Toxicological profile for

Guava extract

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

Not applicable.

1.2. Synonyms

Extract of guava; Psidium guajava extract; EINECS 289-907-2; Guava, Psidium guajava pyrifera, ext. (ChemIDplus)

1.3. Molecular formula

“Phytochemical studies have identified more than 20 compounds in guava extracts (Osman et al., 1974; Begum et al., 2002). The major constituents of its leaves were identified to be tannins, β-sitosterol, maslinic acid, essential oils, triterpenoids and flavonoids (Osman et al., 1974; Arima and Danno, 2002; Begum et al., 2002; 2004).” (Abreu et al., 2006)

“An extract of whole guava puree, after TLC and GLC, showed the presence of two monoterpenes and nine sesquiterpenes. β-Caryophyllene comprised 95% of this fraction.” (WilsonIII & Shaw, 1978)

1.4. Structural Formula

Not applicable.

1.5. Molecular weight (g/mol)

No data available to us at this time.

1.6. CAS registration number

90045-46-8

1.7. Properties

1.7.1. Melting point

(°C): No data available to us at this time.

1.7.2. Boiling point

(°C): No data available to us at this time.

1.7.3. Solubility

No data available to us at this time.

1.7.4. pKa

No data available to us at this time.

1.7.5. Flashpoint

(°C): No data available to us at this time.

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time.

1.7.7. (Auto)ignition temperature

(°C): No data available to us at this time.

1.7.8. Decomposition temperature

(°C): No data available to us at this time.

1.7.9. Stability

No data available to us at this time.

1.7.10. Vapor pressure

No data available to us at this time.

1.7.11. log Kow

No data available to us at this time.

2. General information

2.1. Exposure

Psidium guajava fruit and leaf extracts, and Psidium guajava fruit juice are used as astringent and skin conditioning ingredients in cosmetics in the EU. Psidium guajava fruit is used as a skin conditioning agent and Psidium guajava seed oil as an emollient in cosmetics in the EU. As taken from CosIng (Cosmetic substances and ingredients database). (All materials have CAS RNs given as 91770-12-6). Accessed April 2020, available at: https://ec.europa.eu/growth/tools-databases/cosing/

Guava extract is listed as an ingredient in personal care products by the CPID.

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in JTI Study Report (s).

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area %
formic acid	26100893	2.12	21177415	2.42
acetic acid	96605596	7.83	73275389	8.37
furfural	46990596	3.81	35288816	4.03
furfuryl alcohol	21959491	1.78	15937347	1.82
3-methyl-2,5-furandione	20470152	1.66	14357321	1.64
glycerol + 4,5-dimethylfurfural	60856338	4.93	33613765	3.84
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	153079555	12.41	113038948	12.91
unknown	12202731	0.99	9463586	1.08
5-hydroxymethylfurfural	425547630	34.48	314884394	35.95
unknown	18690278	1.52	9293103	1.06
unknown	15312060	1.24	10442814	1.19
Total area		72.76		74.30

2.3. Ingredient(s) from which it originates

“Psidium guajava (guava), belonging to the family of Myrtaceae, is a native of tropical America and has long been naturalized in Southeast Asia. The positive effects of guava extracts on human ailments have been described (Lozoya, 1999).”

As taken from Abreu et al. 2006. Guava extract (Psidium guajava) alters the labelling of blood constituents with technetium-99m. J. Zhejiang Univ. Sci. B. 7(6). 429–435. PubMed, 2010 available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1474003/

3. Status in legislation and other official guidance

Guava extract (CAS RN 90045-46-8) is included on the FDA’s inventory of “Substances Added to Food (formerly EAFUS)” and is included under 21 CFR section 182.20 (Essential oils, oleoresins (solvent-free), and natural extractives (including distillates)).

As taken from FDA, 2020a,b.

Guava, Psidium guajava pyrifera, ext. (CAS RN 90045-46-8) and Psidium guajava fruit extract (no CAS RN given) are pre-registered under REACH (“envisaged registration deadline 30 November 2010” and “envisaged registration deadline 31 May 2018,” respectively) (ECHA).

Guava, Psidium guajava pyrifera, ext. (CAS RN 90045-46-8) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2020).

According to New Zealand’s Environmental Protection Authority, guava, Psidium guajava pyrifera, ext. (CAS RN 90045-46-8) may be used as a component in a product covered by a group standard but it is not approved for use as a chemical in its own right (NZ EPA, 2006).

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

No data available to us at this time.

4.2. Absorption, distribution and excretion

No data available to us at this time.

4.3. Interactions

“The effects of six Thai fruits, namely banana, guava, mangosteen, pineapple, ripe mango and ripe papaya, on cytochrome P450 (P450) activities were investigated. The median inhibitory concentrations (IC(50) of each of the fruit juices on CYP1A1, CYP1A2, CYP2E1 and CYP3A11 activities were determined. Pineapple juice showed the strongest inhibitory effect against all the evaluated P450 isozyme activities in mouse hepatic microsomes, followed by mangosteen, guava, ripe mango, ripe papaya and banana.…. These observations supported that the six Thai fruits were a feasible cause of food-drug interaction or adverse drug effects owing to their potential to modify several essential P450 activities” (Chatuphonprasert and Jarukamjorn. 2012. Journal of Applied Toxicology 32(12), 994-1001. Abstract available at http://www.ncbi.nlm.nih.gov/pubmed/22499231

“Assessing the bioavailability of non-heme iron and zinc is essential for recommending diets that meet the increased growth-related demand for these nutrients. We studied the bioavailability of iron and zinc from a rice-based meal in 16 adolescent boys and girls, 13-15 y of age, from 2 government-run residential schools. Participants were given a standardized rice meal (regular) and the same meal with 100 g of guava fruit (modified) with (57)Fe on 2 consecutive days. A single oral dose of (58)Fe in orange juice was given at a separate time as a reference dose. Zinc absorption was assessed by using (70)Zn, administered intravenously, and (67)Zn given orally with meals. The mean hemoglobin concentration was similar in girls (129 ± 7.8 g/L) and boys (126 ± 7.1 g/L). There were no sex differences in the indicators of iron and zinc status except for a higher hepcidin concentration in boys ($P < 0.05$). The regular and modified meals were similar in total iron (10-13 mg/meal) and zinc (2.7 mg/meal) content. The molar ratio of iron to phytic acid was >1:1, but the modified diet had 20 times greater ascorbic acid content. The absorption of (57)Fe from the modified meal, compared with regular meal, was significantly ($P < 0.05$) greater in both girls (23.9 ± 11.2 vs. 9.7 ± 6.5%) and boys (19.2 ± 8.4 vs. 8.6 ± 4.1%). Fractional zinc absorption was similar between the regular and modified meals in both sexes. Hepcidin was found to be a significant predictor of iron absorption (standardized $\beta = -0.63$, $P = 0.001$, $R(2) = 0.40$) from the reference dose. There was no significant effect of sex on iron and zinc bioavailability from meals. We conclude that simultaneous ingestion of guava fruit with a habitual rice-

based meal enhances iron bioavailability in adolescents." As taken from Nair KM et al. 2013. J. Nutr. 143(6), 852-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23596161

"BACKGROUND: In-depth information of potential drug-herb interactions between warfarin and herbal compounds with suspected anticoagulant blood thinning effects is needed to raise caution of concomitant administration. The current study aimed to investigate the impact of co-administration of pomegranate peel and guava leaves extracts, including their quality markers namely; ellagic acid and quercetin, respectively, on warfarin's in vivo dynamic activity and pharmacokinetic actions, in addition to potential in vitro cytochrome P450 enzymes (CYP) inhibition. METHODS: Influence of mentioned extracts and their key constituents on warfarin pharmacodynamic and kinetic actions and CYP activity were evaluated. The pharmacodynamic interactions were studied in Sprague Dawley rats through prothrombin time (PT) and International Normalized Ratio (INR) measurements, while pharmacokinetic interactions were detected in vivo using a validated HPLC method. Furthermore, potential involvement in CYP inhibition was also investigated in vitro on isolated primary rat hepatocytes. RESULTS: Preparations of pomegranate peel guava leaf extract, ellagic acid and quercetin in combination with warfarin were found to exert further significant increase on PT and INR values ($p < 0.01$) than when used alone ($p < 0.05$). Pomegranate peel extract showed insignificant effects on warfarin pharmacokinetics ($p > 0.05$), however, its constituent, namely, ellagic acid significantly increased warfarin C_{max} ($p < 0.05$). Guava leaves extract and quercetin resulted in significant increase in warfarin C_{max} when compared to control ($p < 0.01$). Furthermore, guava leaves extract showed a significant effect on changing the AUC, CL and V_z. Significant reduction in CYP2C8, 2C9, and 3A4 was seen upon concomitant use of warfarin with ellagic acid, guava leaves and quercetin, unlike pomegranate that insignificantly affected CYP activities. CONCLUSION: All combinations enhanced the anticoagulant activity of warfarin as the results of in vivo and in vitro studies were consistent. The current investigation confirmed serious drug herb interactions between warfarin and pomegranate peel or guava leaf extracts. Such results might conclude a high risk of bleeding from the co-administration of the investigated herbal drugs with warfarin therapy. In addition, the results raise attention to the blood-thinning effects of pomegranate peel and guava leaves when used alone." As taken from Alnaqeeb M et al. 2019. BMC Complement. Altern. Med. 19(1), 29. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30678660

"Background: Plant-based natural extracts cure several diseases in human. However, the extract of *Psidium guajava* leaf is not yet evaluated on changes of lipid profile in hepatic disease affected rats. Objective: The present study was aimed to evaluate the mitigation effect of the ethanolic extract of *P. guajava* leaf and its isolated quercetin fraction on hepatotoxic rats. Materials and Methods: Carbon tetrachloride (CCl_4) was injected to rats for hepatic disease induction and silymarin drug was used as positive control to compare plant ethanolic extract. The lipid profiles were assessed in both plasma and liver tissue of diseased and control rats. Results: Levels of total cholesterol, triglycerides, free fatty acids, phospholipids, and low-density lipoprotein cholesterol were increased and the level of high-density lipoprotein cholesterol (HDL-C) was decreased in CCl_4-induced hepatotoxic rats. The treatment of*P. guajava*(100, 200, and 300 mg/kg, bw) and isolated quercetin fraction (20 mg/kg, bw) doses decreased the elevated levels of all these parameters in diseased rats and restored the normal concentration of HDL-C. Conclusion: The results of the present study concluded that the*P. guajava*leaf and its isolated quercetin fraction can significantly regulate lipid metabolism in CCl_4-induced hepatotoxic rats and decrease the disease rate.

SUMMARY:*Psidium guajava*leaf extract reduces the hepatotoxicity and disease rate in ratsQuercetin fraction of leaf extract significantly regulates lipid profile in hepatic diseased rats." As taken fromVijayakumar K et al. 2018. Pharmacogn. Mag. 14(53), 4-8. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29576694

"Introduction: Guava leaf contains low calorie, vitamins, minerals antioxidants, polyphenolic, and flavonoid compounds which may play important role in prevention of cancers, aging, cell differentiation, apoptosis and may also have immune enhancing properties while ibuprofen is a nonsteroidal anti inflammatory drug commonly used to relief pain. It reduces hormones that cause inflammation and pain in the body. This project work was therefore designed to evaluate the plasma level of pro (tumor necrosis factor alpha (TNFα)) and anti inflammatory (interleukin 4 [IL 4] and 6) cytokines, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in rabbits overdosed with ibuprofen and supplemented with guava leaf extract. Methods: Fifteen rabbits grouped into three experimental groups labeled A–C (with subgroups B1, B2, C1, and C2) with A as control were investigated. An overdose of ibuprofen of 1600 mg/kgBW was administered to Group B and C rabbits to induce toxicity, while 400 ml/kgBW guava leaf extract was used to reduce and treat ibuprofen toxicity. Plasma tumor necrosis factor alpha (TNFα), IL-4 and 6, AST, and ALT were analyzed biochemically by spectrophotometry and enzyme-linked immunosorbent assay. Results: The result obtained showed a significant increase in the plasma value of TNF-α, IL-4, IL-6, ALT, and AST in rabbits induced with 1600 mg/kgBW of ibuprofen per oral fed with normal meal for 7 days (Group B1 and C1) compared with normal rabbits fed with normal meal and water only for 7 days (Group A) ($P < 0.05$). There was a significant decrease in the plasma value of TNF-α, IL-4, IL-6, and AST in rabbits given 400 ml/KgBW of guava leaf extract daily for 7 days after 7 days of postibuprofen administration (Group B2 and C2) compared to when the rabbits were given 1600 mg/kgBW of ibuprofen per oral with normal meal and water observed for 7 days (B1 and C1) ($P < 0.05$). Conclusion: Guava aqueous extract has been demonstrated to reduce the biochemical alterations in the plasma values of (TNF-α, IL-4, IL-6, AST, and ALT) following ibuprofen overdose as a result of the phytochemical contents of the guava leaf." As taken from Olaniyan MF et al. 2019. Biomed. Biotechnol. Res. J. 2, 254-9. Available at http://www.bmbtrj.org/article.asp?issn=2588-9834;year=2018;volume=2;issue=4;spage=254;epage=259;aulast=Olaniyan;type=0

5. Toxicity

5.1. Single dose toxicity

Oral rabbit TDLo: 4 mL/kg (hypoglycemia) (Psidium guajava, fruit juice).

As taken from RTECS, 2014.

Oral rat: The LD50 of guava leaf extract was more than 5 g/kg, p.o. (Jaiarj et al., 1999).

Oral, rat, LD50: >5 g/kg bw (Psidium guajava Linn., extract).

Oral, mouse LD50: >5 g/kg bw (Psidium guajava Linn., extract).

Intraperitoneal, mouse LD50: 188 mg/kg bw (Psidium guajava Linn., extract).

As taken from RTECS, 2012.

“The emerging resistance of Plasmodium species to currently available anti-malarials remains a public health concern, hence the need for new effective, safe and affordable drugs. Natural products remain a reliable source of drugs. Nefang is a polyherbal anti-malarial of the Cameroonian folklore medicine with demonstrated in vitro antiplasmodial and antioxidant activities. It is composed of Mangifera indica (bark and leaf), Psidium guajava, Carica papaya, Cymbopogon citratus, Citrus sinensis, Ocimum gratissimum (leaves). This study aimed at investigating the suppressive, prophylactic and curative activities of Nefang in Plasmodium infected rodent models. Systemic acute oral toxicity of Nefang aqueous and ethanol extracts was assessed in mice up to a dose of 5,000 mgkg-1 body weight. BALB/c mice and Wistar rats were inoculated with Plasmodium chabaudi chabaudi and Plasmodium berghei, respectively, and treated with Nefang, the Mangifera indica bark/Psidium guajava combination and a Psidium guajava leaf aqueous extracts (75, 150, 300 and 600 mgkg-1 bwt). Their schizonticidal activity was then evaluated using the Peter's 4-day suppressive test). The prophylactic and curative (Rane's Test) activity of Nefang was also evaluated by determining the parasitaemia, survival time, body weight and temperature in pre-treated rodents. Acute oral toxicity of the extract did not cause any observed adverse effects. Percent suppressions of parasitaemia at 600 mgkg-1 bwt were as follows (P. berghei/P. chabaudi): Nefang - 82.9/86.3, Mangifera indica bark/Psidium guajava leaf combination extract - 79.5/81.2 and Psidium guajava leaf - 58.9/67.4. Nefang exhibited a prophylactic activity of 79.5% and its chemotherapeutic effects ranged from 61.2 - 86.1% with maximum effect observed at the highest experimental dose. These results indicate that Nefang has excellent in vivo anti-malarial activities against P. berghei and P. chabaudi, upholding earlier in vitro antiplasmodial activities against multi-drug resistant P. falciparum parasites as well as its traditional use. Hence, Nefang represents a promising source of new anti-malarial agents.” As taken from Arrey Tarkang P et al. 2014. Malar. J. 13(1), 456. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/25421605

“Objective: The main objectives of the research are to investigate the phytochemical screening, histology appearance, and safety of acute oral toxicity study on the extract of the fruit of Psidium guajava Linn. in mice. Methods: Mice that were administered by oral feeding with different and controlled dose were divided into three groups, with dose limits of both 2000 and 5000 mg/kg b.w. We analyzed the P. guajava Linn. extract with specific methods before treating the subject. The methods were followed with acute oral toxicity study of Up-and-Down Procedure Organization for Economic and Development 425. The mice were then observed for signs and symptoms of toxicity. In addition, toxicity in the liver and kidney was analyzed through histology observation. Results: Phytochemical screening revealed the presence of flavonoids, quinone, triterpenoid/steroid, tannins and saponins, and the absence of alkaloids. We found that the treatment with 2000 and 5000 mg/kg b.w. of the extract did not show any differences in body weight changes, number of hepatocyte in liver, and podocyte in kidney compared with control (*p>0.05). Moreover, we noticed all mice lived and were healthy during observation. Conclusion: Our finding indicates that the extract of the fruit of P. guajava Linn. is safe and it was not toxic to the liver and kidney.” As taken from Atik N et al. 2019. Asian Journal of Pharmaceutical and Clinical Research 12(1), 351-355. Available at https://bit.ly/2IJ9Kh8

“The methanol crude extract of the bark of *Psidium guajava* (guava) previously displayed interesting cytotoxic effects on a panel of human cancer cell lines. In the present work, we plan to determine the toxicological effects of this guava botanical in Wistar rats. Acute oral toxicity of the extract was carried out by administration of a single dose of 5000 mg/kg body weight to female rats in 14 days. Subacute toxicity was conducted by oral administration of the extract at daily doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg body weight, respectively, while rats in the control group received no extract. After 28 days of treatment, animals were sacrificed for hematological and biochemical studies. In the acute toxicity study, no mortality or signs of toxicity were recorded; hence, the median lethal dose (LD_{50}) of the *Psidium guajava* bark extract is greater than 5000 mg/kg body weight. For the subacute toxicity study, significant variations in body weight, relative weight of organs, and biochemical parameters were observed in the animals treated at different doses of the plant extract compared to control animals. Histopathological analyses showed minor liver inflammation in females treated at the highest dose (1000 mg/kg). These results suggest that intake of a single high dose of the *Psidium guajava* bark extract is nontoxic, but repeat administration could exhibit mild organ toxicity.” As taken from Manekeng HT et al. 2019. Evid. Based Complement. Alternat. Med. 2019, 8306986. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31885665/

“Recent studies reported interesting ethnopharmacological, antibacterial, and phytochemical data on some medicinal plants used in the traditional treatment of salmonellosis in Benin. Unfortunately, very little data exists on the toxicity of these species. This study aims to evaluate chemical characteristic of six Benin pharmacopoeial plants used in the traditional treatment of salmonellosis in Benin. The acute toxicity of aqueous and ethanolic extracts of Psidium guajava, Vernonia amygdalina, Cajanus cajan, Phyllanthus amarus, Uvaria chamae, and Lantana camara was evaluated according to OECD Guideline 423 at a single dose of 2000 mg/kg body weight on Wistar rats. Histological sections were performed on the liver and kidneys to confirm hematological and biochemical data. The content of aluminum, chromium, cadmium, copper, iron, lead, zinc, arsenic, selenium, and manganese was measured in 10 mg of each extract by the inductively coupled plasma optical emission spectroscopy (ICPOES) method. The results of our study generally show the absence of significant effect of the extracts on the hematological and biochemical parameters of the rats. However, with the exception of the aqueous and ethanolic extracts of Psidium guajava root and the ethanolic extract of Phyllanthus amarus ($P>0.05$), all the extracts have a significant effect on the aspartate aminotransferase (ASAT) level, with a variable threshold of significance ($0.0001< P \leq 0.05$). No mortalities and no renal histological conditions were recorded in the treated rats. In general, the heavy metal contents of the extracts do not exceed the standards set by the WHO/FDA except for a few extracts. Arsenic was not detected in any extract, while aluminum and chromium were detected at levels above the WHO/FDA standards. On the basis of these data, it appears that the six plants studied do not show any toxicity. In view of the pharmacological and chemical data previously available, these plants are good candidates for the development of improved traditional medicines with antibacterial and particularly anti-Salmonella properties.” As taken from Legba B et al. 2019. J. Toxicol. 2019, 3530659. PubMed, 2020 available at https://pubmed.ncbi.nlm.nih.gov/31354814/

5.2. Repeated dose toxicity

Record for Psidium guajava, fruit juice:

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
TDLo-Lowest published toxic dose	Oral	Rodent-rat	875 mg/kg/1W (intermittent)	Endocrine- hypoglycemia	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 49,2189,2011
TDLo-Lowest published toxic dose	Oral	Rodent-rat	3500 mg/kg/4W (intermittent)	Gastrointestinal- changes in structure or function of endocrine pancreas Endocrine- - hypoglycemia Endocrine-other changes	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 49,2189,2011
TDLo-Lowest published toxic dose	Oral	Rodent-rat	7000 mg/kg/4W (intermittent)	Gastrointestinal- changes in structure or function of endocrine pancreas Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels- other oxidoreductases Biochemical-Metabolism (Intermediary) - lipids including transport	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 49,2189,2011

As taken from RTECS, 2014.

