyketide pathway that is selectively toxic to cancer cells, including multidrug-resistant cancer cell lines. Annonaceous acetogenins induce cytotoxicity by inhibiting the mitochondrial complex I, which is involved in adenosine triphosphate (ATP) synthesis. As cancer cells have a higher demand for ATP than the normal cells, mitochondrial complex I inhibitors have potential in cancer therapeutics. The alkaloids (reticulin, coreximine, coclarine and anomurine) and essential oils (β-caryophyllene, δ-cadinene, epi-α-cadinol and α-cadinol), mainly sesquiterpenes derivatives stand out. They have shown cytotoxic activity against MCF-7 (human breast carcinoma) cell line. Phenolic compounds include quercetin and gallic acid. Phenolic compounds are considered as the major phytochemicals responsible for the antioxidant activity.[13,14,15,16,17]

Actions

Anticancer, anti-inflammatory, antioxidant, antispasmodic, anticonvulsant, antidiabetic, antibacterial, antiarthritic, insecticidal, antiparasitic, antimalarial, hepatoprotective, hypotensive, sedative.

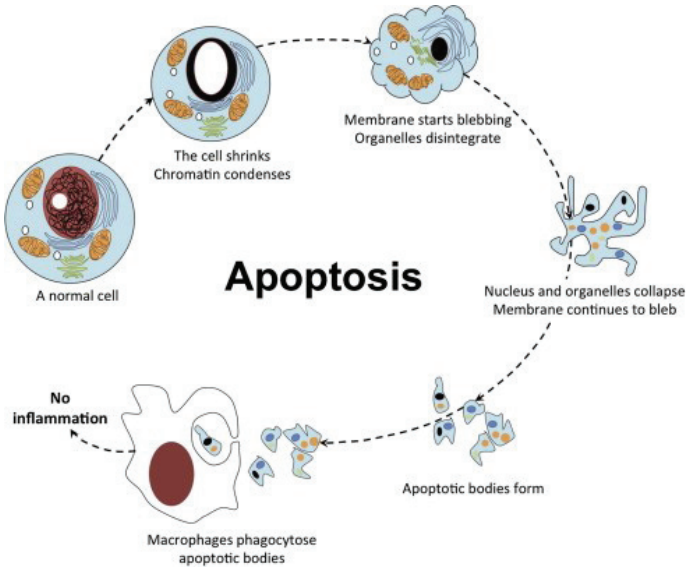
Pharmacological Activity

In vitro and *in vivo* studies support the majority of the traditional uses but lack clinical validation. Mechanisms of action for graviola have been proposed by researchers. Cytotoxicity implies the disruption of the mitochondrial membrane to arrest cells in G0/G1 phase, and the induction of apoptosis, the inhibition of multiple signalling pathways that regulate metabolism, induction of metastasis and necrosis of cancer cells. The mechanism of action of antioxidant activity is by hydrogen donation, while the antimicrobial action is because some phytochemicals have the ability to bind with DNA and inhibit RNA synthesis, and by glycosidase inhibition lacking cytoplasmatic membrane function. The mechanisms of action of antinociception (reduced sensitivity to pain) activity may be by inhibition of cyclooxygenase and lipoxygenase enzymes and other inflammatory mediators. The hypotensive mechanism is thought to be through the blockage of the calcium ion channel. The mechanisms of action of other bioactivities have not been completely elucidated, such as anxiolytic, anti-stress and hypoglycaemic activities.[18]

Anticancer, anti-inflammatory and antioxidant activity

The increasingly popular use of graviola as an anticancer treatment reported ethnobotanically may be related to reports of its selective cytotoxic activity. 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. Plenty of studies report the significant antiproliferative effects of different extracts of graviola and isolated annonaceous acetogenins towards various cancer cell lines however few of these studies have illustrated the underlying mechanism of action. Due to this tremendous antiproliferative effect graviola has been described as “the cancer killer”.[19,20]