5.3. Reproduction toxicity

Subcutaneous, male rats, 10 days pre-mating, TDLo: 1111 mg/kg bw (effects on testes, epididymis, sperm duct, prostate, seminal vesicle, Cowper's gland, accessory glands) (Psidium guajava Linn., extract).

As taken from RTECS, 2012.

5.4. Mutagenicity

Studies on antimutagenic effects of guava (Psidium guajava) in Salmonella typhimurium (Abstract). The water and chloroform extracts of guava were tested for their antimutagenicity. The water extract was effective in inactivating the mutagenicity of direct-acting mutagens, e.g., 4-nitro-o-phenylenediamine, sodium azide, and the S9-dependent mutagen, 2-aminofluorene, in the tester strains of Salmonella typhimurium. The chloroform extract was inactive. Autoclaving of the water extract for 15 min did not reduce its activity appreciably. The enhanced inhibitory activity of the extracts on pre-incubation suggests the possibility of desmutagens in the extracts. Besides ascorbic acid and citric acid, the major constituents of the extracts, the role of other antimutagenic factors in the extracts cannot be ruled out. As taken from Grover and Bala, Mutation Research/Genetic Toxicology, Volume 300, Issue 1, June 1993, Pages 1-3. Science Direct, 2010 available at http://www.sciencedirect.com/

Absence of mutagenicity effects of Psidium cattleyanum Sabine (Myrtaceae) extract on peripheral blood and bone marrow cells of mice (Abstract). Cattley guava (Psidium cattleyanum Sabine) is a native fruit of Brazil that is popular both as a sweet food and for its reputed therapeutic properties. We examined whether it could damage DNA using the alkaline single-cell gel electrophoresis (comet assay) and the micronucleus test in leukocytes and in bone marrow cells of mice. P. cattleyanum leaf extract was tested at concentrations of 1000, 1500 and 2000 mg/kg. N-nitroso-N-ethylurea was used as a positive control. Peripheral blood leukocytes were collected 4 and 24 h after the treatments for the comet assay, and bone marrow cells were collected after 24 and 48 h for the micronucleus test. Unlike N-nitroso-N-ethylurea, P. cattleyanum extract failed to induce a significant increase in cell DNA damage, in micronucleated cell frequency, and in bone marrow toxicity. The lack of mutagenicity and cytotoxicity with high doses of this plant extract means that it can be safely used in traditional medicine. As taken from Costa et al., Genet. Mol. Res. 7 (3): 679-686 (2008), available at http://www.funpecrp.com.br/gmr/year2008/vol7-3/pdf/gmr475.pdf

“Psidium guajava Linn. (family Myrtaceae; PG) is a tropical fruit with a blood-glucose-lowering effect in diabetic rats, but its mechanism of action is still unknown. We investigated the antihyperglycemic efficacy and mechanisms of action of PG in streptozotocin (STZ)-induced diabetic rats. After 4 weeks of PG supplementation (125 and 250 mg/kg), PG significantly restored the loss of body weight caused by STZ and reduced blood glucose levels in a dose-dependent manner compared with that in diabetic control rats. Mechanistically, PG protected pancreatic tissues, including islet β-cells, against lipid peroxidation and DNA strand breaks induced by STZ, and thus reduced the loss of insulin-positive β-cells and insulin secretion. Moreover, PG also markedly inhibited pancreatic nuclear factor-kappa B protein expression induced by STZ and restored the activities of antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase. We conclude that PG has a significant antihyperglycemic effect, and that this effect is associated with its antioxidative activity”. As taken from Huang CS et al. 2011. Fd Chem. toxicol. 49, 2189-2195. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/21679740

“The use of medicinal herbs has been a common practice in Asia but their genotoxic properties are little known. In the present study, genotoxic effects of three antidiarrheal herbs, guava leaf, mangosteen peel and pomegranate peel, were examined using established human cell lines, Raji and P3HR-1. Cells were treated with boiled-water extract

of the herbs at various concentrations for 24 and 48 hours in vitro. Cell growth and viability were dose dependently reduced. No apparent chromosomal aberrations were induced by the treatment. Administration of pomegranate extract induced apoptotic DNA fragmentation. This genotoxicity test system is simple and convenient for the primary screening." As taken from Settheetham W & Ishida T. 1995. Southeast Asian J. Trop. Med. Public Health 26, 306-310. PubMed, 2014 available at https://www.ncbi.nlm.nih.gov/pubmed/8629131

"Cancer is one of the leading causes of deaths worldwide. The agents capable of causing damage to genetic material are known as genotoxins and, according to their mode of action, are classified into mutagens, carcinogens or teratogens. Genotoxins are involved in the pathogenesis of several chronic degenerative diseases including hepatic, neurodegenerative and cardiovascular disorders, diabetes, arthritis, cancer, chronic inflammation and ageing. In recent decades, researchers have found novel bioactive phytocompounds able to counteract the effects of physical and chemical mutagens. Several studies have shown potential antigenotoxicity in a variety of fruits. In this review (Part 1), we present an overview of research conducted on some fruits (grapefruit, cranberries, pomegranate, guava, pineapple, and mango) which are frequentl consumed by humans, as well as the analysis of some phytochemicals extracted from fruits and yeasts which have demonstrated antigenotoxic capacity in various tests, including the Ames assay, sister chromatid exchange, chromosomal aberrations, micronucleus and comet assay." As taken from Izquierdo-Vega JA et al. 2017. Nutrients 9(2), E102. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28157162

"Fruits, vegetables and medicinal herbs rich in phenolics antioxidants contribute toward reduced risk of age-related diseases and cancer. In this study, Psidium guajava leaf extract was fractionated in various organic solvents viz. petroleum ether, benzene, ethyl acetate, ethanl and methanol and tested for their antioxidant and antimutagenic properties. Methanolic fraction showed maximum antioxidant activity comparable to ascorbic acid and butylated hydroxyl toluene (BHT) as tested by DPPH free radical scavenging, phosphomolybdenum, FRAP ($Fe3+$ reducing power) and CUPRAC (cupric ions ($Cu2+$) reducing ability) assays. The fraction was analyzed for antimutagenic activities against sodium azide ($NaN3$), methylmethane sulfonate (MMS), 2-aminofluorene (2AF) and benzo(a)pyrene (BP) in Ames Salmonella tester strains. The methanol extracted fraction at 80 µg/ml concentration inhibited above 70% mutagenicity. Further, phytochemical analysis of methanol fraction that was found to be most active revealed the presence of nine major compounds by gas chromatography-mass spectrometry (GC-MS). This data suggests that guava contains high amount of phenolics responsible for broad-spectrum antimutagenic and antioxidant properties in vitro and could be potential candidates to be explored as modern phytomedicine." As taken from Zahin M et al. 2017. Drug Chem. Toxicol. 40(2), 146-153. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27268266

5.5. Cytotoxicity

Molecular Action Mechanism against Apoptosis by Aqueous Extract from Guava Budding Leaves Elucidated with Human Umbilical Vein Endothelial Cell (HUVEC) Model (Abstract).

Chronic cardiovascular and neurodegenerative complications induced by hyperglycemia have been considered to be associated most relevantly with endothelial cell damages (ECD). The protective effects of the aqueous extract of Psidium guajava L. budding leaves (PE) on the ECD in human umbilical vein endothelial cell (HUVEC) model were investigated. Results revealed that glyoxal (GO) and methylglyoxal (MGO) resulting from the glycative and autoxidative reactions of the high blood sugar glucose (G) evoked a huge production of ROS and NO, which in turn increased the production of peroxynitrite, combined with the activation of the nuclear factor kappaB (NFkappaB), leading to cell apoptosis. High plasma glucose activated p38-MAPK, and high GO increased the expressions of p38-MAPK and JNK-MAPK, whereas high MGO levels induced the activity of ERK-MAPK. Glucose and dicarbonyl compounds were all found to be good inducers of intracellular PKCs, which together with MAPK acted as the upstream triggering factor to activate NFkappaB. Conclusively, high plasma glucose together with dicarbonyl compounds can trigger the signaling pathways of MAPK and PKC and induce cell apoptosis through ROS and peroxynitrite stimulation and finally by NFkappaB activation. Such effects of PE were ascribed to its high plant polyphenolic (PPP) contents, the latter being potent ROS inhibitors capable of blocking the glycation of proteins, which otherwise could have brought forth severe detrimental effects to the cells. As taken from Hsieh et al., J. Agric. Food Chem., 2007, 55 (21), pp 8523–8533, available at http://pubs.acs.org/

“Aim. To determine the antimicrobial potential of guava (Psidium guajava) leaf extracts against two gram-negative bacteria (Escherichia coli and Salmonella enteritidis) and two gram-positive bacteria (Staphylococcus aureus and Bacillus cereus) which are some of foodborne and spoilage bacteria. The guava leaves were extracted in four different solvents of increasing polarities (hexane, methanol, ethanol, and water). The efficacy of these extracts was tested against those bacteria through a well-diffusion method employing 50 μL leaf-extract solution per well. According to the findings of the antibacterial assay, the methanol and ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, whereas the gram-negative bacteria were resistant to all the solvent extracts. The methanol extract had an antibacterial activity with mean zones of inhibition of 8.27 and 12.3 mm, and the ethanol extract had a mean zone of inhibition of 6.11 and 11.0 mm against B. cereus and S. aureus, respectively. On the basis of the present finding, guava leaf-extract might be a good candidate in the search for a natural antimicrobial agent. This study provides scientific understanding to further determine the antimicrobial values and investigate other pharmacological properties.” As taken from Biswas B et al. 2013. Int. J. Microbiol. 2013, 746165. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24223039

“In the present study, we evaluated the antimicrobial activity of 16 Jordanian medicinal plant extracts against four reference bacteria; Staphylococcus aureus, Enterobacter faecalis, Escherichia coli, and Salmonella typhi. For that purpose, whole plants were extracted and antimicrobial susceptibility testing and minimum inhibitory concentration (MIC) were determined. Ethanolic extracts of most medicinal plants exerted a dose-dependent cytotoxiciy against different reference bacteria. Origanum syriaca, Varthemia iphionoides, Psidium guajava, Sarcopoterium spinosa plant extracts were most active against S. aureus (MIC; 70 μg/mL), E. faecalis (MIC; 130 μg/mL), E. coli (MIC; 153 μg/mL), and S. typhi (MIC; 110 μg/mL), respectively. Results indicate that medicinal plants grown in Jordan might be a valuable source of starting materials for the extraction and/or isolation of new antibacterial agents.” As taken from Masadeh MM et al. 2013. Pak. J. Pharm. Sci. 26(2), 267-70. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23455195

“Psidium guajava (Myrtaceae) is an evergreen shrub growing extensively throughout the tropical and subtropical areas. Four new compounds, guavinoside C, D, E and F (1-3, 10) were isolated from the leaves of P. guajava, along with six known ones (4-9). Their structures were elucidated by spectroscopic analysis. Compounds 1, 4 and 10 showed significant cytotoxic activities on HeLa, SGC-7901 and A549 cell lines, respectively. Compounds 1 and 4-10 showed antioxidant activities in DPPH, ABTS and FRAP assays, and five of them (1, 4-6, 10) exhibited stronger activities than that of vitamin C.” As taken from Feng XH et al. 2015. Bioorg. Med. Chem. Lett. 25(10), 2193-8. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25862199

"Context Psidium guajava L. (Myrtaceae) leaves are used in traditional medicines for the treatment of cancer, inflammation and other ailments. Objective The current study explores scientific validation for this traditional medication. Materials and methods We used ferric-reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picryl hydrazil (DPPH) assays to estimate antioxidant activity of P. guajava leaf extracts (methanol, hexane and chloroform). Antitumour and in vivo cytotoxic activities were determined using potato disc assay (PDA) and brine shrimp lethality assay, respectively. Three human carcinoma cell lines (KBM5, SCC4 and U266) were incubated with different doses (10-100 µg/mL) of extracts and the anticancer activity was estimated by MTT assay. NF-κB suppressing activity was determined using electrophoretic mobility shift assay (EMSA). Chemical composition of the three extracts was identified by GC-MS. Total phenolic and flavonoid contents were measured by colorimetric assays. Results and discussions The order of antioxidant activity of three extracts was methanol > chloroform > hexane. The IC50 values ranged from 22.73 to 51.65 µg/mL for KBM5; 22.82 to 70.25 µg/mL for SCC4 and 20.97 to 89.55 µg/mL for U266 cells. The hexane extract exhibited potent antitumour (IC50 value = 65.02 µg/mL) and cytotoxic (LC50 value = 32.18 µg/mL) activities. This extract also completely inhibited the TNF-α induced NF-κB activation in KBM5 cells. GC-MS results showed that pyrogallol, palmitic acid and vitamin E were the major components of methanol, chloroform and hexane extracts. We observed significant ($p < 0.05$) difference in total phenolic and flavonoid contents of different solvent extracts. Conclusion The present study demonstrates that P. guajava leaf extracts play a substantial role against cancer and down-modulate inflammatory nuclear factor kB." As taken from Ashraf A et al. 2016. Pharm. Biol. 54(10), 1971-81. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26841303

Origin	Extraction Method	Major Constituent	Cells	Assay	Main Results	Ref.
Korea	Maceration in MeOH:H_2O 70% (*v*/*v*) (5 days)	-	LPS-stimulated RAW 264.7 (Mouse macrophage)	Griess, MTT, ELISA kit, western blot, transient transfection, and luciferase assays	At 125 µg/mL: no cytotoxic effect, ← 44–62% inhibition rates. ↓ LPS-induced NO and PEG_2 ↓ iNOS and COX-2 (↓ I-κBα degradation, ↓ activation NF-κB).	[48]

Korea	Extraction in MeOH:H_2O 70% (*v*/*v*) (6 h)	TPC: 426.84 mg (GAE)/g	LPS-stimulated RAW 264.7	MTT, Griess, and ELISA test assays	At 30 µg/mL: no cytotoxic effect. ↓ LPS-induced NO (52.58%) and the production of PGE_2 (43.45).	[49]
Korea	Extraction in EtOH:H_2O 55% (*v*/*v*) (4.9 h, 47 °C)	Gallic acid (0.2) and catechin (4.4) in mg/g	LPS-stimulated RAW 264.7	MTT, Griess, ELISA test, RT-PCR, and total western blot assays	At 50 µg/mL: no cytotoxic effect. ↓ LPS-induced NO (>65%) by ↓ iNOS, ↓ PGE_2 (to basal level) via ↓ COX-2 mRNA. ↓ IL-6. ↓ iNOS and COX-2 due to the down-regulation of ERK1/2 pathway, because no effect was found to other proteins at the dose tested.	[50]
Korea	Soxhlet with EtOH:H_2O 55% (*v*/*v*) (4.9 h, 47 °C)	Gallic acid (0.09) and catechin (0.72) in mg/g	LPS-stimulated RAW 264.7	MTT, Greiss and ELISA test assays	At 30 µg/mL: no cytotoxic effect. ↓ LPS-induced NO (47.5%) and PGE_2 (45.8).	[52]

Díaz-de-Cerio E et al. (2017b). Health Effects of Psidium guajava L. Leaves: An Overview of the Last Decade. Int. J. Mol. Sci. 18(4), E897. PubMed, 2017 available at: https://www.ncbi.nlm.nih.gov/pubmed/28441777

“The aim of this study is to investigate the biopotency of methanolic extracts of Vitex mollis, Psidium guajava, Dalbergia retusa, and Crescential alata leaves against various staphylococcal strains isolated from cattle and rabbits. Methicillin-resistant S. aureus strains were isolated from cattle, while other strains were isolated from rabbits using standard methodology. The total phytochemical phenolic and saponins contents were obtained being the main groups of the antinutritional factors. The antimicrobial activity of the extracts against the standard culture of S. aureus (control) and S. aureus isolated from cattle and rabbits were investigated comparatively relative to that of oxacillin. It was found that both the control S. aureus and the isolated S. aureus are susceptible to all the four plant extracts, and sensitive to oxacillin. Of all the S. aureus including the control, MRSA2 is the most susceptible to all the extracts at 1000 µg/mL, except that of V. mollis where it is the least susceptible. Among all the plant extracts, P. guajava is the most active against MRSA2 and SOSA2. Therefore, the isolates from cattle (MRSA1 and MRSA2) are more susceptible to all the plant extracts than the isolates from rabbits. Among all the rabbit isolates, CoNS3 is the least susceptible to the extracts. Since all the plant extracts exhibit remarkable inhibitory activities against all the S. aureus strains, they are promising towards the production of therapeutic drugs.” As taken from Medina MFE et al. 2017. Microb. Pathog. 113, 286-294. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29101063