Graviola (*Annona muricata*) full monograph



Cytology (the study of cells) of apoptosis. The different stages of apoptotic cell death start by cellular shrinkage and chromatin (a complex of DNA and proteins that forms chromosomes within the nucleus of eukaryotic cells) condensation, concomitant with formation of membrane blebs. Organelles and nucleus fragment and the blebs begin formation of apoptotic bodies which are eventually engulfed by macrophages or neighbouring cells by endocytosis/phagocytosis. The lack of release of cellular components to the extracellular fluid results in the absence of inflammation. Photo credit: https://www.researchgate.net/figure/274013152_fig3_Cytology-of-apoptosis-The-different-stages-of-apoptotic-cell-death-start-by-cellular

Graviola was the most popular plant in a 2016 survey identifying the most common herbal remedies and functional foods used among prostate, breast and colorectal cancer patients in Trinidad. A descriptive, cross-sectional survey was conducted from June to August 2012 at two speciality treatment centres on the island. Among the 150 patients who reported use of herbal remedies/functional foods, graviola was the most popular with 80.7% using the leaves, bark, fruit and seeds on a regular basis. The leaves, bark and seeds were used to make infusions and decoctions, and the ripe fruit was eaten raw, juiced or blended. Two patients consumed either boiled or steeped graviola seeds. In a recent nationwide survey in Trinidad the researchers also found that the leaves of soursop were used traditionally as a "cooling/cleanser" and for the treatment of hypertension. Other common herbal remedies/functional foods included wheatgrass (*Triticum aestivum* L.), saffron (*Crocus sativus* L.) and *Aloe vera* (L.) Burm. f. The most commonly used functional foods were beetroot (*Beta vulgaris* L.), carrots (*Daucus carata* L.) and papaya (*Carica papaya* L.) used by 43.3% of patients; and these were mostly blended as a mixture. Herbal remedies and functional foods were used on a daily basis and patients believed that this modality was equally or more efficacious than conventional treatment.[21]

Two cases of anticancer evaluations have been reported. In one of them tumour markers showed that a breast cancer patient has been stable, and had no side effects, after therapy for five years. Therapy consisted of taking 227g of graviola leaf decoction (10 to 12 dry leaves in water for 5 to 7min) daily and an orally-administered chemotherapeutic agent (2500mg), two weeks on one week off. The other study involved the disappearance of the malignancy with substantial regression of colon tumour cells in a patient who combined lifestyle modifications with the intake of some herbal extracts and nutraceuticals. The therapy included the daily ingestion of 5g of powered leaf and seed of graviola.[22,23]

Graviola represents a source of anticancer drugs a 2016 study found. Graviola and *Passiflora edulis* are good cytotoxic plants that could be used to develop phytomedicine to fight mostly haematological cancers including multi-drug resistance (MDR) phenotypes. Cancer cells rapidly acquire resistance leading to treatment failures. In this study researchers evaluated the cytotoxicity of 17 methanol extracts from 11 Cameroonian medicinal plants against sensitive leukaemia cells and the best ones were further tested on a panel of eight other human cancer cell lines, including various MDR phenotypes as well as against the normal hepatocytes. In an initial screening using leukaemia cells, ten extracts from five plants namely graviola, *Alchornea floribunda, Euphorbia prostata, Pachypodanthium staudtii* and *Passiflora edulis* displayed IC50 values below 20µg/mL. They were further tested in eight other cell lines as well as in normal hepatocytes. According to the U.S. National Cancer Institute plant screening program, plant extracts with IC50 values below 20µg/mL following incubation between 48 and 72 hours are recognized as potential cytotoxic substances.[24]

Results of a 2016 study indicate that the ethanol extract of leaves of graviola causes apoptosis of liver cancer cells through the endoplasmic reticulum stress pathway, which supports the ethnomedicinal use of this herb as an alternative or complementary therapy for cancer. Graviola extracts have been shown to cause apoptosis of various cancer cells *in vitro*, and inhibit tumour growth *in vivo* in animal models. However, the molecular mechanisms underlying its anti-cancer and apoptotic effects of the herb remain to be explored. This study investigated the molecular mechanisms underlying liver cancer cell apoptosis triggered by the ethanol extract of graviola. Experiments showed that the extract was able to reduce viability and trigger apoptosis of the cancer cells.[25]