“BACKGROUND: Pomegranate, Grape seed and Guava extracts have much been reviewed in Ayurveda and has been proven to have antibacterial action Aim: The objective of the study is to investigate and compare the mouthwash prepared from pomegranate, grape seed and guava extracts on salivary streptococci levels at the end of 48 hr and 7 days, of twice a day usage. STUDY DESIGN: 40 school going children aged 8-10 yrs,

randomly allocated into 4 groups (n=10 for experimental group) were asked to rinse with a) Mouthwash prepared from Pomegranate extract, 15 ml twice a day b) Mouthwash prepared from Grape seed extract, 15 ml twice a day, c) Mouthwash prepared from guava extract, 15 ml twice a day, d) Control- Distil water, twice a day. The oral streptococci colony forming units/ml (CFU/ml) was assessed by inoculating the salivary samples on blood agar media at the end of 48 hrs, and 7 days. RESULTS AND CONCLUSION: the aqueous extracts of the chosen herbal plants showed an acceptable antibacterial efficacy against oral streptococci."As taken from Singla S et al. 2018. J. Clin. Pediatr. Dent. 42(2), 109-113. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/29087796

"BACKGROUND: This study evaluated the antibacterial activity of some plants used in folklore medicine to treat diarrhoea in the Eastern Cape Province, South Africa. METHODS: The acetone extracts of Acacia mearnsii De Wild., Aloe arborescens Mill., A. striata Haw., Cyathula uncinulata (Schrad.) Schinz, Eucomis autumnalis (Mill.) Chitt., E. comosa (Houtt.) Wehrh., Hermbstaedtia odorata (Burch. ex Moq.) T.Cooke, Hydnora africana Thunb, Hypoxis latifolia Wight, Pelargonium sidoides DC, Psidium guajava L and Schizocarphus nervosus (Burch.) van der Merwe were screened against Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, multi-resistant Salmonella enterica serovar Isangi, S. typhi, S. enterica serovar Typhimurium, Shigella flexneri type 1b and Sh. sonnei phase II. A qualitative phytochemical screening of the plants extracts was by thin layer chromatography. Plants extracts were screened for antibacterial activity using serial dilution microplate technique and bioautography. RESULTS: The TLC fingerprint indicated the presence of terpenoids and flavonoids in the herbs. Most of the tested organisms were sensitive to the crude acetone extracts with minimum inhibitory concentration (MIC) values ranging from 0.018-2.5 mg/mℓ. Extracts of A. striata, C. uncinulata, E. autumnalis and P. guajava were more active against enteropathogens. S. aureus and Sh. flexneri were the most sensitive isolates to the crude extracts but of significance is the antibacterial activity of A. arborescens and P. guajava against a confirmed extended spectrum betalactamase positive S. enterica serovar Typhimurium. CONCLUSION: The presence of bioactive compounds and the antibacterial activity of some of the selected herbs against multidrug resistant enteric agents corroborate assertions by traditional healers on their efficacies." As taken from Bisi-Johnson MA et al. 2017. BMC Complement. Altern. Med. 17(1), 321. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/28629407

Record for Psidium guajava L., leaf, decoction

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor Concentration 50	In vitro	Human - lung fibroblast	32.94 mg/L	In Vitro Toxicity Studies - cell viability (cell death), unspecified assay	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 141,301,2012

As taken from RTECS, 2019a

"OBJECTIVE: This study examined the antimicrobial activity of Cannabis sativa, Thuja orientalis and Psidium guajava against methicillin-resistant Staphylococcus aureus (MRSA) and used a standardized purification protocol to determine the presence and abundance of

bioactive compounds in the leaf extracts. METHODS: In vitro antimicrobial activities of the ethanolic extracts of C. sativa, T. orientalis and P. guajava were tested against MRSA. The presence of bioactive molecules in these three leaves was evaluated using biochemical assays and high-performance thin-layer chromatography (HPTLC). RESULTS: Resistance to methicillin, penicillin, oxacillin and cefoxitin was observed in each of the clinical and nonclinical MRSA isolates. However, they were still vulnerable to vancomycin. Used individually, the 50% extract of each plant leaf inhibited MRSA growth. A profound synergism was observed when C. sativa was used in combination with T. orientalis (1:1) and when P. guajava was used in combination with T. orientalis (1:1). This was shown by larger zones of inhibition. This synergism was probably due to the combined inhibitory effect of phenolics present in the leaf extracts (i.e., quercetin and gallic acid) and catechin, as detected by HPTLC. CONCLUSION: The leaf extracts of C. sativa, T. orientalis and P. guajava had potential for the control of both hospital- and community-acquired MRSA. Moreover, the inhibitory effect was enhanced when extracts were used in combination." As taken from Chakraborty S et al. 2018.J. Integr. Med. 16(5), 350-357. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30120078

"AIM: The present study was undertaken to assess the inhibitory effect of guava extracts on Porphyromonas gingivalis and Aggregatibacteractinomycetemcomitans, to assess the time-kill curve of P. gingivalis and A. actinomycetemcomitans, and to determine the antiproteolytic activity of guava on P. gingivalis. MATERIALS AND METHODS: Kanamycin blood agar was used to isolate P. gingivalis and A. actinomycetemcomitans. Ethanolic guava extract (EGE) and aqueous guava extract (AGE) were prepared and the inhibitory effects of these extracts for two periodontal pathogens were tested by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) procedures. Antibacterial activity of guava extracts was determined by well diffusion method. Antiproteolytic activity of guava on protease of P. gingivalis was determined by gelatin liquefaction test. RESULTS: The MIC determined for AGE and EGE was at 75 µL/mL concentration for P. gingivalis, whereas EGE exhibited the activity at 75 µL/mL on P. gingivalis. The MIC determined for AGE was at 50 µL/mL for A. actinomycetemcomitans, whereas MIC determined for EGE was at 3.12 µL/mL for A. actinomycetemcomitans. Porphyromonas gingivalis was susceptible to EGE compared with AGE. Aggregatibacter actinomycetemcomitans was more susceptible to guava extracts compared with P. gingivalis. CONCLUSION: Guava extract may be a potential therapeutic agent for periodontitis as it shows significant activity against both P. gingivalis and A. actinomycetemcomitans.CLINICAL SIGNIFICANCE:Guava leaves extract can be used as economical and suitable adjuvant to synthetic drugs and can be a potential therapeutic agent for periodontitis." As taken from Shetty YS et al. 2018.J. Contemp. Dent. Pract. 19(6), 690-697. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29959298

"Introduction: The spread of drug-resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Extracts of plants such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots have evoked interest as sources of natural products. Irrigation with a broad-spectrum antiseptic substance and inter-appointment intracanal medication has become a standard regimen in root canal therapy. Aim: The aim of this study is to compare the antimicrobial efficacy of different natural extracts such as guava leaf extract, *Aloe vera* extract, papaya leaf extract, and cashew apple extract against *Enterococcus faecalis* and *Candida albicans*. Materials and Methods: The antimicrobial activity was determined using agar diffusion test. The solutions were divided into four groups: Group I - guava leaf extract, Group II - *A. vera* extract, and Group

III - papaya leaf extract, and Group IV - cashew apple extract. The zones of inhibition of growth were recorded. The strains used for this study were *E. faecalis* ATCC 29212 and *C. albicans* ATCC 90028. Results and Conclusion: Sodium hypochlorite had demonstrated the best results among the tested solutions. Among the herbal extracts, cashew apple extract and guava leaf extract had shown statistically significant activity against *E. faecalis* and *C. albicans*." As taken from Noushad MC et al. 2018. Contemp. Clin. Dent. 9(2), 177-181. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29875557

"We screened a total of 672 plant-tissue extracts to search for phytochemicals that inhibit the function of the type III secretion system (T3SS) of enteropathogenic Escherichia coli (EPEC) and enterohemorrhagic E. coli (EHEC). Among candidates examined, we found that an extract from the leaves of Psidium guajava (guava) inhibited the secretion of the EspB protein from EPEC and EHEC without affecting bacterial growth. The guava extract (GE) also inhibited EPEC and EHEC from adhering to and injecting EspB protein into HEp-2 cells. GE seemed to block the translocation of EspB from the bacterial cells to the culture medium. In addition to EPEC and EHEC, GE also inhibited the T3SS of Yersinia pseudotuberculosis and Salmonella enterica serovar Typhimurium. After exposure to GE, Y. pseudotuberculosis stopped the secretion of Yop proteins and lost its ability to induce the apoptosis of mouse bone marrow-derived macrophages. S. Typhimurium exposed to GE ceased the secretion of Sip proteins and lost its ability to invade HEp-2 cells. GE inhibited EspC secretion, the type V secretion protein of EPEC, but not Shiga toxin2 from EHEC. Thus, our results suggest that guava leaves contain a novel type of antimicrobial compound that could be used for the therapeutic treatment and prevention of gram-negative enteropathogenic bacterial infections." As taken from Nakasone N et al. 2018. Microbiol. Immunol. Epub ahead of print. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29790584

"The study aimed to evaluate the antimicrobial activity of medicinal plant extracts against the bacterial pathogens prominent in dental caries. A total of 20 plant species (herbs, shrubs and trees) belonging to 18 genera and 15 families were documented for dental caries. Antimicrobial activity of solvent extracts and essential oil from plants were determined by zone of inhibition on the growth of *Streptococcus mutans* (MTCC 497) and *Lactobacillus acidophilus* (MTCC 10307) using the agar well diffusion method. The results of in vitro antimicrobial assay prove that methanol is more successful in the extraction of phytochemicals from plant samples than aqueous solvent, as methanol extracts show higher antimicrobial activity than aqueous extracts against both the test pathogens. Methanol extracts of *Nigella sativa, Psidium guajava* and *Syzygium aromaticum* were the most effective among all 20 plant samples and have potent inhibitory activity against both dental caries pathogens with minimum inhibitory concentration of 0.2 mg mL^{-1}. *N. sativa* seed methanol extract was more effective with 22.3 mm zone of inhibition at 0.2 mg mL^{-1} against *S. mutans* (MTCC 497), while *L. acidophilus* (MTCC 10307) was more sensitive to *S. aromaticum* bud methanol extract at 11.3 mm zone of inhibition at concentration 0.1 mg mL^{-1}. Essential oil extracted from plants also possesses strong antimicrobial activity for both test pathogens, with a minimum inhibitory concentration range of 0.05-0.16 mg mL^{-1}. *Syzygium aromaticum* bud essential oil at 0.05 mg mL^{-1} was most active against *S. mutans* (MTCC 497). Plant extracts viewing antimicrobial activity with minimum inhibitory concentration show the efficacy of the plant products that could be considered as a good indicator of prospective plants for discovering new antimicrobial agents against dental caries pathogens. The findings of this study provide a lead to further polyherbal formulations for the treatment of dental caries malaise." As taken from Besra M and Kumar V 2018. 3

Biotech. 8(5), 257. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29765815

“This study investigated a lycopene-rich extract from red guava (LEG) for its chemical composition using spectrophotometry, mass spectrometry, attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR), and computational studies. The cytotoxic activity of LEG and the underlying mechanism was studied in human breast adenocarcinoma cells (MCF-7), murine fibroblast cells (NIH-3T3), BALB/c murine peritoneal macrophages, and sheep blood erythrocytes by evaluating the cell viability with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method and flow cytometry. Spectrophotometry analysis showed that LEG contained 20% of lycopene per extract dry weight. Experimental and theoretical ATR-FTIR suggests the presence of lycopene, whereas MS/MS spectra obtained after fragmentation of the molecular ion $[M]^{+\cdot}$ of 536.4364 show fragment ions at m/z 269.2259, 375.3034, 444.3788, and 467.3658, corroborating the presence of lycopene mostly related to all-trans configuration. Treatment with LEG (1600 to 6.25μg/mL) for 24 and 72h significantly affected the viability of MCF-7 cells (mean half maximal inhibitory concentration [IC_{50}]=29.85 and 5.964μg/mL, respectively) but not NIH-3T3 cells (IC_{50}=1579 and 911.5μg/mL, respectively). Furthermore LEG at concentrations from 800 to 6.25μg/mL presented low cytotoxicity against BALB/c peritoneal macrophages (IC_{50}≥800μg/mL) and no hemolytic activity. LEG (400 and 800μg/mL) caused reduction in the cell proliferation and induced cell cycle arrest, DNA fragmentation, modifications in the mitochondrial membrane potential, and morphologic changes related to granularity and size in MCF-7 cells; however, it failed to cause any significant damage to the cell membrane or display necrosis or traditional apoptosis. In conclusion, LEG was able to induce cytostatic and cytotoxic effects on breast cancer cells probably via induction of an apoptotic-like pathway.” As taken from Dos Santos RC et al. 2018. Food Res. Int. 105, 184-196. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29433206

“This study was performed to evaluate the in vivo anticancer activity against ehrlich ascites carcinoma (EAC) cells and in vitro antimicrobial activity of Psidium guajava bark extracts. By soxhlet apparatus, the P. guajava bark extracts were obtained using four solvents (n-hexane, petroleum benzene, chloroform, and methanol) according to their increasing solubility. In case of in vivo anticancer activity of the sample extracts, mice were seeded with approximately 1x105 ehrlich ascites carcinoma (EAC) cells. After seven days of consecutive treatment, the negative and positive control groups (n=8 each group) showed an average EAC cell count of 2.4x108 and 1.8x108 respectively, and the experimental groups showed the cell count of 2.2x 108, 2.1x108, 1.9x108, and 1.41x108 when mice received h-hexane, petroleum benzene, chloroform, and methanol extract respectively. Experimental group that received methanol extract showed percent increase of life span (% ILS) of 33.3 when compared with the negative control. However, treatment in a cyclic manner of the mice showed % ILS of 52.15 for experimental group when compared negative control. In antimicrobial activity experiment, an intermediate zone of sensitivity of the crude methanol extract was found against Escherichia coli, Shigella flexneri, and Staphylococcus aureus when compared with amoxicillin. All these results indicated the anticancer activity and antimicrobial activity of the methanol extract of P. guajava barks on different experimental models.” As taken from Hossain MJ et al. 2018. Bangladesh Journal of Microbiology 35(1), 79-81. Available at https://www.banglajol.info/index.php/BJM/article/view/39808

“Background: The control of biofilm adherence on tooth surface has always been the keystone of periodontal therapeutic systems. However, prevalence of gingivitis suggest inadequacy of self-performed oral hygiene measures and need for adjunctive aid for mechanical plaque control. Oral rinses containing chlorhexidine, has been widely used however, with certain limitations. Herbal products have been used widely reflecting its action as alternative and complementary remedy. Hence, the purpose of the present study was to evaluate the antimicrobial and antioxidant efficacy of a Guava leaf extract based mouthrinse in patients with chronic generalized gingivitis as an adjunct to oral prophylaxis. **Methods:** Sixty subjects (n = 20) in compliance with the inclusion criteria were randomly assigned to one of the 3 study groups i.e. Group A- 0.15% Guava mouth rinse, Group B- 0.2% Chlorhexidine (CHX) mouth rinse, Group C- Distilled water (placebo). All the participants received professional oral prophylaxis and were dispensed with experimental mouth rinses and instructed to use for period of 30 days. Clinical parameters such as gingival index, plaque index along with microbial colony forming units using plaque samples and antioxidant levels in saliva were estimated at baseline, 30 and 90 days' time intervals.**Results:** All 3 groups showed gradual reduction in GI, PI and microbial counts. Considering the mean scores of recorded parameters at the scheduled time intervals, notable changes were observed between chlorhexidine and guava mouth rinse compared to placebo group. Although there was improvement in the antioxidant status in all study participants, yet there was no statistically significant difference observed.**Conclusion:** Guava mouth rinse can be used as an empirical adjunct to professional oral prophylaxis owing to its multifactorial properties and favourable acceptance. However, long term studies need to be conducted to validate its use for an extended period of time.” As taken from Nayak N et al. 2019. BMC Complement. Altern. Med. 19(1), 327. PubMed, 2020 available athttps://pubmed.ncbi.nlm.nih.gov/31752836/

“Objective: To investigate the Phytochemical screening, antioxidant and antimicrobial activities of the essential oil and ethanol extract of *Psidium guajava*.Methods: The leaf of *Psidium guajava*belongs to the myrtle family (Myrtacease) which is used as herbal remedies for the cure of many ailments by natives in northern part of Nigeria, was collected in June, 2018 from the Professor’s Quarters of Modibbo Adama University of Technology (MAUTECH) Yola. The leaf was air dried, pulverized and extracted by simple overnight maceration technique and then analyzed. Fresh leaf of the aforementioned was extracted using modified steam distillation. The phytochemical screening of the ethanol extract was carried out using standard method.Results: The result revealed the present of Tannin, Flavanoid, Alkaloid, Volatile oil, Triterpene, Saponin, Glycoside while phenolic compound was absent in the ethanol extract of *Psidium guajava*. The result of the antioxidant activity of the essential oil was screened using DPPH method and the IC_{25} values of ascorbic acid (standard drug) was 57.92 µl/m and *Psidium guajava* of the essential oil was 46.55 µl/ml respectively. Antibacterial activity was carried out using dics diffusion method and the results showed reasonable zone of inhibition against tasted organisms, with *Staphylococcus epidemidis* being the most inhibited (23 mm) and *Proteus vulgari* being the least inhibited (2 mm) with the ethanol extract of *Psidium guajava*. In contrast, *Staphylococcus aureus* was the most inhibited (13 mm) and *Salmonella typhi* showed the least inhibition (9mm) in the essential oil of *Psidium guajava*.Conclusion: The result, thus support the use of the plants traditionally to treat chronic diarrhea, fever, diabetes, malaria and suggest its usage in the formulation of new antioxidant and antibacterial drugs.” As taken from Emmanuel A et al. 2019. Asian Journal of Physical and Chemical Sciences 7(4), 1-8. Available athttp://www.journalajopacs.com/index.php/AJOPACS/article/view/30102

“Escherichia coli is a Gram-negative bacteria that has a natural habitat in the human digestive tract. This bacteria is a normal flora but have a potency to be pathogenic. Escherichia coli is the most common cause of diarrhea. Guava leaves and bay leaves contain essential oils, tannins, flavonoids, and saponins which have antimicrobial effects. The purpose of this study was to determine the difference in the effectiveness of inhibition between ethanol extract of guava leaves and bay leaves on the growth of Escherichia coli bacteria. This research was an experimental laboratory study. The inhibitory test was carried out by measuring the clear zone around the paper disc using calipers. The results of data analysis using one way ANOVA showed that there were significant differences between groups of extracts with F output = 49.83 and p = 0.00 (p≤ 0,05). Guava leaf extract concentration of 10%, 30% was significantly different from bay leaf extract concentration of 10%, 20%, 30%, with a p-value ≤ 0.05. The conclusion of this study is Ethanol extract of guava leaves has a higher inhibitory ability than the ethanol extract of bay leaves.” As taken from Witari NPD et at. 2019. Journal of Physics: Conference Series 1402, 055085. Available at https://iopscience.iop.org/article/10.1088/1742-6596/1402/5/055085/pdf

“Psidium guajava is a small tree native to South and Central America. Guava leaves have traditionally been used for treating different illnesses. These benefits can be attributed to phenolics and flavonoids produced by guava. The chemical composition of guava leaf extracts was correlated with biological activity. Total phenolics, total flavonoids, ABTS/DPPH, TZMbl, plaque reduction, XTT, spectrophotometric and Kirby-Bauer assays were used to test phenols, flavonoids, antioxidant properties, antiviral activity, cytotoxicity, and antibacterial activity, respectively. The median cytotoxicity concentration and half-maximal effective concentration values were obtained in order to determine antiviral selectivity against human immunodeficiency virus type 1 and herpes simplex virus type 1. Antibacterial activity against Escherichia coli and Bacillus subtilis were evaluated using a spectrophotometric assay and Kirby-Bauer test. The guava leaf extracts had a high phenol (0.8 to 2.1 GAE mg/mL) and flavonoid (62.7 to 182.1 Rutin Eq mg/g DW) content that correlated with high antioxidant capacity and selective antiviral activity (therapeutic index values above 10). Results of antibacterial tests indicated that the extracts have activity against gram-negative and gram-positive bacteria.” As taken from Melo C et al. 2020. Journal of Medicinally Active Plants 9(1), 1-13. Available at https://scholarworks.umass.edu/cgi/viewcontent.cgi?article=1137&context=jmap

5.6. Carcinogenicity

Subcutaneous, rat, TDLo: 10 gm/kg/72W (intermittent) (equivocal tumorigenic agent by RTECS criteria; tumors at site of application) (Psidium guajava Linn., extract).