The findings of a 2015 *in vivo* study substantiate the usage of graviola in ethnomedicine against cancer and highlight annomuricin E as one of the contributing compounds in its anticancer activity. Graviola has been used in traditional medicine for the treatment of cancer and tumours. This study evaluated the chemopreventive properties of an ethyl acetate extract of graviola on rodents. An increase in the levels of enzymatic antioxidants and a decrease in the malondialdehyde (a marker for oxidative stress) level of the colon tissue homogenates were observed suggesting the suppression of lipid peroxidation. The apoptotic effect of graviola appears to be an important mechanism for its anti-cancer activity as it has been demonstrated under different experimental conditions.[26]

A 2015 study demonstrated the phytochemical synergy among the constituents of graviola compared to its flavonoid-enriched (FEF) and acetogenin-enriched (AEF) fractions. Isolation of the 'most-active fraction', or single constituents, from whole

extracts may not only compromise the therapeutic efficacy but also render toxicity thus emphasizing the importance of preserving the natural composition of whole extracts. The leaves of graviola are known to be rich in flavonoids, isoquinoline alkaloids and annonaceous acetogenins. Comparative quantitation of flavonoids revealed enrichment of rutin (~7-fold) and quercetin-3-glucoside (Q-3-G, ~3-fold) in FEF compared to graviola. *In vivo* pharmacokinetics and *in vitro* absorption kinetics of flavonoids revealed enhanced bioavailability of rutin in FEF compared to graviola. However, graviola was more effective in inhibiting *in vitro* prostate cancer proliferation, viability and clonogenic capacity compared to FEF. Oral administration of 100mg/kg of graviola showed 1.2-fold higher tumour growth-inhibitory efficacy than FEF in human prostate tumour xenografts although the concentration of rutin and Q-3-G was more in FEF. Contrarily, AEF, despite its superior *in vitro* and *in vivo* efficacy, resulted in death of the mice due to toxicity. The data indicates that despite lower absorption and bioavailability of rutin, maximum efficacy was achieved in the case of graviola, which is also comprised of other phytochemical groups including acetogenins that make up its natural complex environment. Hence, this study emphasises evaluating the nature of interactions among graviola leaf phytochemicals for developing favourable dose regimen for prostate cancer management to achieve optimal therapeutic benefits. Recent advances in understanding mechanistic roles played by constituent phytochemicals of whole foods continue to reveal the importance of synergistic interactions among themselves in their natural complex environment. This has spurred several studies to address a new paradigm, i.e. the imperative need to consume whole foods to attain maximum therapeutic and chemopreventive benefits. Solubility, absorption, pharmacokinetics and toxicity parameters of single agents lend support to the emergence of this paradigm shift. The loss of therapeutic efficacy upon isolation of a single agent or a fraction as compared with the parent underlies the mystifying phytocomplexity of whole foods, which led to the formulation of this study.[27]

Data from a 2014 study showed for the first time that the ethyl acetate extract of graviola inhibited the proliferation of lung cancer cells, leading to cell cycle arrest and programmed cell death through activation of the mitochondrial-mediated signalling pathway with the involvement of the NF-kB signalling pathway. Graviola leaves have been reported to have antiproliferative effects against various cancer cell lines however the detailed mechanism has yet to be defined. Cell viability analysis revealed the selective cytotoxic effect of graviola towards lung cancer cells.[28]

The findings of a 2014 study provide a scientific basis for the use of graviola in the treatment of cancer although further *in vivo* studies are still required. The purpose of this study was to investigate the anticancer properties of graviola on colon cancer cells and the underlying mechanisms. Graviola exerted significant cytotoxic effects on colon cancer cells. Analysis confirmed the induction of apoptosis.[29]