As taken from RTECS, 2012.

“Anticancer drug research based on natural compounds enabled the discovery of many drugs currently used in cancer therapy. Here, we report the in vitro, in vivo and in silico anticancer and estrogen-like activity of Psidium guajava L. (guava) extracts and enriched mixture containing the meroterpenes guajadial, psidial A and psiguadial A and B. All

samples were evaluated in vitro for anticancer activity against nine human cancer lines: K562 (leukemia), MCF7 (breast), NCI/ADR-RES (resistant ovarian cancer), NCI-H460 (lung), UACC-62 (melanoma), PC-3 (prostate), HT-29 (colon), OVCAR-3 (ovarian) and 786-0 (kidney). Psidium guajava's active compounds displayed similar physicochemical properties to estradiol and tamoxifen, as in silico molecular docking studies demonstrated that they fit into the estrogen receptors (ERs). The meroterpene-enriched fraction was also evaluated in vivo in a Solid Ehrlich murine breast adenocarcinoma model, and showed to be highly effective in inhibiting tumor growth, also demonstrating uterus increase in comparison to negative controls. The ability of guajadial, psidial A and psiguadials A and B to reduce tumor growth and stimulate uterus proliferation, as well as their in silico docking similarity to tamoxifen, suggest that these compounds may act as Selective Estrogen Receptors Modulators (SERMs), therefore holding significant potential for anticancer therapy." As taken from Rizzo LY et al. 2014. Curr. Med. Chem. 21(20), 2322-30. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24438525

"Context Psidium guajava L. (Myrtaceae) leaves are used in traditional medicines for the treatment of cancer, inflammation and other ailments. Objective The current study explores scientific validation for this traditional medication. Materials and methods We used ferric-reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picryl hydrazil (DPPH) assays to estimate antioxidant activity of P. guajava leaf extracts (methanol, hexane and chloroform). Antitumour and in vivo cytotoxic activities were determined using potato disc assay (PDA) and brine shrimp lethality assay, respectively. Three human carcinoma cell lines (KBM5, SCC4 and U266) were incubated with different doses (10-100 µg/mL) of extracts and the anticancer activity was estimated by MTT assay. NF-κB suppressing activity was determined using electrophoretic mobility shift assay (EMSA). Chemical composition of the three extracts was identified by GC-MS. Total phenolic and flavonoid contents were measured by colorimetric assays. Results and discussions The order of antioxidant activity of three extracts was methanol > chloroform > hexane. The IC50 values ranged from 22.73 to 51.65 µg/mL for KBM5; 22.82 to 70.25 µg/mL for SCC4 and 20.97 to 89.55 µg/mL for U266 cells. The hexane extract exhibited potent antitumour (IC50 value = 65.02 µg/mL) and cytotoxic (LC50 value = 32.18 µg/mL) activities. This extract also completely inhibited the TNF-α induced NF-κB activation in KBM5 cells. GC-MS results showed that pyrogallol, palmitic acid and vitamin E were the major components of methanol, chloroform and hexane extracts. We observed significant ($p < 0.05$) difference in total phenolic and flavonoid contents of different solvent extracts. Conclusion The present study demonstrates that P. guajava leaf extracts play a substantial role against cancer and down-modulate inflammatory nuclear factor kB." As taken from Ashraf A et al. 2016. Pharm. Biol. 54(10), 1971-81. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26841303

Only one study is available on the anti-tumor effect that could be related to the phenolic composition of guava leaves. An ethanol extract of the leaves was administrated to B6 mice after inoculation of melanoma cells. The results suggested that the extract had a vaccine effect, but not a therapeutic effect, against tumors through by depressing T regulatory cells [104].

Moreover, the meroterpene-enriched fraction of guava leaves, containing guajadial, psidial A, and psiguadial A and B, was evaluated in vivo in a solid Ehrlich murine breast-adenocarcinoma model. The results suggested that these compounds may act as

phytoestrogens, presenting tissue-specific antagonistic and agonistic activity on estrogen receptors [43]. These data partially confirmed the results in vitro obtained by Ryu et al. [47].

In vitro studies against neoplasm

Origin	Extraction Method	Major Constituent	Cell Line	Assay	Main Results	Ref.
Japan	Maceration in EtOH:H_2O 50% (*v*/*v*)	TPC: 71 g/100 g	Human colon adenocarcinoma (COLO320DMA)	Cyclooxygenase and cell proliferation assays	At 1 mg/mL: ↓ human cyclooxygenase activity (IC_{50} 55 and 560 µg/mL PGHS-1 and 2, respectively), ↓ IC_{50} 5.1 µg/mL (PGSH) and 4.5 µg/mL (cyclooxygenase).	[37]
					At 100 µg/mL: Quercetin ↓ IC_{50} = 5.3 (PGSH-1) and 250 µg/mL (PGSH-2), ↓ DNA synthesis rate.	
Malaysia	Soxhlet with-ether, MeOH, and H_2O	-	Cervical cancer (HeLa), breast cancer (MDA-MB-231) and osteosarcoma (MG-63). Control: non-malignant Madin-Darby canine kidney (MDCK)	Methylene blue assay	At 10 mg/mL: HeLa: No anti-proliferative activity.	[38]
					MDA-MB-231: IC_{50} ether extract (4.2 µg/mL) > MeOH (18.6 µg/mL) > H_2O (55.7 µg/mL).	
					MG-63: same order (IC_{50} of 5.42, 23.25, and 61.88 µg/mL, respectively).	
					MDCK: cytotoxic effect of ether and MeOH extract (IC_{50} = 5.03 and 11.55 µg/mL, respectively).	
Brazil	Maceration in EtOH	TPC: 766.08 mg/g, TFC: 118.90 mg/g	HeLa, colorectal carcinoma (RKO-AS45-1), and lung fibroblast (Wi-26VA4)	MTT assay	At 1 mg/mL: IC_{50} = 15.6 µg/mL (HeLa), 21.2 (RKO) µg/mL, and 68.9 µg/mL (Wi-26VA4).	[39]
Palestine	Maceration in-DCM:MeOH 50% (*v*/*v*) (24 h)	-	Murine fibrosarcoma (L929sA), and human breast cancer (MDA-	MTT assay	IC_{50} = 55 µg/mL (L929sA), 820 µg/mL (MCF7 cells), no cytotoxic effect on MDA-MB-231 cells.	[40]

			MB-231 and MCF-7)			
Taiwan	Decoction (30 min)	-	Human prostate carcinoma (DU-145)	MTT, ELISA, gelatinolytic zymography, wound scratch, and chicken chorioallantoic membrane assays	At 0.25 mg/mL: cell suppression (IC_{50} 0.57 mg/mL). ↓ Expressions of VEGF (76.9%), IL-6 (98.8%) and IL-8 (98%), and MMP-2 (100%) and MMP-9 (100%). Suppressed the cell migration (30.9%) and the angiogenesis.	[41]
Taiwan	Decoction (1 h)	TPC: 470.0 mg/g Individual compounds: gallic acid (348), catechin (102), epicatechin (60), rutin (100), quercetin (102), and rutin (100) in mg/g	Human prostate epithelial (PZ-HPV-7) and DU-145	MTT assay	At 1 mg/mL: 100% suppression DU-145 cells. PZ-HPV-7 cells followed an auto-decaying process. Cell-killing rate coefficient (kapp) = 0.03 × 10^3 phenolic compounds cells/mg h.	[42]
Brazil	Soxhlet with DCM. Maceration with EtOH	Guajadial, psidial A, and psiguajadial A and B	Leukemia (K-562), MCF-7, ovarian cancer (NCI/ADR-RES), lung (NCI-H460), melanoma (UACC-62), prostate (PC-3), colon (HT-29), ovarian (OVCAR-3), and kidney (786-0)	Protocol established by NCI (ELISA test)	At 250 µg/mL: Anti-proliferative activity DCM > EtOH, inhibition growths: 26 (OVCAR-3)-65 (UACC-62) µg/mL due to the major compounds.	[43]
Japan	Maceration with sonication in MeOH:H_2O 80% (*v*/*v*) (3 h) and isolation	CPO	PC-3 and MCF-7	MTT, annexin V antibody, TUNEL, and western blot assays	At 50 µg/mL: ↓ cell proliferation, ← early and late apoptotic effect, down-regulation of PI3K/AKT/mTOR/S6K1 pathway, up-regulation of MAPKs, JNK, ERKs, and p38 MAPK.	[44]
Thailand	Hydrodistillation		Human mouth epidermal carcinoma (KB) and murine	MTT assay	At 0.15 mg/mL: KB: 75% cytotoxic effect, IC_{50} = 0.04 mg/mL; At 0.08 mg/mL: P388:	[45]

			leukemia (P388)		80% cytotoxic effect, IC_{50} = 0.05 mg/mL.	
Jamaica	Maceration in-hexane (4 days)		Leukemia (Kasumi-1)	MTT assay	IC_{50} = 200 µg/mL.	[46]
Japan	Maceration with sonication in MeOH:H_2O 80% (*v*/*v*) (3 h). Fractionation with hexane	60 compounds (in hexane fraction): β-eudesmol (11.98%), α-copaene (7.97%), phytol (7.95%), α-patchoulene (3.76%), and CPO (3.63%)	Human prostate cancer (PC-3 and LNCaP)	MTT, annexin V antibody, TUNEL, and western blot assays	At 150 µg/mL: PC-3: ← apoptotic effect of the hexane fraction (15%), ↓ effect on early apoptotic cells, ← effect for late apoptosis, via the suppression of PI3K/AKT/mTOR/S6K1 and MAPK signalling cascades in both cell lines.	[47]

Díaz-de-Cerio E et al. (2017b). Health Effects of Psidium guajava L. Leaves: An Overview of the Last Decade. Int. J. Mol. Sci. 18(4), E897. PubMed, 2017 available at: https://www.ncbi.nlm.nih.gov/pubmed/28441777

5.7. Irritation/immunotoxicity

“Atopic dermatitis (AD) is a chronic, relapsing, and inflammatory skin disease associated with eczematous symptoms and IgE hyperproduction. Psidium guajava is an important food crop and medicinal plant with anti-oxidant, anti-inflammatory, and anti-allergic activities, supporting its traditional uses. Our previous studies have shown that P. guajava extract inhibits Th2 chemokine expression by suppressing the activation of NF-κB and STAT1 co-stimulated with TNF-α and INF-γ. In this study, we investigated the inhibitory effect of P. guajava water extract (PGW) on 2,4-dinitrochlorobenzene (DNCB)-induced AD-like skin lesions in NC/Nga mice. Treatment of cream containing PGW onto DNCB-induced AD-like skin lesions in NC/Nga mice ameliorated lesion intensity scores, levels of IgE, thymus and activation-regulated chemokine (TARC), TNF-α, and IL-4 in serum and ears. In contrast, PGW increased level of the immunosuppressive cytokine IL-10. Histological analyses demonstrated decreased thickening of the epidermis/dermis as well as dermal infiltration by inflammatory cells. These results suggest that cream containing PGW may be a potential therapeutic modality for AD and adjunctive agent to control pruritus in AD”. As taken from Choi JH et al. 2012. Fd Chem. Toxicol. 50, 2923-2929. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22609491

"Context Psidium guajava L. (Myrtaceae) leaves are used in traditional medicines for the treatment of cancer, inflammation and other ailments. Objective The current study explores

scientific validation for this traditional medication. Materials and methods We used ferric-reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picryl hydrazil (DPPH) assays to estimate antioxidant activity of P. guajava leaf extracts (methanol, hexane and chloroform). Antitumour and in vivo cytotoxic activities were determined using potato disc assay (PDA) and brine shrimp lethality assay, respectively. Three human carcinoma cell lines (KBM5, SCC4 and U266) were incubated with different doses (10-100 µg/mL) of extracts and the anticancer activity was estimated by MTT assay. NF-κB suppressing activity was determined using electrophoretic mobility shift assay (EMSA). Chemical composition of the three extracts was identified by GC-MS. Total phenolic and flavonoid contents were measured by colorimetric assays. Results and discussions The order of antioxidant activity of three extracts was methanol > chloroform > hexane. The IC50 values ranged from 22.73 to 51.65 µg/mL for KBM5; 22.82 to 70.25 µg/mL for SCC4 and 20.97 to 89.55 µg/mL for U266 cells. The hexane extract exhibited potent antitumour (IC50 value = 65.02 µg/mL) and cytotoxic (LC50 value = 32.18 µg/mL) activities. This extract also completely inhibited the TNF-α induced NF-κB activation in KBM5 cells. GC-MS results showed that pyrogallol, palmitic acid and vitamin E were the major components of methanol, chloroform and hexane extracts. We observed significant ($p < 0.05$) difference in total phenolic and flavonoid contents of different solvent extracts. Conclusion The present study demonstrates that P. guajava leaf extracts play a substantial role against cancer and down-modulate inflammatory nuclear factor kB." As taken from Ashraf A et al. 2016. Pharm. Biol. 54(10), 1971-81. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26841303

Guava leaves have been suggested as a therapeutic agent to control pruritus in atopic dermatitis. The improvement of the skin lesions was due to a reduction in serum immunoglobulin E level and in the eczematous symptoms [136]. Moreover, the epithelium was repaired with connective tissue and absence or moderate presence of inflammatory cells by the leaves. As a result, the leaves exhibited wound healing properties [137]. Furthermore, guava leaf extract was tested on rat skin, and exhibited inhibitory activity towards an active cutaneous anaphylaxis reaction [138]. Díaz-de-Cerio E et al. (2017b). Health Effects of Psidium guajava L. Leaves: An Overview of the Last Decade. Int. J. Mol. Sci. 18(4), E897. PubMed, 2017 available at: https://www.ncbi.nlm.nih.gov/pubmed/28441777

Record for Psidium guajava L., leaf, decoction

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	50 mg/kg	Biochemical - Metabolism (Intermediary) - effect on inflammation or mediation of inflammation	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 156,88,2014

As taken from RTECS, 2019a

Record for Psidium guajava L., leaf, 70% acetone extract

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	50 mg/kg	Behavioral - analgesia Biochemical - Metabolism (Intermediary) - effect on inflammation or mediation of inflammation	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 156,88,2014

As taken from RTECS, 2017

5.8. All other relevant types of toxicity

Antioxidant activity and free radical-scavenging capacity of extracts from guava (Psidium guajava L.) leaves (Abstract). The objectives of this study were to study the antioxidant activity and free radical-scavenging effects of extracts from guava leaves and dried fruit. The results indicated that 94.4–96.2% of linoleic acid oxidation was inhibited by the addition of guava leaf and guava tea extracts at a concentration of 100 µg/ml. The guava dried fruit extracts exhibited weaker antioxidant effects than did the leaf extracts. The results also demonstrated that the scavenging effects of guava leaf extracts on ABTS+ radicals and superoxide anion increased with increasing concentrations. The guava leaf extracts displayed a significant scavenging ability on the peroxyl radicals. However, the scavenging effects were decreased when the extract concentration was greater than 10 µg/ml. The extracts from leaves of various guava cultivars exhibited more scavenging effects on free radicals than did commercial guava tea extracts and dried fruit extracts. The chromatogram data indicated that guava extracts contained phenolic acids, such as ferulic acid, which appeared to be responsible for their antioxidant activity. Correlation analysis indicated that there was a linear relationship between antioxidant potency, free radical-scavenging ability and the content of phenolic compounds of guava leaf extracts. As taken from Chen & Yen, Food Chemistry, Volume 101, Issue 2, 2007, Pages 686-694. Science Direct, 2010 available at http://www.sciencedirect.com/

“Based on the traditional use in popular medicine, the effect of extracts from Psidium guajava L. leaves and of the main flavonol-glycoside components on dipeptidyl-peptidase IV (DP-IV), a key enzyme of blood glucose homoeostasis, has been investigated in-vitro. An ethanolic extract was prepared from dried, powdered leaves of guava and was found to contain seven main flavonol-glycosides, which were isolated by semipreparative HPLC and tested individually. The ethanolic guava leave extract was shown to exert a dose-dependent inhibition of DP-IV, with an IC50 of 380 µg/ml test assay solution. Also the individual flavonol-glycosides inhibited DP-IV dose-dependently, with variations of the effects by a factor of 10, and an overall effect accounting for 100% of that observed for the total guava extract. The recovery of individual flavonol-glycosides in CaCo-2 epithelial cells, a model of

gastrointestinal tract absorption, amounted to 2.3-5.3% of the amount available for absorption over 60 min at 37°C." As taken from Eidenberger T et al. 2013. Fitoterapia 89, 74-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23707747