Extracts made from graviola have strong antiproliferation potential and can induce apoptosis of human leukaemia cells. The aim of this 2014 study was to determine the *in vitro* anti-proliferative effects and apoptotic events of graviola extracts on the test cells as well as to quantify its phenol content.[30]

The results of a 2014 study show that graviola is a promising new antioxidant and anticancer agent. Phytochemical screening of graviola revealed it is rich in secondary class metabolite compounds such as alkaloids, saponins, terpenoids, flavonoids, coumarins and lactones, anthraquinones, tannins, cardiac glycosides, phenols and phytosterols. The ethanolic leaves extract was found to be selectively cytotoxic *in vitro* to tumour cell lines.[31]

Ethanolic extract of graviola was suggested to have apoptosis-inducing potential against leukemic cells, although the detailed mechanism of action has not been explained in a 2013 study. Phytochemical screening verified the presence of alkaloids, tannins, flavonoids, saponins, anthraquinones and cardiac glycosides.[32]

Graviola has potent anticancerous acetogenins which are potent inhibitors of NADH oxidase (an enzyme that catalyses the production of superoxide, a reactive free radical) in the plasma membranes of cancer cells a 2013 study found. In the experimental analysis graviola leaves were collected and the extracted components were tested against various cancer cell lines. Test cells treated with 75µg of a crude leaf extract of graviola showed 80% cell inhibition.[33]

A 2012 study is the first to demonstrate that graviola extract reduces the viability of pancreatic cancer cells and tumours by inducing necrosis and cell cycle arrest, and by inhibiting pancreatic cancer cell motility (i.e. cytoskeleton rearrangement), migration and metabolism. *In vitro* experiments revealed that the compounds present in the extract inhibited several pathways involved in pancreatic cancer cell proliferation and metabolism, simultaneously. Such inhibitions ultimately led to a decrease in tumour growth and metastasis *in vivo*. Two studies below in antidiabetic activity suggest an additional benefit of graviola against pancreatic cancer given that diabetes has been classified as a risk factor of the malignant disease.[34,35]

The presence of therapeutically active antineoplastic compounds in graviola have been confirmed in a 2012 study. The study was aimed to evaluate the cytotoxic potential of graviola on normal human hepatic cells, human breast carcinoma cells and human immortalized keratinocyte cells lines. Most of the observed chemo-preventive potential correlated with the amount of total phenolics present in the extract. Correlation studies indicated a strong and significant positive correlation of phenolic compounds with free radical scavenging potential. The results revealed that the extract was moderately cytotoxic to normal cells when compared with those obtained for cancerous cells.[36]

Graviola has been found to be a promising new antitumor agent in numerous *in vitro* studies and showed greater anti-tumour activity in murine models than curcumin, a known natural chemopreventive. Chemoprevention of cancer can be defined as the use of natural, synthetic or biological substances

that intervene in the early precancerous stages and therefore reverse and suppress the formation of tumour. Human beings have been exposed to a variety of carcinogenic agents which may act as an initiator and promoter of tumour formation. In fact, the initiation of carcinogenesis may occur many years before it is being promoted. Thus chemopreventive agents are preferable to slow, reverse or completely halt multiple steps in the carcinogenesis process. Therefore a new science of chemoprevention has appeared as an attractive alternative to control malignancy. A 2012 *in vivo* study investigated the chemopreventive effects of an ethanolic extract of graviola. The results suggest that graviola was able to suppress tumour initiation as well as tumour promotion even at lower dosage.[37]

Results of a 2010 study suggest that graviola can be a source of substances with antinociceptive (reduced sensitivity to pain) and anti-inflammatory activities. Antinociceptive and anti-inflammatory activities of graviola ethanol extract were investigated in animal models. Orally the extract reduced the number of abdominal contortions.[38]

Significant anti-inflammatory and antinociceptive effects were demonstrated in another 2010 study. Ethanolic extract of graviola was used to investigate its anti-inflammatory and antinociceptive activities *in vivo*. Graviola exhibited significant and dose-dependent anti-inflammatory activity when administered orally to rats.[39]

A 2010 study suggests that graviola presents notable anti-arthritic activities that may be mediated by suppressing pro-inflammatory cytokines. The study was carried out to investigate the anti-arthritic effects of graviola ethanolic extract in induced arthritis *in vivo*. According to the results, oral administration of the extract reduced the oedema in a dose-dependent manner after two weeks of injection. Because the extract at higher doses significantly suppressed TNF-α and IL-1β expression in local tissue, the anti-arthritic activity of graviola leaves contributed to the suppression of pro-inflammatory cytokines. Hence the anti-arthritic potential of graviola was substantiated by the findings of this *in vivo* study.[40]

Graviola possesses potent *in vitro* antioxidant activity suggesting its role as an effective free radical scavenger and augmenting its therapeutic value. Antioxidant potential of leaves of three different species of *Annona* were studied by using different *in vitro* models. The ethanolic extract of *Annona muricata* showed maximum scavenging activity. This may be due to presence of acetogenins.[41]

Antispasmodic and anticonvulsant activity

In African countries a decoction of graviola leaves is traditionally used to control fever and convulsive seizures. To substantiate the anticonvulsant activity of the leaves in ethnomedicine researchers investigated the effect of the ethanolic extract of the leaves *in vivo*. The result showed that the plant extract at 100 and 300mg/kg doses significantly decreased the incidence and the mortality rate of tonic seizures in mice. Administration of the extract to mice also lengthened the onset of clonic seizures. This study showed that a subsequent bioassay-guided investigation may lead to the isolation of a bioactive compound that can be used as an anticonvulsant drug.[42]

Antidiabetic activity

Ethanolic extracts of graviola leaves have been clinically evaluated in relation to their hypoglycaemic activity. A randomised, parallel grouped, double blind phase II clinical trial, in patients with type 2 diabetes mellitus was conducted recently. Groups of patients were given 1, 2 or 3 capsules of ethanol extract from graviola leaves (180mg) plus 5mg of glibenclamide (an antidiabetic drug) for 30 days, and another group only received glibenclamide. The results of this study showed a decrease in the blood glucose or glycaemia level in patients receiving graviola compared to patients who did not receive it. Compounds responsible for the hypoglycaemic activity found in graviola could be flavonoids and alkaloids.[43]

A South African study was undertaken to investigate the possible protective effects of graviola in rat experimental paradigms of diabetes mellitus. The findings of this laboratory animal study suggest that graviola has a protective, beneficial effect on hepatic tissues subjected to induced oxidative stress, possibly by decreasing lipid peroxidation and indirectly enhancing production of insulin and endogenous antioxidants.[44]

A 2010 study has shown the ability of graviola to regenerate pancreatic islet β cells in diabetic rats. Microanatomical changes in the pancreatic islet cells of induced diabetic rats were studied after treatment with methanolic extracts of graviola. The results revealed regeneration of the beta-cells of islets of pancreatic islet of rats treated with graviola. The management of diabetes mellitus depends on continuous hypoglycaemic therapy which may not be consistently adhered to by the patient. This research therefore investigated whether or not graviola could provide lasting hypoglycaemic control through regeneration of the destroyed β -cells of the pancreatic islets of experimentally induced diabetic rats.[45]

Another study has shown the ability of graviola to regenerate pancreatic islet β cells in diabetic rats. Treatment with graviola has beneficial effects on pancreatic tissues subjected to induced oxidative stress by directly quenching lipid peroxides and indirectly enhancing production of endogenous antioxidants. An African study was undertaken to investigate the effects of graviola on the morphology of pancreatic β-cells and oxidative stress in induced diabetic rats.[46]

Antibacterial, antiparasitic, anthelmintic and insecticidal activity

Graviola could be developed as an alternative to sodium hypochlorite for root canal irrigants the results of a 2016 study show. The study was done to determine the antimicrobial effect of water extracts of graviola leaves and *Simarouba glauca* on *Enterococcus faecalis* using agar diffusion method. Graviola showed similar effectiveness as that of sodium hypochlorite. *Enterococcus faecalis* is the most common pathogen found in the root canals.[47]