"Fruits, vegetables and medicinal herbs rich in phenolics antioxidants contribute toward reduced risk of age-related diseases and cancer. In this study, Psidium guajava leaf extract was fractionated in various organic solvents viz. petroleum ether, benzene, ethyl acetate, ethanl and methanol and tested for their antioxidant and antimutagenic properties. Methanolic fraction showed maximum antioxidant activity comparable to ascorbic acid and butylated hydroxyl toluene (BHT) as tested by DPPH free radical scavenging, phosphomolybdenum, FRAP (Fe3 + reducing power) and CUPRAC (cupric ions (Cu2+) reducing ability) assays. The fraction was analyzed for antimutagenic activities against sodium azide (NaN3), methylmethane sulfonate (MMS), 2-aminofluorene (2AF) and benzo(a)pyrene (BP) in Ames Salmonella tester strains. The methanol extracted fraction at 80 µg/ml concentration inhibited above 70% mutagenicity. Further, phytochemical analysis of methanol fraction that was found to be most active revealed the presence of nine major compounds by gas chromatography-mass spectrometry (GC-MS). This data suggests that guava contains high amount of phenolics responsible for broad-spectrum antimutagenic and antioxidant properties in vitro and could be potential candidates to be explored as modern phytomedicine." As taken from Zahin M et al. 2017. Drug Chem. Toxicol. 40(2), 146-153. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27268266

"BACKGROUND/AIM: Elevated uric acid level, an index of gout resulting from the over-activity of xanthine oxidase (XO), increases the risk of developing hypertension. However, research has shown that plant-derived inhibitors of XO and angiotensin 1-converting enzyme (ACE), two enzymes implicated in gout and hypertension, respectively, can prevent or ameliorate both diseases, without noticeable side effects. Hence, this study characterized the polyphenolics composition of guava leaves extract and evaluated its inhibitory effect on XO and ACE in vitro. MATERIALS AND METHODS: The polyphenolics (flavonoids and phenolic acids) were characterized using high-performance liquid chromatography (HPLC) coupled with diode array detection (DAD). The XO, ACE, and Fe(2+)-induced lipid peroxidation inhibitory activities, and free radicals (2,2-diphenylpicrylhydrazyl [DPPH]* and 2,2´-azino-bis-3-ethylbenzthiazoline-6-sulphonic [ABTS]*(+)) scavenging activities of the extract were determined using spectrophotometric methods. RESULTS: Flavonoids were present in the extract in the order of quercetin > kaempferol > catechin > quercitrin > rutin > luteolin > epicatechin; while phenolic acids were in the order of caffeic acid > chlorogenic acid > gallic acids. The extract effectively inhibited XO, ACE and Fe(2+)-induced lipid peroxidation in a dose-dependent manner; having half-maximal inhibitory concentrations (IC50) of 38.24 ± 2.32 µg/mL, 21.06 ± 2.04 µg/mL and 27.52 ± 1.72 µg/mL against XO, ACE and Fe(2+)-induced lipid peroxidation, respectively. The extract also strongly scavenged DPPH* and ABTS*(+). CONCLUSION: Guava leaves extract could serve as functional food for managing gout and hypertension and attenuating the oxidative stress associated with both diseases." As taken from Irondi EA et al. 2016. J. Intercult. Ethnopharmacol. 5(2), 122-30. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27104032

"ETHNOPHARMACOLOGICAL RELEVANCE: The use of popular plants has guided pharmaceutical research aimed at combating pathogenic microorganisms. Psidium guajava L. is a plant of great versatility and it has been used both as food and as a therapeutic agent. Root, bark, leaves, fruits, flowers and seeds are used for medicinal purposes, especially in infusions and decoctions for oral and topical use. P. guajava is utilized in

symptomatology treatment related to organ malfunction and of diseases caused by the action of pathogenic and/or opportunistic microorganisms. Many pharmacological studies have been conducted to scientifically assess its therapeutic potential. AIMS OF STUDY: The aim of the current study is to relate the popular use of this plant and its bioscientific assessment as a therapeutic agent in the treatment of diseases and symptoms caused by the action of protozoa, fungi, bacteria and viruses, and also evaluate the safety for the usage and the interaction with drugs. MATERIALS AND METHODS: A bibliographic database the ethnobiology of Psidium guajava (2005-2015) and the pharmacological infections and parasitic diseases (2010-2015). Searches were done in scientific disclosure databases such as PubMed, Web of Science, and Scopus. RESULTS: P. guajava leaf extracts were scientifically investigated for the treatment of diseases caused by protozoa (leishmaniasis, malaria, giardiasis, amoebiasis and trichomoniasis), fungi (dermatosis, systemic and mucocutaneous diseases), bacteria (respiratory, mucocutaneous and gastrointestinal infections, cholera, gastritis and stomach ulcers, oral and periodontal infections, venereal diseases and urinary infections) and viruses (herpes, influenza, rotavirus disease and AIDS). The toxicity assays indicates the safet for usage. CONCLUSIONS: Highlight and elucidate the therapeutic potential and versatility of P. guajava. They also justify using ethnobiology efficiency to guide pharmacological studies. Some limitations can be observed in this kind of study, as the lack for ethnobiological informations and the absence of some controls in the assays." As taken from Morais-Braga MF et al. 2016. J. Ethnopharmacol. 194, 1140-1152. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27845266

"Psidium guajava is a Myrtaceae plant whose medicinal properties are recognized in several locations. The use of teas and tinctures prepared from their leaves has been used to combat infections caused by fungi of the genus Candida. In this study, aqueous extracts of leaves and hydroethanolic were tested to verify the antifungal potential and its chemical composition has been investigated. The microbiological assays were performed by broth microdilution to determine the minimum inhibitory concentration (MIC) and from these the minimum fungicidal concentration was performed (MFC) by subculturing on solid media. A cell viability curve was obtained for demonstration of inhibition of fungal growth of strains of Candida albicans and Candida tropicalis. Tests to check morphological changes by the action of the extracts were performed in microcultive cameras depleted environment at concentrations of MIC/2, MIC and MIC × 2. Extracts analyzed by high performance liquid chromatography demonstrated flavonoids and phenolic acids. The extracts showed fungistatic effect and no fungicide with MIC >8192 µg/mL, MFC above 8192 µg/mL. The IC50 was calculated ranging from 1803.02 to 5623.41 µg/mL. It has been found that the extracts affect the morphological transition capability, preventing the formation of pseudohyphae and hyphae. Teas and tinctures, therefore, have the potential antifungal, by direct contact, causing inhibition of fungal multiplication and its virulence factor, the cell dimorphism, preventing tissue invasion. Further studies are needed to elucidate the biochemical pathways and genes assets involved in these processes." As taken from Morais-Braga MF et al. 2017. Saudi J. Biol. Sci. 24(2), 302-313. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28149166

"OBJECTIVE: The aim of this study was to assess the antimicrobial potency of aqueous extract of Psidium guajava leaves in two different concentrations as a toothbrush disinfectant against three oral bacterial species. MATERIALS AND METHODS: Aqueous extracts of P. guajava leaves were prepared at 20% and 30% concentrations and 0.2% chlorhexidine was used as control. The toothbrushes were equally divided into 9 groups

with 10 toothbrushes per disinfectant, which were contaminated with Streptococcus mutans, Lactobacillus acidophilus, and Enterococcus faecalis. Microbial culture was done after 5 min and 3 h of decontamination. RESULTS: Group Ia and Ib showed that the presence of E. faecalis was observed in 8 (40%) of 20 toothbrushes. Group IIa and IIb showed a significant reduction in colony forming unit/toothbrush during 3 h evaluation. Group IIIa and IIIb showed nil growth during 3 h evaluation. Nil growth was observed with the control group for all three organisms. Statistically significant values were obtained for 5 min ($P < 0.001$) and 3 h ($P < 0.001$) disinfection period against L. acidophilus at two different concentrations. CONCLUSION: Aqueous extracts of guava leaves can be used as an alternative organic product for disinfection of toothbrushes." As taken from Vignesh R et al. 2017. Eur. J. Dent. 11(1), 111-116. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28435376

"In our previous study, we have found that persimmon, guava, and sweetsop owned considerably high antioxidant activity and contained high total phenolic contents as well. In order to further supply information on the antibacterial and antioxidant activity of these three tropic fruits, they were extracted by 80% methanol. We then examined the extractions about their phenolic compounds and also studied the extractions and phenolic contents about their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against twelve targeted pathogens including 8 standard strains (Staphylococcus aureus, Bacillus cereus, Staphylococcus epidermidis, Monilia albican, Escherichia coli, Salmonella typhimurium, Shigella flexneri, and Pseudomonas aeruginosa) and 4 multidrug-resistant strains (methicillin-resistant Staphylococcus aureus, ESBLs-producing Escherichia coli, carbapenems-resistant Pseudomonas aeruginosa, and multidrug-resistant Acinetobacter baumannii), which are common and comprehensive in clinic. We also employed two ways, that is, FRAP and TEAC, to evaluate their antioxidant activities, using ultraviolet and visible spectrophotometer. Our study indicated that the three tropical fruits possessed obvious antioxidant and antibacterial activity, which supported the possibility of developing the fruits into new natural resource food and functional food as well as new natural antimicrobial agent and food preservatives. Moreover, phenolic compounds detected in the fruits could be used as a potential natural antibacterial agent and antioxidant." As taken from Fu L et al. 2016. Biomed. Res. Int. 2016, 4287461. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27648444

"BACKGROUND: The aim of this in vitro study was to assess antimicrobial efficacy of Acacia nilotica, Murraya koenigii (L.) Sprengel, Eucalyptus hybrid, Psidium guajava extracts, and their combination on Streptococcus mutans and Lactobacillus acidophilus. MATERIALS AND METHODS: The branches of four plants were collected, identified, and authenticated by a taxonomist. The plants were rinsed in water, healthy leaves were separated and shade dried over a period of 3-4 weeks. Soxhlet apparatus using ethanol was employed for extraction procedure. The combinations of plant extracts were prepared by mixing equal quantities of 10% solutions of each of these extracts. 0.2% chlorhexidine and dimethyl sulfoxide were used as positive and negative controls, respectively. The antimicrobial efficacy testing was done using agar well-diffusion method under anaerobic conditions. The mean diameter of inhibition zone was computed and compared between different categories using one-way analysis of variance and Tukey's post-hoc test. A qualitative assay was carried out to identify the various phytochemical constituents in the plants. The data was assessed by SPSS version 20. The statistical significance was fixed at 0.05. RESULTS: All the plants extracts and their combinations inhibited S. mutans and L. acidophilus. However, the quadruple combination of A. nilotica + M. koenigii (L.) Sprengel +

Eucalyptus hybrid + P. guajava produced the maximum inhibition zone (23.5 ± 2.2 mm) against S. mutans. Although, 0.2% chlorhexidine produced the highest inhibition zone against L. acidophilus (18.8 ± 1.2 mm), A. nilotica extract produced maximum inhibition among the various plant extracts and their combinations (14.1 ± 1.8 mm). CONCLUSION: All the individual plant extracts and their combinations were effective against S. mutans and L. acidophilus. These could be tried as herbal alternates to chlorhexidine. However, these in vitro results have to be further evaluated for any toxicity of the polyherbal combinations in animal models and effectiveness has to be assessed using in vivo studies on humans." As taken from Chandra Shekar BR et al. 2016. Dent. Res. J. (Isfahan). 13(2), 168-73. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27076832

"INTRODUCTION: Debridement and disinfection of the root canal system is a critical step in endodontic treatment. Most of the irrigants presently used in the endodontic treatment can have an impact on the microbes surviving in the biofilm but none of them are able to do all of the required tasks. Researches are going on its full swing in order to produce an endodontic irrigant having ideal properties. AIM: To compare the antimicrobial efficacy of different irrigants like QMiX, guava leaf extract, aloevera extract, 2.5% sodium hypochlorite and 2% chlorhexidine gluconate against Enterococcus faecalis and Candida albicans. MATERIALS AND METHODS: The antimicrobial activity was determined using agar diffusion test. The solutions were divided into five groups: Group I- QMiX, Group II- Guava leaf extract and Group III-Aloevera extract, Group IV-2.5% Sodium hypochlorite and Group V-2% Chlorhexidine. The zones of inhibition of growth were recorded. RESULTS: Statistical analysis was performed using one way ANOVA with post-hoc Tukey's HSD. Values obtained were statistically analyzed ($p<0.05$). QMiX showed maximum inhibitory effect against Enterococcus faecalis and Candida albicans followed by, 2% chlorhexidine, 2.5% sodium hypochlorite, guava leaf extract and aloevera extract. Results obtained were statistically significant. CONCLUSION: Guava leaf extract showed significant inhibitory effects against Enterococcus faecalis and Candida albicans. QMiX demonstrated the best results among the tested solutions and can be considered as a potential alternative to existing root canal irrigants." As taken from Jose J et al. 2016. J. Clin. Diagn. Res. 10(5), ZC20-3. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27437354

"The fungus Beauveria bassiana is naturally found in poultry houses and causes high rates of mortality in Alphitobius diaperinus. Laboratory and field experiments have shown the potential of this fungus as an insect control agent. However, in poultry houses, bacteria as Salmonella, can be found and have been studied alternative control methods for this pathogen. Thus, this study aimed to evaluate the effect of plant extracts and a disinfectant on the fungus Beauveria bassiana (strain Unioeste 4). Conidial viability, colony-forming unit (CFU) counts, vegetative growth, conidia production, insecticidal activity of the fungus and compatibility were used as parameters in the evaluation of the effect of these products on the fungus. Alcoholic and aqueous extracts of jabuticaba (Myrciaria cauliflora (Mart.), guava (Psidium guajava (L.)), and jambolan (Syzygium cumini (L.), at concentrations of 10% as well as the commercial disinfectant, Peroxitane® 1512 AL, were evaluated at the recommended concentrations (RC), 1:200 (RC), 0.5 RC and 2 RC. There was a negative influence of alcoholic and aqueous extracts of jabuticaba, guava and three dilutions of Peroxitane on the viability of conidia. The CFUs and vegetative growth of the fungus were affected only by the Peroxitane (all dilutions). For conidial production, the aqueous extract of guava had a positive effect, increasing production, while the Peroxitane at the R and RC concentrations resulted in a negative influence. The mortality of A. diaperinus, caused by the fungus after exposure to these products, was 60% for the peracetic acid at 0.5 RC, and

above 80% for the extracts. Thus, the results showed that all the extracts and Peroxitane at RC 0.5 are compatible with the fungus B. bassiana Unioeste 4, however only the extracts had a low impact on inoculum potential." As taken from Martins CC et al. 2016. Braz. J. Biol. 76(2) 420-7. PubMed, 2017 available at: https://www.ncbi.nlm.nih.gov/pubmed/27143049

In vitro assays against diseases of the blood and immune system.

Origin	Extraction Method	Major Constituent	Cells	Assay	Main Results	Ref.
Korea	Maceration in MeOH:H_2O 70% (*v*/*v*) (5 days)	-	LPS-stimulated RAW 264.7 (Mouse macrophage)	Griess, MTT, ELISA kit, western blot, transient transfection, and luciferase assays	At 125 µg/mL: no cytotoxic effect, ← 44–62% inhibition rates. ↓ LPS-induced NO and PEG_2 ↓ iNOS and COX-2 (↓ I-κBα degradation, ↓ activation NF-κB).	[48]
Palestine	Maceration in DCM:MeOH 50% (*v*/*v*) (24 h)	-	L929sA fibroblast	Transfection and luciferase assays	At 62.5 µg/mL: ↓ expression of IL-6 and NF-κB luciferase reporter gene construct via the NF-κB transactivation level, since no ↓ inhibition of NF-κB/DNA binding.	[40]
Korea	Extraction in MeOH:H_2O 70% (*v*/*v*) (6 h)	TPC: 426.84 mg (GAE)/g	LPS-stimulated RAW 264.7	MTT, Griess, and ELISA test assays	At 30 µg/mL: no cytotoxic effect. ↓ LPS-induced NO (52.58%) and the production of PGE_2 (43.45).	[49]
Korea	Extraction in EtOH:H_2O 55% (*v*/*v*) (4.9 h, 47 °C)	Gallic acid (0.2) and catechin (4.4) in mg/g	LPS-stimulated RAW 264.7	MTT, Griess, ELISA test, RT-PCR, and total western blot assays	At 50 µg/mL: no cytotoxic effect. ↓ LPS-induced NO (>65%) by ↓ iNOS, ↓ PGE_2 (to basal level) via ↓ COX-2 mRNA. ↓ IL-6. ↓ iNOS and	[50]

					COX-2 due to the down-regulation of ERK1/2 pathway, because no effect was found to other proteins at the dose tested.	
India	Maceration in-MeOH:H_2O 90% (*v*/*v*) (x3)	-	LPS-stimulated in Labeo rohita head-kidney macrophages	MTT, Greiss, ELISA, RT-PCR, and western blot assays	At 200 µg/mL, ↓ LPS-induced NO (75%) by ↓ iNOS-mRNA, ↓ PGE2 (45%) via ↓ production COX-2-mRNA, TNF-α, IL-1β, IL-10, and mRNA expression. Suppressed phosphorylation of MAPK (↓ I-κBα degradation ↓ activation NF-κB).	[51]
Korea	Soxhlet with EtOH:H_2O 55% (*v*/*v*) (4.9 h, 47 °C)	Gallic acid (0.09) and catechin (0.72) in mg/g	LPS-stimulated RAW 264.7	MTT, Greiss and ELISA test assays	At 30 µg/mL: no cytotoxic effect. ↓ LPS-induced NO (47.5%) and PGE_2 (45.8).	[52]
India	Maceration with-agitation in MeOH and EtOH (24 h)	-	Human blood	HRBC membrane stabilization method	At 200 µg/mL: ← 13.8–14.4% prevention of lysis of the membrane.	[53]
Indonesia	Maceration with agitation in EtOH:H_2O 96% (*v*/*v*) (6 h)	TPC: 101.93 mg GAE/g	Human lymphocyte	MTT assay	0.5 µg/mL: Stimulation index 1.54%.	[54]

Compounds in guava leaves with anti-diabetic properties in in vitro assays.