The results of a 2015 study confirm the use of graviola in traditional medicine for the treatment of bacterial infections and diseases associated with oxidative stress such as diabetes. The purpose of the study was to evaluate the *in vitro* antibacterial activity of hydro-ethanol extracts of stems, barks and leaves of graviola against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabillis* and *Bacillus cereus,* followed by the evaluation of their antioxidant activities. *Pseudomonas aeruginosa* was the most sensitive strain obtained from leaves extract. The phytochemical screening revealed that the plant contains bioactive compounds such as steroids, triterpenes and alkaloids.[48]

The antibacterial and phytochemical activities of a methanolic and aqueous leaf extract of graviola was evaluated on *Staphylococcus aureus, Escherichia coli Streptococcus pyogenes, Bacillus subtilis, Salmonella typhimurium* and *Klebsiella pneumonia*. The most inhibited gram positive bacteria were *Bacillus subtilis* and *Staphylococcus aureus*, while the most inhibited gram negative bacteria was *Escherichia coli. Klebsiella pneumonia* was inhibited at almost every concentration of the methanolic extract. Both extracts showed antibacterial properties but the methanolic extract was more effective as it inhibited a wide range of organisms at varying concentrations. There was a significant difference between the methanolic and aqueous extract. Antibacterial activity of extracts was compared with the standard antibiotic, streptomycin. The results obtained in this 2014 study suggest that graviola can be used as an antibacterial substance. The researchers suggested graviola can be employed in the treatment of various bacterial infectious diseases like pneumonia, diarrhoea, urinary tract infection and even some skin disease.[49]

Graviola and some of its isolated compounds have shown effectiveness against protozoans responsible for human diseases such as the genera *Plasmodium, Leishmania, Biomphalaria, Trypanosoma* and *Entamoeba*, responsible for malaria, leishmaniasis, schistosomiasis, Chagas and amebiasis diseases respectively. The antiplasmodic effect has particular interest due to the necessity for antimalarial drugs in tropical areas. As a natural agent graviola has been subjected to various pathogenic parasites to determine its cytotoxic effects. The ethyl acetate leaf extract of graviola was assayed against three *Leishmania* species and *Trypanosoma cruzi*. Promising activity was reported with IC50 values lower than 25µg/mL.[50,51]

The same promising antileishmanial effect was reported against *Leishmania braziliensis* and *Leishmania panamensis* species with a toxicity effect higher than Glucantime, which was used as a positive control.[52]

Graviola exerted significant antimalarial activity with no toxicity and prolonged survival time a 2016 *in vivo* study found. Graviola inhibited parasitemia (parasites in the blood) dose dependently. Survival time was prolonged in infected mice treated with the extract.[53]

A recent *in vitro* investigation on graviola aqueous leaf extract was performed against *Haemonchus contortus*, a gastrointestinal parasite. The result showed 89.08% and 84.91% toxicity against larvae and eggs as assessed by larval motility and egg hatch tests. The immobilisation of adult worms within six to eight hours of exposure to different doses of the extract revealed a promising anthelmintic activity in the leaves.[54]

Eighteen plants originating from the Ivory Coast were selected by an ethnobotanical survey as plants commonly used by traditional healers for the treatment of malaria. The pentane leaf extract of graviola was assayed against two strains of *Plasmodium falciparum*, a malaria causing parasite transmitted by mosquitos. A promising antiplasmodial effect was obtained. These findings substantiated the traditional use of graviola as an antimalarial agent.[55]

Mosquito controlling activity for the ethanolic extract of graviola against *Culex quinquefasciatus* has been reported.[56]

Hepatoprotective activity

A 2012 study investigated the hepatoprotective effect of the aqueous extract of graviola leaves against induced liver damage. It was reported that the extract was effective against hyperbilirubinemia, or jaundice, with similar effects to silymarin *(Silybum marianum)*. The extract reduced the harmful effects and preserved the hepatic physiological mechanism of the liver damaged by a hepatotoxins such as paracetamol (Acetaminophen), a drug widely used as antipyretic and analgesic which can cause liver damage if taken in excess. This study suggests that graviola reduces bilirubin levels due the glucosides present in the extract which might be converted into glucuronic acid. Pretreatment with different concentrations of the extract (50, 100, 200, and 400mg/kg) for seven days prior to liver damage restored liver function toward normal haemostasis. These findings substantiated the traditional use of graviola against jaundice and showed the potential hepatoprotective activity.[57]

In addition to the above study due to its use as a treatment for jaundice a study was conducted to determine the *in vivo* bilirubin-lowering potential of the aqueous extract of graviola. This study was performed on induced jaundice in rats, and the levels of direct and total bilirubin were measured in rats orally treated with 50 and 400mg/kg of the extract. The extract at both doses caused a significant reduction to hyperbilirubinemia, which was close to normal levels.[58]

Gastroprotective activity

Graviola showed protective gastric effects like omeprazole (known as Losec) in induced ulcerogenesis in rats. The effect of the extracts on gastric tissues was accompanied with an increase in the activity of enzymatic antioxidants and suppression of lipid peroxidation representing the preservative effect against gastric wall mucus.[59]

The gastroprotective activity of graviola was examined against induced gastric injury *in vivo*. The results of the oral

administration of the extract (200 and 400mg/kg) showed significant antiulcer potential which was mediated through protective effects against gastric wall mucosal damages.[60]

The ethanolic extract of graviola was used to investigate its antinociceptive and anti-ulcerogenic activities. Graviola exhibited significant and dose-dependent antinociceptive activity. It also significantly decreased the ulcerative lesion produced by ethanol in rats in a dose-dependent manner. The 2012 study concluded that the antiulcer potential of graviola is probably through its antioxidant compounds that increase the mucosal nonprotein sulfhydryl group content. The study also showed that graviola had antinociceptive activity in both neurogenic and inflammatory phases.[61]

Hypotensive activity

The hypotensive effects of graviola are mediated through peripheral mechanisms involving antagonism of calcium ions. A 2012 *in vivo* study investigated the blood pressure reducing effect of graviola and the possible mechanisms that may be responsible. Graviola caused significant dose-dependent reduction in blood pressure without affecting the heart rates of rats. The hypotensive effect has been attributed to alkaloids such as coreximine, anomurine and reticulin and some essential oil components such as β-caryophyllene.[62]

Wound healing activity

Graviola has shown elevation in wound contraction compared with wounds without treatment. The wound healing activity of graviola against excisional wound healing in rats was investigated in 2015. Wound healing consists of four complex phases: coagulation, inflammation, proliferation and maturation. Graviola accelerates 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. Graviola induced upregulation of Hsp70 in wound tissues. In this phase the inflammatory cells produce cytokines and free radicals that in large quantities can produce lipid peroxidation in the wound. Antioxidants also fortified the wound healing activity of graviola. The same experiment using the alcoholic extract of the stem bark also showed a significant reduction in the wound area from the 4th day after injury onwards.[63,64]

Indications

- Cancer adjuvant therapy
- Diabetes
- Hypertension
- Gastric upset, indigestion, diarrhoea
- Jaundice
- Fever
- Pain
- Parasitic and bacterial infections

Toxicity

None know.

Use in Pregnancy

Information regarding safety and efficacy in pregnancy is lacking.

Contraindications and cautions

There is some concern that graviola might exacerbate Parkinson's symptoms because acetogenin constituents are neurotoxins *in vivo*. Neurotoxicity studies of Annonacin suggest that there is a need for a long exposure to this molecule to observe the effect in rodent models, while pharmacokinetic studies estimated low bioavailability of this compound. Nevertheless patients with established Parkinson disease should avoid consumption of the fruit or decoctions made from the leaves.[65,66]

Drug Interactions

None known.

Administration and Dosage

Liquid extract 1:2 in 45% alcohol 40 to 80mL weekly.[66]

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