Origin	**Compound**	**Assay**	**Main Results**	**Ref.**

India	Ethyl acetate fraction	In vitro glycation of BSA-fluorescence measurement	In vitro AGEs formation with IC_{50} of 38.95 ± 3.08 µg/mL.	[55]
Taiwan	Gallic acid, catechin and quercetin	In vitro glycation of BSA-fluorescence measurement; Fructosamine assay and Girard-T assay	At 100 µg/mL: 80% inhibitory effects on the formation of α-dicarbonyl compounds at a concentration of 50 µg/mL, inhibitory effects on AGEs formation in BSA glycation systems.	[56]
China	Quercetin, kaempferol, myricetin	Rat intestinal sucrase and maltase inhibitory activities; Porcine pancreatic α-amylase inhibitory activity	At 1.5 mg/mL: inhibitory activities with IC_{50} values of 3.5, 5.2 and 3.0 mM against sucrase, with IC_{50} values of 4.8, 5.6 and 4.1 mM against maltase and with IC_{50} values of 4.8, 5.3 and 4.3 mM against α-amylase, respectively. Synergistic effect against α-glucosidase.	[57]
China	Water-soluble polysaccharides, including GP90 and P90	α-Glucosidase inhibition assay	α-Glucosidase inhibition activity with an EC_{50} of 2.27 µg/mL and 0.18 mg/mL.	[59]
-	Peltatoside, hyperoside, isoquercitrin, guaijaverin and flavonol-glycosides	Spectrophotometric assay; absorption assay into CaCo-2 cells	Concentration of the compounds (0.01 to 0.06 µmol/mL). Individual flavonol-glycosides inhibited DP-IV dose-dependently. The ethanolic guava leaves extract (380 µg/mL) showed a dose-dependent inhibition of DP-IV, with an IC_{50} of 380 µg/mL test assay solution; the highest uptake was from Guaijaverin.	[60]
Korea	Quercetin and catechin	Fructose transport in CaCo-2 cell systems	At 1 mg/mL: inhibition of fructose uptake (55%). At 30 µg/mL: quercetin contributed to both, GLUT2 and 5 transporters, and catechin to GLUT5-mediated fructose uptake inhibition.	[61]
India	Guavanoic acid	Spectrophotometric assay	At 27 µg/mL: remarkable PTP1B inhibitory activity (90%) and in vitro stability in various physiological medium including saline, histidine, cysteine, BSA, HSA and buffers (pH 5, 7 and 9). IC_{50} = 1.14 µg/mL.	[64]
India	n-Hexane, methanol, ethanol and aqueous leaf extracts	Inhibitory glucose diffusion	At 50 g/L: the methanol extract was the most potent with the lowest mean glucose concentration of 201 ± 1.69 mg/dL at the end of 27 h (↓ 93% uptake).	[63]
Japan	70% Ethanol extract	Oil Red O Assay; Real-Time RT	At 100 µg/mL: inhibition of 3T3-L1 differentiation via down-regulation of adipogenic transcription factors and	[65]

			markers (mRNA levels of PPAR-γ, C/EBP-α, and aP2), and suppression of mitotic clonal expansion (at day 4 and 8).	
Taiwan	Aqueous extract	Glucose uptake test; bicinchonic acid method; Western-blot analysis	At 400 µg/mL: ← IR (25.1%), p-IR (46.2%), p-IRS (51.2%), PI3K (32.2%), Akt (46.1%), p-Akt (36.3%), GLUT-2 (46.8%), and total glycogen synthase (45.5%).	[66]
Taiwan	Vescalagin	Glucose-uptake test	At 100 µg/mL: Enhancement of glucose uptake in TNF-α-induced insulin-resistant.	[67]

Advanced glycation end products (AGEs); bovine serum albumin (BSA), dipeptidyl peptidase (DP); effective concentration (EC50); glucose transporter 2 and 5 (GLUT-2; GLUT-5); human serum albumin (HSA); inhibitory concentration (IC50); insulin receptor (IR); insulin receptor substrate (p-IRS (Tyr)); p85 regulatory subunit of phospho-inositide 3 kinase (PI3K (p85)); phosphorylation of the insulin receptor (p-IR (Tyr)); protein kinase B (p-Akt (Ser)); tumor necrosis factor (TNF); ↓ decreases the effect.

Díaz-de-Cerio E et al. (2017b). Health Effects of Psidium guajava L. Leaves: An Overview of the Last Decade. Int. J. Mol. Sci. 18(4), E897. PubMed, 2017 available at: https://www.ncbi.nlm.nih.gov/pubmed/28441777

“This work assessed the effects of a 28-day treatment with lycopene-rich extract (LRE) from red guava fruit (*Psidium guajava* L.) on the lipid profile and oxidative stress in an experimental model of dyslipidemia. Male hamsters (116.5 ± 2.16 g) were fed with the AIN 93G diet containing casein (20%), coconut fat (13.5%) and cholesterol (0.1%). The animals were divided into four groups: normolipidemic control (standard feed; NC, $n = 7$); hypercholesterolemic control (HC, $n = 7$); LRE 25 mg/kg/day (LRE-25, $n = 7$) and LRE 50 mg/kg/day (LRE-50, $n = 9$). After treatment, plasma concentrations of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL) cholesterol (LDL-c), high-density lipoprotein (HDL) cholesterol (HDL-c), malondialdehyde (MDA-p) and myeloperoxidase (MPO), as well as erythrocytic superoxide dismutase (SOD-e) and the atherogenic index, were determined. Malondialdehyde (MDA-h), catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD-h) levels were assessed. Feed intake (FI) and weight gain (WG) were also determined. The LRE-25 group presented significantly lower TG levels and atherogenic index than did the HC group ($p < 0.05$). Both LRE-25 and LRE-50 groups presented lower levels of MDA-p and MPO than did the HC group ($p < 0.05$). LRE demonstrated a promising effect against dyslipidemia and oxidative stress.” As taken from Brito AKDS et al. 2019. Nutrients 11(2), E393. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30781884

“Diabetes mellitus is characterized by hyperglycaemia that results from defects in insulin secretion or insulin action and is accompanied by general disturbances metabolism. Psidium guajava (PG) leaf is known to have antidiabetic effects that include lowering of blood glucose. The aim of the study was to investigate the effect of PG leaf extract on tissue activity of glycogen synthase (GS) and glycogen phosphorylase (GP); tissue activity of hormone sensitive lipase (HSL); serum lipid profile; and serum enzyme biomarkers of tissue damage. Diabetes was induced in male Sprague-Dawley rats with a single dose of

40 mg/kg body weight streptozotocin. The aqueous extract of PG leaves was used to treat both normal and diabetic animals (400 mg/kg body weight) for 2 weeks while control animals were treated with the vehicle. At the end of the treatment period, blood, liver and adipose tissue samples were collected from the euthanized animals. The results show that PG extract significantly decreased ($P < 0.05$) HSL activity in adipose tissue and liver of diabetic animals which was accompanied by increased glycogen levels, reduced serum triglycerides, total cholesterol, LDL-cholesterol and increased HDL-cholesterol. This study demonstrates that P. guajava has significant anti-diabetic effects that include increased glycogen storage and reduced HSL activity in the liver and adipose tissue with an improved serum lipid profile." As taken from Tella T et al. 2019. Biomed. Pharmacother. 109, 2441-2446. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/30551504

"Psidium guajava (PG) is a short shrub or tree cultivated in tropical and subtropical regions around the world. The leaf extract of PG (guava leaf) has been used historically to cure many ailments. However, mechanisms of action of guava leaf in treating diabetes are not fully understood. Effects and underlying mechanisms of guava leaf on gluconeogenesis and glycogenesis in hepatocytes, insulin signaling proteins, liver function markers, and lipid profile in streptozotocin (STZ) injected diabetic Wistar rats were investigated within the current study. PG was given orally at the dose of 100, 200, and 400 mg/kg b.w to diabetic rats for the period of 45 days. The results reveal that oral administration of PG (200 mg/kg b.w) has considerably raised the levels of insulin, glycogen, hexokinase, glucose-6-phosphatase dehydrogenase and significant ($p < 0.05$) belittled hepatic markers, gluconeogenic enzymes, and OGTT fasting blood glucose levels. OGTT has shown least statistical significance between the group 5 (200 mg/kg b.w) and group 6 and vital difference between group 5 and group 4 (400 mg/kg). PG has attenuated the triglycerides, total cholesterol, phospholipids, free fatty acid, and LDL levels and raised HDL levels. PG considerably ($p < 0.05$) activated IRS-1, IRS-2, Akt, p-Akt, PI3K, GLUT2, AMPK, p-AMPK, and p-ACC, which are the key effector molecules of the PI3K/Akt pathway in STZ rats. The results of our study specify that treatment with PG ameliorated glucose-metabolism and lipid profile in STZ evoked diabetic rats; the rationale ought to be the activation of PI3K/Akt, phosphorylation of AMPK pathway in liver and therefore has beneficial anti-diabetic activity." As taken from Vinayagam R et al. 2018. Biomed. Pharmacother. 103, 1012-1017. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29710658

"SCOPE: Known pharmacological activities of guava (Psidium guajava) include modulation of blood glucose levels. However, mechanistic details remain unclear in many cases. METHODS AND RESULTS: This study investigated the effects of different guava leaf and fruit extracts on intestinal glucose transport in vitro and on postprandial glucose levels in vivo. Substantial dose- and time-dependent glucose transport inhibition (up to 80%) was observed for both guava fruit and leaf extracts, at conceivable physiological concentrations in Caco-2 cells. Using sodium-containing (both glucose transporters, sodium-dependent glucose transporter 1 [SGLT1] and glucose transporter 2 [GLUT2], are active) and sodium-free (only GLUT2 is active) conditions, we show that inhibition of GLUT2 was greater than that of SGLT1. Inhibitory properties of guava extracts also remained stable after digestive juice treatment, indicating a good chemical stability of the active substances. Furthermore, we could unequivocally show that guava extracts significantly reduced blood glucose levels (≈fourfold reduction) in a time-dependent manner in vivo (C57BL/6N mice). Extracts were characterized with respect to their main putative bioactive compounds (polyphenols) using HPLC and LC-MS. CONCLUSION: The data demonstrated that guava leaf and fruit extracts can potentially contribute to the regulation of blood glucose levels." As taken from Müller U

et al. 2018. Mol. Nutr. Food Res. 62(11), e1701012. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29688623

“Traditional Chinese medication has been utilized by Chinese medical practitioners to treat the varied symptoms of diabetes mellitus (DM). Notably, guava leaf has been used to treat diabetes in Asia. Our present study has been designed to analyze the action of guava leaf extract (GLE) at the molecular level in treating DM. A low dose of streptozotocin (STZ) was used to induce experimental diabetes in animals. Rats were treated with GLE at different concentrations (100, 200, and 400 mg/kg b.w.). The standard drug glibenclamide (GB) (600 μg/kg b.w.) was used for comparison. The diabetic rats showed a reduced level of insulin, accompanied by exaggerated levels of blood glucose, lipid peroxidation product, and augmented expressions of inflammatory cytokines, and showed reduced levels of antioxidants compared to the control rats. Supplementation with GLE counteracted the consequences of STZ. It suppresses the oxidative stress and inhibits the state of inflammation and the results are almost similar to that of standard drug group (GB group 5). Our present research, therefore, provides useful data concerning guava leaf extract by a thorough assessment in diabetes management. Being a natural product, additional analysis on GLE can shed light on finding effective phytochemicals within the field of diabetes mellitus.” As taken from Jayachandran M et al. 2018. Biomed. Res. Int. 4601649. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29670899

Records for Psidium guajava L., leaf, decoction and Psidium guajava L., ethanol extract.

Type of Test	**Route of Exposure or Administration**	**Species/Test System**	**Dose Data**	**Toxic Effects**	**Reference**
ICLo - Inhibitor Concentration Low	In vitro	Monkey - kidney	5000 mg/L/48H	In Vitro Toxicity Studies - cell morphology: overgrowth of cell appendixes etc.	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 141,975,2012
ICLo - Inhibitor Concentration Low	In vitro	Human - fibroblast	250 mg/L/24H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 119,275,2018

As taken from RTECS, 2019a and b

Record for Psidium guajava L., bark, methanol extract

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
IC50 - Inhibitor	In vitro	Human - leukemia cells	1.29 mg/L/72H	In Vitro Toxicity Studies -	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub.

Concentration 50				cell metabolic activity: Alamar Blue assay etc.	Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 222,21,2018
IC50 - Inhibitor Concentration 50	In vitro	Human - breast tumor	27.24 mg/L/72H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 222,21,2018
IC50 - Inhibitor Concentration 50	In vitro	Human - gastrointestinal tumor	18.63 mg/L/72H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 222,21,2018
IC50 - Inhibitor Concentration 50	In vitro	Human - other brain tumors	28.84 mg/L/72H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 222,21,2018
IC50 - Inhibitor Concentration 50	In vitro	Human - liver tumor	24.63 mg/L/72H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 222,21,2018

IC50 - Inhibitor Concentration 50	In vitro	Human - liver	38.05 mg/L/72H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 222,21,2018

As taken from RTECS, 2019c

Record for Psidium guajava L., leaf, infusion (no CAS RN given)

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
ICLo - Inhibitor Concentration Low	In vitro	Human - fibroblast	500 mg/L/24H	In Vitro Toxicity Studies - cell metabolic activity: Alamar Blue assay etc.	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 119,275,2018

As taken from RTECS, 2019d

6. Functional effects on

6.1. Broncho/pulmonary system

Anticough and antimicrobial activities of Psidium guajava Linn. leaf extract (Abstract). The anticough activity of Psidium guajava Linn. (guava) leaf extract was evaluated in rats and guinea pigs. The results showed that water extract of the plant at doses of 2 and 5 g/kg, p.o. decreased the frequency of cough induced by capsaicin aerosol by 35 and 54%, respectively, as compared to the control, within 10 min after injection of the extract, ($P<0.01$). However, the anticough activity is less potent than that of 3 mg/kg dextromethorphan which decreased frequency of cough by 78% ($P<0.01$). An experiment on isolated rat tracheal muscle showed that the extract directly stimulated muscle contraction and also synergized with the stimulatory effect of pilocarpine. This effect was antagonized by an atropine. Moreover, growth of Staphylococcus aureus and beta-streptococcus group A, as determined by the disc diffusion method, was inhibited by water,

methanol and chloroform extract of dry guava leaves (P<0.001). The LD50 of guava leaf extract was more than 5 g/kg, p.o. These results suggest that guava leaf extract is recommended as a cough remedy. As taken from Jaiarj et al., Journal of Ethnopharmacology, Volume 67, Issue 2, November 1999, Pages 203-212. Science Direct, 2010 available at http://www.sciencedirect.com/

6.2. Cardiovascular system

Hypoglycemic effect of guava juice in mice and human subjects Abstract). Guava is a plentiful fruit in Taiwan and it was taken from the plants of Psidium guajava Linn. (Myrtaceae). According to the folklore in Chinese Medicine, gauva was useful in the treatment of diabetes mellitus. In the present study, acute i.p. treatment with 1 g/kg guava juice produced a marked hypoglycemic action in normal and alloxan-treated diabetic mice. Although effective duration of guava is more transient and it is less potent than chlorpropamide and metformin, blood glucose lowering effect of guava also can be obtained by oral administration in maturity-onset diabetic and healthy volunteers. Thus, it is suggested that guava may be employed to improve and/or prevent the disease of diabetes mellitus." As taken from Cheng JT and Yang RS Am J Chin Med. 1983; 11(1-4):74-6. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/6660217

Guava leaf extract and topical haemostasis (Abstract). The effects of guava leaf extract on the bleeding time and the three main mechanisms of haemostasis: vasoconstriction, platelet aggregation and blood coagulation, were investigated. The water extract of guava leaves did not shorten bleeding times in rats. Guava leaf extract potentiated the vascular muscle contraction induced in rabbits by phenylephrine, and when given alone it stimulated human platelet aggregation in vitro in a dose-dependent manner. On the other hand, it significantly prolonged blood coagulation; activated partial thromboplastin time (APTT) test ($p < 0.05$). The higher the concentration of the extract, the longer APTT was observed. Thus, a water extract of guava leaves showed ambiguous effects on the haemostatic system. Guava leaf extract did not affect bleeding times, it stimulated vasoconstriction and platelet aggregation but it inhibited blood coagulation. Therefore, guava leaf extract is not recommended as a haemostatic agent. Copyright © 2000 John Wiley & Sons, Ltd. As taken from Jaiarj et al., Phytotherapy Research, Volume 14 Issue 5, Pages 388 – 391, Published Online: 28 Jul 2000 available at http://www3.interscience.wiley.com/

"Non enzymatic glycosylation (glycation) between reducing sugar and protein results in the formation of advanced glycation end products (AGEs), which is believed to play an important role in diabetes associated cardiovascular complications. Thus agents that inhibit the formation of AGEs are believed to have therapeutic potential against diabetic complications. In the present study we evaluated the antiglycative potential of ethyl acetate fraction of Psidium guajava leaves (PGEt) by administering the extract into streptozotocin induced diabetic rats. Daily administration of the extract for a period of one month significantly decreased the blood glucose, glycated hemoglobin and fructosamine levels in a dose dependent manner. Evaluation of the toxicity markers like SGOT and SGPT revealed the non toxic nature of the extract. Apart from this we evaluated the presence of cardiac

isoform of liver alpha 2 macroglobulin, which is a major protein associated with earlier stages of cardiac hypertrophy. SDS-PAGE analysis showed that the level of this protein decreased significantly in extract treated groups compared to diabetic control. These findings support that the administration of PGEt extract may be beneficial for preventing cardiovascular complications associated with diabetes". As taken from Soman S et al. 2013. Expt. Toxicol. Path. 65, 91-95. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/21742475

"The present study attempts to investigate the effects of Psidium guajava (P. guajava) when administered in combination with sodium arsenite @ 20 ppm in drinking water with the aim of achieving normalization of altered biochemical, hematological parameters suggestive of hepatic damage and depletion of inorganic arsenic following chronic arsenic exposure. Thirty adult Wistar rats were given 20 ppm arsenic for eight weeks along with hydro alcoholic leaf extract of P. guajava at a dose of 100 mg/kg body weight wt. (orally) (once daily for eight weeks). Arsenic exposure led to significant depletion of hemoglobin, red blood cells (RBC) and packed cell volume (PCV) but elevated leucocyte count (TLC). There was a significant increase ($P<0.01/P<0.05$) in serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphotase (ALP), acid phosphotase (ACP) and blood glucose whereas decrease in total protein level in arsenic-exposed untreated animals. The changes were accompanied by a significant elevation in blood and soft-tissue arsenic concentration. Co-administration of P. guajava was most effective not only in reducing arsenic-induced hematological and biochemical alterations but also in depleting arsenic from blood and soft tissues following arsenic exposure. We thus recommend combined leaf extract of P. guajava for achieving optimum effects of chelation therapy". As taken from Tandon N et al. 2012a. Toxicol. Int. 19, 121-124. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22778508

"Obesity is associated with a low-grade inflammatory status that affects vascular function. Previous studies have reported the beneficial effects of Psidium guajava L. (guava) on diabetes. Here we evaluate the how guava leaf extract at the dose of 5 mg/kg, affects vascular dysfunction in obese mice fed a high-fat diet for 7 weeks. Extract intake did not alter weight over time, although it reduced glycemia and insulin resistance, improving the serum lipid profile in obese mice. Additionally, guava leaf extract reversed the endothelial dysfunction found in obese mice in terms of endothelium- and NO (nitric oxide)-dependent vasodilatation induced by acetylcholine in aortic rings. In conclusion, the beneficial effects of guava leaf extract in obese mice were associated with improved vascular functions altered by obesity, probably due to its phenolic content." As taken from Díaz-de-Cerio E et al. 2017a. Food Res. Int. 96, 64-71. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28528109

"CONTEXT: Antiglycative potential of Psidium guajava L. (Myrtaceae) leaves has been established. However, the molecular basis of its antiglycative potential remains unknown. OBJECTIVE: The ethyl acetate fraction of P. guajava leaves (PGEt) was evaluated to determine the cardioprotective effect and its mechanism of action compared to quercetin. MATERIALS AND METHODS: After the induction of diabetes by streptozotocin (55 mg/kg body weight), PGEt and quercetin (50 mg/kg body weight) was administered for 60 days. Rats were grouped as follows: Group C: Control, Group D: Diabetic, Group D + E: Diabetic rats treated with PGEt, Group D + Q: Diabetic rats treated with quercetin. The antiglycative potential was evaluated by assaying glycosylated haemoglobin, serum fructosamine and advanced glycation end product levels. The differential receptor for advanced glycation end

products and nuclear factor kappa B (NFκB) protein levels was determined by western blot and the transcript level changes of connective tissue growth factor (CTGF), brain natriuretic peptide (BNP) and TGF-β1 in heart tissue were assessed by RT-PCR analysis. RESULTS: Glycated haemoglobin and serum fructosamine levels were found to be enhanced in diabetic rats when compared with control. Administration of PGEt significantly reduced the glycated haemoglobin and fructosamine levels to a larger extent than quercetin treated diabetic rats. PGEt reduced the translocation of NFκB from cytosol to nucleus when compared with diabetic rats. Expression of TGF-β1, CTGF and BNP was downregulated in PGEt treated groups compared with diabetic controls. DISCUSSION AND CONCLUSION: Administration of PGEt ameliorated diabetes associated changes in the myocardium to a greater extent than quercetin." As taken from Soman S et al. 2016. Pharm. Biol. 54(12), 3078-3085. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27418019

In vivo studies against diseases of the blood and immune system.

Origin	**Extraction Method**	**Subject**	**Treatment**	**Main Results**	**Ref.**
Nigeria	Maceration with agitation in EtOH:H_2O 80% (*v*/*v*) (24 h)	Wistar rats	*T. b. brucei*/no infected	Treatment (1–7 days) at 150 mg/kg: ← Hb (6.5 to 10.7 g/dL), PCV (28.6 to 34.4%), RBCC (4.1 to 5.0 × 10^{12}/L), MCV (53.6 to 64.3 fL), and MCHC (21.4 to 31.4 g/dL); ↓ WBC (23.2 to 19.4 × 10^{9}/dL) and neutrophil levels (28.9 to 27.3 × 10^{3}/mL).Compared to no infected subjects: similar values that obtained in treated-infected animals but with opposite conclusions.	[105]
Nigeria	Extraction in chloroform (24 h)	Mice	No infected	Treatment (28 days) at 45.9 mg/mL: no differences in Hb (12 to 11 g/dL), PCV (37 to 35%), RBCC (6.1 to 5.1 × 10^{6}/L), and MCHC (33 to 32 g/dL), and neutrophil levels (13 to 12%); ← lymphocyte levels (85 to 92%) and MCV (61 to 69 fL).	[106]
Korea	Extraction in EtOH:H_2O 55% (*v*/*v*) (4.9 h, 47 °C)	Sprague-Dawley rats and mice	Freund's complete adjuvant-induced hyperalgesia/LPS-induced endotoxic shock	At 400 mg/kg: PWL restored; ← 67% survival rate (72 h) by ↓ TNF-α (500 to 325 pg/mL) and IL-6 (80 to 58 ng/mL).	[50]
Brazil	Turbo-extraction in water and acetone: H_2O 70% (*v*/*v*) (20 min)	Swiss mice	Carrageenan-induced peritonitis, acetic acid-induced abdominal writhing and hot plate test	At 50mg/kg: number of leukocyte migration into the peritoneal cavity H_2O < H_2O -acetone extract. No central analgesic activity. Peripheral analgesic activity: ↓ number writhing response (from 50 to 15 count).	[19]

India	Maceration in EtOH (7 days)	Wistar rats	Acetic acid-induced writhing	At 2 mg/kg ↓ 66% number writhing response (from 67 to 54 count). Comparable to diclofenac sodium (75%).	[107]
India	Distillation with MeOH and H_2O	Wistar rats	Acetic acid-induced writhing and hot plate test	At 10 and 30 mg/kg ↓ responses time (at 9.4 and 10.6 s) compared to the analgesic drug Pentazocine (14 s).	

Cardiovascular disorders have been related to the endothelial cell damage that causes atherosclerosis. In this sense, extracts from budding guava leaves demonstrated a protective, in vitro, effect in bovine aortal endothelial cells, delaying low-density lipoprotein oxidation and preventing oxidized low-density lipoprotein cytotoxicity [69]. A similar effect was also noted in human umbilical-vein endothelial cell due to the ability of saving cell-viability reduction, suppressing reactive oxygen species production and nitric oxide release, as well as inhibiting the expression of NF-κB [70]. Moreover, budding guava leaves also showed their ability as an anticoagulant in plasma, since they reduced thrombin clotting time and inhibited the activity of antithrombin III. Thus, they could help to reduce the development of cardiovascular complications [71].

In addition, flavonoids and phenolic acids in the leaves could contribute to the prevention and amelioration of gout and hypertension, since, in rat-tissues homogenates, they inhibit the activity of two enzymes related to the development of both diseases (xanthine oxidase and angiotensin 1-converting enzymes) [72].

Ademiluyi et al. [118] assessed the lipid peroxidation in rats after checking the antihypertensive effect, in vitro, of red and white guava leaves. The work concluded that the activity may be related to rosmarinic acid, eugenol, carvacrol, catechin, and caffeic acid since they were the major constituents of their extracts. In addition, this activity was supported by the biphasic and contractile effect on rat vascular smooth muscles [119, 120].

In addition, atherosclerosis development was reduced in apoE-knockout mice by guava leaf extracts. In fact, the effect was connected to the presence of ethyl gallate and quercetin [121, 122]. In streptozotocin-induced diabetic rats, vascular reactivity to vasoconstrictor agents was reduced, as was vessel atherosclerosis [112]. Furthermore, Soman et al. [123] found that an ethyl acetate fraction of guava leaves reduced cardiac hypertrophy in streptozotocin-induced diabetic rats due to an anti-glycative effect.

Díaz-de-Cerio E et al. (2017b). Health Effects of Psidium guajava L. Leaves: An Overview of the Last Decade. Int. J. Mol. Sci. 18(4), E897. PubMed, 2017 available at: https://www.ncbi.nlm.nih.gov/pubmed/28441777

“Background: Angiogenesis is the process of formation of new blood vessels from the existing one. Pathological angiogenesis is widely implicated in many diseases, including cancer, diabetic neuropathy, retinopathy, obesity, and arthritis. Objective: The present study was aimed to evaluate the in vitro antioxidant and in ovo antiangiogenic activity of aqueous extract of Psidium guajava leaves (AEPG). Materials and Methods: Psidium guajava commonly known as guava reported to contain polyphenols and flavonoids such as gallic acid, epigallocatechin, catechin, rutin, and quercetin in glycosidic forms in its leaves. The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-

bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), nitric oxide, hydrogen peroxide, hydroxyl, and superoxide radical scavenging assays (RSAs) and antiangiogenic activity was evaluated using vascular endothelial growth factor (VEGF)-induced chick chorioallantoic membrane (CAM).The correlation between the antioxidant and antiangiogenic activity was correlated with total phenolic content (TPC) of AEPG. Results: The TPC of AEPG was found to be 493.8 ± 8.9 mg of GAE/g. The total flavonoid content of AEPG was found to be 254.9 ± 13.7 mg of CE/g. In vitro antioxidant activity of AEPG showed IC50 values of 19.4 ± 1.9, 25.5 ± 0.2, 4.9 ± 0.5, 29.9 ± 2.06, 39.5 ± 2.07, and 29.9 ± 0.9 μg/ml, respectively, for DPPH, ABTS, nitric oxide, hydrogen peroxide, hydroxyl, and superoxide RSAs. Significant reduction in angiogenesis in the AEPG treated groups when compared to untreated VEGF groups and the Pearson's correlation coefficient between TPC of AEPG and total length, area, branches of blood vessels and CAM thickness were −0.9261, −0.9807, −0.9637, and −0.9597, respectively. Conclusion: The results revealed potent antiangiogenic activity of AEPG leaves and exhibit significant correlation between the antioxidant and antiangiogenic activity of AEPG and its TPC." As taken from Latha S et al. 2018. Pharmacognosy Magazine 14(57), 284-293. Available at http://www.phcog.com/article.asp?issn=0973-1296;year=2018;volume=14;issue=57;spage=284;epage=293;aulast=Latha;type=0

6.3. Nervous system

In other animal studies, guava leaf extracts have shown central nervous system (CNS) depressant activity (Shaheen, 2000).

The present work examines the effects of hexane, ethyl acetate and methanol extracts of Psidium guajava leaves (20,100,500 and 1250 mg/kg) on the central nervous system in mice. The three extracts exhibited mostly dose-dependent antinociceptive effects in chemical and thermal tests of analgesia. The extracts also produced dose-dependent prolongation of pentobarbitone-induced sleeping time. However, they had variable and mostly non-significant effects on locomotor coordination, locomotor activity or exploration. In the pharmacological tests used, the ethyl acetate extract seemed to be the most active, followed by the hexane and then the methanol extracts. As taken from Shaheen HM, Effect of Psidium gua java leaves on some aspects of central nervous system in mice, Phytother.Res, 14(2), 2000, 107-111. Available at http://www3.interscience.wiley.com/

Record for Psidium guajava L., leaf, 70% acetone extract

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	50 mg/kg	Behavioral - analgesia Biochemical - Metabolism (Intermediary) - effect on inflammation or mediation	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 156,88,2014

				of inflammation	

As taken from RTECS, 2017

“Context: The search for biologically active compounds from natural source has always been of great interest to researchers looking for new source of drugs useful in infectious diseases. Higher plants have played a vital role as the source of important therapeutic agents. Objective: The present investigation was aimed to find novel analgesic agent from herbal origin. For the purpose, Psidium guajava stem extracts was screened for its analgesic potential. Materials and Methods: Animal model of acetic acid induced writhing was followed. Three different extracts were used to study the activity. Results: The methanolic extract at the dose tested was shown to possess analgesic activity. The significant reduction in acetic acid-induced writhings suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of prostaglandins (PGs) and other endogenous substances. Discussion: The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics. In general acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, PGs bradykinins and substance P, which stimulate nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response. Conclusion: It can be concluded that the crude extract of stem of P. guajava have given positive results for analgesic activity. These medicinal herbs may afford lead compounds which could be beneficial for future drug development.” As taken from Satija S 2018. International Journal of Green Pharmacy 12(2), 53-57. Available at https://www.greenpharmacy.info/index.php/ijgp/article/view/1830

6.4. Other organ systems, dependent on the properties of the substance

“The antioxidant activity of some compounds could be used to prevent various chronic diseases such as heart-disease, diabetes, cancer, arterial thrombosis, cataract and may provide health-promoting effects (Kimura et al., 1985; Qian and Nihorimbere, 2004).” As taken from Abreu et al., Guava extract (Psidium guajava) alters the labelling of blood constituents with technetium-99m; J Zhejiang Univ Sci B. 2006 June; 7(6): 429–435; PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1474003/

Guava Fruit (Psidium guajava L.) as a New Source of Antioxidant Dietary Fiber (Abstract). Guava (Psidium guajava L.) is a tropical fruit, widely consumed fresh and also processed (beverages, syrup, ice cream, and jams). Pulp and peel fractions were tested, and both showed high content of dietary fiber (48.55−49.42%) and extractable polyphenols (2.62−7.79%). The antioxidant activity of polyphenol compounds was studied, using three complementary methods: (i) free radical DPPH· scavenging, (ii) ferric reducing antioxidant power assay (FRAP), and (iii) inhibition of copper-catalyzed in vitro human low-density lipoprotein (LDL) oxidation. All fractions tested showed a remarkable antioxidant capacity, and this activity was correlated with the corresponding total phenolic content. A 1-g (dry matter) portion of peel contained DPPH· activity, FRAP activity, and inhibition of copper-induced in vitro LDL oxidation, equivalent to 43 mg, 116 mg, and 176 mg of Trolox,

respectively. These results indicate that guava could be a suitable source of natural antioxidants. Peel and pulp could also be used to obtain antioxidant dietary fiber (AODF), a new item which combines in a single natural product the properties of dietary fiber and antioxidant compounds. As taken from Jiménez-Escrig et al. J. Agric. Food Chem., 2001, 49 (11), pp 5489–5493. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/pubmed/11714349

“Method: Seven-week-old male SHRSP/ZF rats were divided into two groups, a control group and a guava leaf extract (GLE) group. We gave 2 g/kg/day GLE or water by forced administration for 6 weeks. After the experimental period, the rats were sacrificed and organ weight, hepatic lipids, serum aminotransferase and liver pathology were examined. To search for a possible mechanism, we examined the changes of key enzyme and transcriptional factors involved in hepatic fatty acid beta-oxidation. Results: The triglyceride content of the liver significantly decreased in the GLE group in comparison with the control group, and decreased fat-drop formation in the liver tissue graft in the GLE group was observed. In addition, the improvement of liver organization impairments with fat accumulation restriction was suggested because blood AST and ALT in the GLE group significantly decreased. Furthermore, it was supposed that the activity of AMPK and PPARα significantly increased in the GLE group via the increase of adiponectin receptors. These were thought to be associated with the decrease of the triglyceride content in the liver because AMPK and PPARα in liver tissue control energy metabolism or lipid composition. On the other hand, insulin resistance was suggested to have improved by the fatty liver improvement in GLE. Conclusion: Our results indicate that administration of GLE may have preventive effects of hepatic accumulation and ameliorated hepatic insulin resistance by enhancing the adiponectin beta-oxidation system. Guava leaf may be potentially useful for hepatic steatosis without the side effects of long-term treatments” (Yoshitomi et al., 2012).

”The hypolipidemic effect of 10% fruit fibers in rats fed with high-fat diet (HFD) was evaluated. This study was conducted on a total of 50 male Albino rats divided into 10 equal groups fed with different types of dietary fruits. The feeding period lasted for 24 weeks. Fasting blood samples were collected and sera separated and subjected to lipid profile assay and atherogenic index. In addition, total antioxidant activity of different fruits was determined. The results obtained showed that pomegranate had higher content of antioxidants followed by apple, strawberry and guava compared with other fruits. ….. Histological examination revealed that there was a large lipid and cholesterol deposition in the livers of rats fed with HFD. The potential in lowering the levels of plasma total cholesterol and triglyceride is in the following order: pomegranate > apple > strawberry > guava > papaya > mandarin and orange. Accumulation of hepatic lipid droplets was diminished when compared with the HFD group. Also, antiatherogenic is better than the untreated groups. Accordingly these hypolipidemic effects may be due to high-fiber content and antioxidant activity of these fruits.” As taken from Esmael OA et al. 2015. Toxicol. Ind. Health 31(3), 281-8. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/23315090

“This study analyzed the content of phenolic acids and flavonoids in extracts of guava fruit (Psidium guajava L.), and examined the renal protective effects of guava aqueous extract (GAE) and ethanol extract (GEE) in diabetic mice. GAE had more caffeic acid, myricetin, and quercetin; and GEE had more cinnamic, coumaric and ferulic acids. GAE or GEE at 1 and 2 % was supplied in diet for 12 weeks. GAE or GEE intake at 2 % significantly reduced

glucose and blood urea nitrogen levels, increased insulin level in plasma of diabetic mice ($p < 0.05$). GAE or GEE treatments dose-dependently reserved glutathione content, retained activity of catalase and glutathione peroxidase, and decreased reactive oxygen species, interleukin (IL)-6, tumor necrosis factor-α and IL-1β levels in kidney ($p < 0.05$). GAE and GEE treatments at 2 % significantly declined renal N (ε)-(carboxymethyl)lysine, pentosidine and fructose levels ($p < 0.05$), and suppressed renal activity of aldose reductase ($p < 0.05$). These findings support that guava fruit could protect kidney against diabetic progression via its anti-oxidative, anti-inflammatory and anti-glycative effects" (Lin and Yin, 2012. Plant Foods for Human Nutrition. 67(3), 303-8. Abstract taken from http://www.ncbi.nlm.nih.gov/pubmed/22581156).

"Psidium guajava is an important plant of high medicinal value and has been used in traditional systems of medicine against various ailments. The antidiabetic effect of the ethanolic extract of Psidium guajava leaves and also its protective effect on altered glucose metabolism was evaluated in streptozotocin (stz)-induced diabetic rat model. Diabetes was induced in rats by means of intraperitoneal injection of 50-mg/kg body weight (b.wt.) of stz. Diabetes-induced rats were randomly divided into two groups. One group of rats was treated with Psidium guajava leaf extract at a dosage of 300-mg/kg b.wt. and the other group of rats was treated with the standard drug glyclazide at a dosage of 5-mg/kg b.wt. for 30 days. The blood glucose levels, plasma insulin, Hb, HbA1c were measured. The effect on the drug on altered glucose metabolizing enzymes were also studied. Treatment with Psidium guajava extract showed a significant reduction in blood glucose and HbA1c levels and a significant increase in plasma insulin levels. The drug also significantly restored the activities of carbohydrate metabolizing enzymes. This suggests that the potential antidiabetic effect of the ethanolic extract of the Psidium guajava leaves may be due to the presence of flavonoids and other phenolic components present in the drug." As taken from Khan HB et al. 2013. J. Diet Suppl. 10(4), 335-44. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24237189

"BACKGROUND: Metabolic syndrome (MS) and type 2 diabetes mellitus (T2DM) have been associated with insulin-resistance; however, the effective therapies in improving insulin sensitivity are limited. This study is aimed at investigating the effect of Guava Leaf (GL) extracts on glucose tolerance and insulin resistance in SHRSP.Z-Leprfa/Izm rats (SHRSP/ZF), a model of spontaneously metabolic syndrome. METHODS: Male rats at 7 weeks of age were administered with vehicle water or treated by gavage with 2 g/kg GL extracts daily for six weeks, and their body weights, water and food consumption, glucose tolerance, and insulin resistance were measured. RESULTS: Compared with the controls, treatment with GL extracts did not modulate the amounts of water and food consumption, but significantly reduced the body weights at six weeks post treatment. Treatment with GL extracts did not alter the levels of fasting plasma glucose and insulin, but significantly reduced the levels of plasma glucose at 60 and 120 min post glucose challenge, also reduced the values of AUC and quantitative insulin sensitivity check index (QUICKI) at 42 days post treatment. Furthermore, treatment with GL extracts promoted IRS-1, AKT, PI3Kp85 expression, then IRS-1, AMKP, and AKT308, but not AKT473, phosphorylation, accompanied by increasing the ratios of membrane to total Glut 4 expression and adiponectin receptor 1 transcription in the skeletal muscles. CONCLUSIONS: These data indicated that GL extracts improved glucose metabolism and insulin sensitivity in the skeletal muscles of rats by modulating the insulin-related signaling."As taken from Guo X et

al. 2013. BMC Complement. Altern. Med. 13, 52. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23452929

“We report that the P. guajava extract exerted anti-cancer control on both haematological and solid neoplasias. P. guajava extract's anti-tumour properties were found to be tightly bound to induction of apoptosis and differentiation. Use of ex vivo myeloid leukaemia blasts corroborated that P. guajava was able to induce cell death but did not exhibit anti-cancer effects on all malignant cells investigated, indicating selective activity against certain types of tumour. Analyses of P. guajava pulp, peel and seeds identified the pulp as being the most relevant component for causing cell cycle arrest and apoptosis, whereas peel was responsible for causing cell differentiation. P. guajava itself and its pulp-derived extract were found to induce apoptosis accompanied by caspase activation and p16, p21, Fas ligand (FASL TNF super-family, member 6), Bcl-2-associated agonist of cell death (BAD) and tumour necrosis factor receptor super-family, member 10b (DR5), overexpression. Our findings showed that P. guajava L. extract was able to exert anti-cancer activity on cultures in vitro and ex vivo” (Bontempo et al. 2012. Cell Proliferation 45, 22-31. Abstract available at http://www.ncbi.nlm.nih.gov/pubmed/22172154).

“To investigate the nephro-protective effects of total triterpenoids from Psidium guajava leaves (TTPGL) on type 2 diabetic rats. METHODS: Diabetic rats were induced by intraperitoneal injection of streptozotocin (STZ, 35 mg/kg) and a high-fat diet. Diabetic rats were divided into five groups: diabetic model control, low-dose TTPGL-treated (60 mg/kg, L-TTPGL), medium-dose TTPGL-treated (120 mg/kg, M-TTPGL), high-dose TTPGL-treated (240 mg/kg, H-TTPGL) and rosiglitazone-treated (3 mg/kg, RSG). The rats received daily treatment for six weeks. At the end of the period,the levels of fasting blood glucose (FPG), fasting insulin (FINS), creatinine (Cr) and blood urea nitrogen (BUN) in serum were measured. Kidneys for histopathological evaluation were stained with Hematoxylin and Eosin (HE). RESULTS: Compared with normal control group, the level of FPG was increased, the insulin and insulin sensitivity index were decreased in the model group; The levels of BUN and Cr were increased with histopathological changes related to diabetic nephropathy in the kidney, which were the glomerular endothelium and mesangial cell proliferation, capillary narrowed, the base-membrane incrassation, glomerular swelling, cysts narrowed and tubules edema. Compared with the model group, the levels of FPG were decreased, serum insulin and insulin sensitivity index were increased significantly in M-TTPGL and H-TTPGL groups ($P<0.01$ or $P<0.05$); The levels of BUN and Cr were decreased significantly ($P<0.01$ or $P<0.05$) and the renal structural damages were improved significantly. CONCLUSION: TTPGL could decrease the level of blood glucose of diabetic rat effectively, increase the insulin sensitivity index and protect renal lesions in diabetic rats”. As taken from Kuang QT et al. 2012. Zhong Yao Cai. 35(1), 94-7. (In Chinese). PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/22734419

“This study was undertaken to evaluate the protective effect of aqueous extract of Psidium guajava leaves against sodium arsenite-induced toxicity in experimental rats. Animals were divided into four groups. Control group received arsenic free distilled water and three treatment groups (II, III, and IV) exposed to the arsenic (NaAsO(2)) (20 mg/kg b.wt) through drinking water. Group III and IV were administered a daily oral dose of P. guajava leaf extract 50 and 100 mg/kg b.wt. (AEPG(50) and AEPG(100)) for the period of 6 weeks. Blood samples and organs were collected at the end of the experiment. Arsenic exposure resulted in significant rise in lipid peroxidation (LPO) levels in erythrocyte, liver, kidney, and brain. In addition toxin decreased ($P<0.05$) the level of reduced glutathione (GSH),

superoxide dismutase (SOD), and catalase (CAT) activities in the studied tissues. Residual effect of arsenic in various tissues was also observed. Histopathological results revealed mild to severe type of necrosis and degenerative changes in kidney and liver of arsenic intoxicated animals. Cytological alteration in brain tissue was also observed. Treatment with AEPG(100) (aqueous extract of P. guajava) @100 mg/kg body weight) significantly restored activities of oxidative stress markers like LPO levels, GSH levels, SOD, and CAT activities but having the limited protective activity of the herbal extract was observed on tissues architecture. It is therefore concluded that prophylactic co-administration of AEPG could provide specific protection from oxidative injury and to some extent on tissue damage". As taken from Tandon N et al. 2012b. Toxicol. Int. 19, 245-249. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/23293461

"Psidium guajava Linn. (family Myrtaceae; PG) is a tropical fruit with a blood-glucose-lowering effect in diabetic rats, but its mechanism of action is still unknown. We investigated the antihyperglycemic efficacy and mechanisms of action of PG in streptozotocin (STZ)-induced diabetic rats. After 4 weeks of PG supplementation (125 and 250 mg/kg), PG significantly restored the loss of body weight caused by STZ and reduced blood glucose levels in a dose-dependent manner compared with that in diabetic control rats. Mechanistically, PG protected pancreatic tissues, including islet β-cells, against lipid peroxidation and DNA strand breaks induced by STZ, and thus reduced the loss of insulin-positive β-cells and insulin secretion. Moreover, PG also markedly inhibited pancreatic nuclear factor-kappa B protein expression induced by STZ and restored the activities of antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase. We conclude that PG has a significant antihyperglycemic effect, and that this effect is associated with its antioxidative activity". As taken from Huang CS et al. 2011. Fd Chem. Toxicol. 49, 2189-2195. PubMed, 2013 available at http://www.ncbi.nlm.nih.gov/pubmed/21679740

"Furtherance to a previous report on the anti-trypanosomal properties of Psidium guajava aqueous leaf extract in rats experimentally infected with Trypanosoma brucei brucei, we have evaluated the effects of the daily intraperitoneal administration of P. guajava leaf extract to rats on the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and acid phosphatase (ACP) in the kidney, liver and serum. The results obtained revealed that the administration of the extract produced significant increase in the serum activities of AST, ALT, ALP and ACP when compared with the control ($p < 0.05$). Also AST, ALT and ALP and ACP activities in the tissues of animals administered the extract revealed inconsistent changes ($p < 0.05$) relative to control. The increase in the serum activity of ALP may be an indicator that there was a likely compromise to the integrity of the plasma membrane as a result of the ethanolic extract administration. This could have caused leakages of the other enzymes investigated, which may explain the corresponding increases in the serum activities of AST, ALT and ACP observed." As taken from Adeyami OS & Okanji MA. 2011. Human Expt. Toxic. 30, 1266-1274. PubMed, 2013 available at _ http://www.ncbi.nlm.nih.gov/pubmed/21056949

"The aim of this study was to evaluate the skeletal effect of guava triterpene-enriched extract (GE) in rats and identify osteogenic compounds thereof, and determine their modes of action. In growing female rats, GE at 250 mg/kg dose increased parameters of peak bone mass including femur length, bone mineral density (BMD) and biomechanical strength, suggesting that GE promoted modeling-directed bone growth. GE also stimulated bone regeneration at the site of bone injury. In adult osteopenic rats (osteopenia induced by

ovariectomy, OVX) GE completely restored the lost bones at both axial and appendicular sites, suggesting a strong osteoanabolic effect. Serum metabolomics studies showed changes in several metabolites (some of which are related to bone metabolism) in OVX compared with ovary-intact control and GE treatment to OVX rats reversed those. Out of six abundantly present triterpenes in GE, ursolic acid (UA) and 2α-hydroxy ursolic acid (2α-UA) induced osteogenic differentiation in vitro as did GE by activating Wnt/β-catenin pathway assessed by phosphorylation of GSK-3β. Over-expressing of constitutively active GSK-3β (caGSK-3β) in osteoblasts abolished the differentiation-promoting effect of GE, UA and 2α-UA. All three increased both glycolysis and mitochondrial respiration but only rotenone (inhibitor of mitochondrial electron transfer) and not 2-deoxyglucose (to block glycolysis) inhibited osteoblast differentiation. In addition, caGSK-3β over-expression attenuated the enhanced mitochondrial respiration caused by GE, UA and 2α-UA. We conclude that GE has osteoanabolic effect which is contributed by UA and 2α-UA, and involve activation of canonical Wnt signaling which in turn modulates cellular energy metabolism leading to osteoblast differentiation." As taken from Porwal K et al. 2017. J. Nutr. Biochem. 44, 22-34. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28343085

"Ultraviolet (UV) irradiation is a major environmental factor affecting photoageing, which is characterized by skin wrinkle formation and hyperpigmentation. Although many factors are involved in the photoageing process, UV irradiation is thought to play a major role in melanogenesis. Tyrosinase is the key enzyme in melanin synthesis; therefore, many whitening agents target tyrosinase through various mechanisms, such as direct interference of tyrosinase catalytic activity or inhibition of tyrosinase mRNA expression. Furthermore, the highly selective calcium channel ORAI1 has been shown to be associated with UV-induced melanogenesis. Thus, ORAI1 antagonists may have applications in the prevention of melanogenesis. Here, we aimed to identify the antimelanogenesis agents from methanolic extract of guava leaves (Psidium guajava) that can inhibit tyrosinase and ORAI1 channel. The n-butanol (47.47%±7.503% inhibition at 10 μg/mL) and hexane (57.88%±7.09% inhibition at 10 μg/mL) fractions were found to inhibit ORAI1 channel activity. In addition, both fractions showed effective tyrosinase inhibitory activity (68.3%±0.50% and 56.9%±1.53% inhibition, respectively). We also confirmed that the hexane fraction decreased the melanin content induced by UVB irradiation and the ET-1-induced melanogenesis in murine B16F10 melanoma cells. These results suggest that the leaves of P. guajava can be used to protect against direct and indirect UV-induced melanogenesis." As taken from Lee DU et al. 2016. Exp. Dermatol. 25(12), 977-982. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27488812

Endocrine and metabolic in vivo assays with guava leaves.

Origin	Subject	Treatment	Main Results	Ref.
Nigeria	Rabbits	High-cholesterol diet	At 250 mg/kg: ↓ TC (15%); ← HDL (69%); ↓ LDL (74%); ↓ hyperglycemia 43%.	[109]
Brazil	Wistar rats	High-cholesterol diet	At 369.89 mg phenolic compound in the extract/g: ↓ TC (29–35%), TG (59–73%); ← HDL (46%); ↓ VLDL+LDL; ↓ enzyme activity (SOD (6.2 to 5.7 U/mg protein), GP (4.6 to 2.3 μmol/g protein).	[110]

Korea	*Lepr*^{db}/*Lepr*^{db} juvenile and adult mice	Diabetes spontaneous mutation	At 10 mg/kg: 87% inhibition PTP1B; ↓ glucose levels 31% and 42% respectively.	[111]
Iran	Wistar rat	Streptozotocin-induced diabetes	At 1mg/L: ↓ Ca/Mg ratio (18 to 12), glucose level, TG (100 to 65 mg/dL), TC (68 to 48 mg/dL), ← HDL (18 to 40 mg/dL), ↓ LDL, and VLDL to normal levels; ↓ alteration in vascular reactivity (110 to 50 mmHg).	[112]
Taiwan	Sprague-Dawley rats	Low-dose streptozotocin and nicotinamide-induced diabetes	At 400 mg/kg: ↓ blood glucose level (230 to 140 mg/dL); ← plasma insulin level and glucose utilization (normal levels); ← enzyme activity (hepatic hexokinase (8 to 11 U/mg protein), phosphofructokinase (18 to 25 U/mg protein) and glucose-6-phosphate dehydrogenase (11 to 25 U/mg protein).	[113]
India	Sprague-Dawley rats	Streptozotocin-induced diabetes	At 100 mg/kg: ↓ blood glucose level (4 to 1 mg/mL) and lipid peroxidation (2 to 1 mmol/100 g tissue); ← enzyme activity (CAT (6 to 10 × 10^3 U/mg protein), SOD (6 to 10 U/mg protein), GPx (0.4 to 0.6 U/mg protein), GRd (0.1 to 0.3 U/mg protein).	[55]
Nigeria	Albino rats	Alloxan-induced diabetes	At 200 mg/kg: ← average weight (99 to 209g); ↓ blood glucose level (15 to 8 mmol/L); ↓ alanine aminotransferase activity (32 to 24 U/L).	[114]
India	Albino rats	Alloxan-induced diabetes	At 500 mg/kg: ↓ blood glucose level, TC (231 to 163 mg/dL), TG (133 to 69 mg/dL), LDL (186 to 126 mg/dL), VLDL (26 to 13 mg/dL); ← HDL (18 to 23 mg/dL).	[115]
Nigeria	Wister rats	-	At 150 mg/kg: ← ALP (300, 175and 650 IU), AST (500, 400, 450 IU), ALT (1200, 1200, 1800 IU), ACP (750, 650, 900 IU) activity in the kidney, liver, and serum, respectively.	[116]
Nigeria	Mice	-	At 49.3 mg/mL: ← AST (93 to 126 iμ/L), ALT (30 to 35 iμ/L), ALP (57 to 66 iμ/L), conjugate bilirubin (0.2 to 0.3 mg/dL) and creatinine (0.9 to 1.2 mg/dL).	[106]
Nigeria	Albino rats	-	At 150 mg/kg: ← serum urea (2.9 to 6 mmol/L) and creatinine (2.7 to 4 mmol/L); ↓ concentration of serum Na^+ (122 to 99 mmol/L).	[117]

acid phosphatase (ACP); alanine aminotransferase (ALT); alkaline phosphatase (ALP); aspartate aminotransferase (AST); catalase (CAT); glutathione peroxidase (GPx); glutathione reductase (GRd); high-density lipoprotein (HDL) cholesterol; low-density lipoprotein (LDL) cholesterol; protein tyrosine phosphatase 1B (PTP1B); superoxide

dismutase enzyme (SOD); total cholesterol (TC); triglycerides (TG); very low-density lipoprotein (VLDL) cholesterol; ← increases the affect; ↓ decreases the effect.

In vitro assays against diseases related to the digestive system.

Origin	Extraction Method	Microorganism(s)/Cells	Assay	Main Results	Ref.
India	Soxhlet with MeOH (4.5 h)	*S. mutans* strains	Agar well diffusion assay, effect on acid production, on sucrose-dependent adherence to smooth glass surfaces, and on sucrose-induced cellular aggregation, and MATH assays	MIC > 5 mg/mL (MeOH). MIC = 2–4 mg/mL (guaijaverin) At sub-MIC (0.125–2 mg/mL): ← pH (5 to 6–7), hydrophobicity indexes (3.2–72%), ↓ sucrose-dependent adherence (34–84%) and aggregation.	[73]
Malaysia	Decoction	*S. sanguinis* and *S. mutans*	NAM model system	At 60.95 mg/mL: MIC = 7.62 (*S. sanguinis*) and 3.81(*S. mutans*.) mg/mL. MBC values = 15.24 and 30.48 mg/mL, respectively. At 0.5 mg/mL: ↓ adherence 57 and 60% (single-species) and 88–89% (dual-species).	[74]
Malaysia	Sonication with H_2O (10 min)	*S. sanguinis*, *S. mitis*, and *Actinomyces* spp.	MATH assay	At 1 mg/mL: ↓ 54.1%, 49.9% and 40.6%, respectively, cell-surface hydrophobicity. At 20 mg/mL: was 64.7, 60.5, and 55.5%, respectively.	[75]
Malaysia	Decoction	*S. sanguinis*, *S. mitis*, and *Actinomyces* spp.	Bacterial growth and generation time rates determinations	At 4 mg/mL: Time growth = 1.22 (*S. sanguinis*, *Actinomyces* spp) and 2.06 h (*S. mitis*) ↓ growth 42.6%, 51.2% and 55%.	[76]
India	Maceration with stirring in EtOH (2 days)	*S. mutans*, *S. sanguinis*, and *S. salivarius*	Agar well diffusion assay	At 10 mg/mL: inhibition zones of 21.17, 18.58, and	[77]

				23.00 mm, respectively.	
India	Maceration (2 days) and Soxhlet (6 h) with EtOH, H_2O, and EtOH:H_2O 50% (*v*/*v*)	*S. mutans* and *S. mitis*	Agar well diffusion assay, sucrose-dependent adherence and cellular co-aggregation activities, and biofilm formation sterile acrylic tooth determinations	At 15 mg/mL: inhibition zone for H_2O (11.8 mm) to EtOH:H_2O (25 mm), both by Soxhlet. MIC = 1 mg/mL. EtOH:H_2O extract: at >0.05 mg/mL: ↓ adherence and co-aggregation, at MIC, ↓ the viable count of dental biofilm (3.50 log_{10} CFU/mL).	[78]
India	Soxhlet with EtOH:H_2O 50% (*v*/*v*) (6 h)	*S. mutans* and *S. mitis*	MATH assay	At >1 mg/mL ↓ hydrophobicity (index < 40%).	[79]
India	Maceration with stirring (2 days) and Soxhlet with EtOH	*S. mutans*, *S. sanguinis*, and *S. salivarius*	Agar well diffusion assay	At 10 mg/mL: ← inhibition zones for maceration extracts (19–23 mm).	[80]
Ghana	Maceration with agitation in EtOH:H_2O 70% (*v*/*v*) (24 h)	*Aggregatibacter actinomycetemcomitans* strains	Agar well diffusion assay, release of the cytosol enzyme lactate dehydrogenase, fluorescence assisted cell sorter, and ELISA assays	No growth inhibitory effect, although neutralized the cell death and pro-inflammatory response, and restored the morphological alterations induced by the leukotoxin. These effects were due to the direct binding of guava compounds and the leukotoxin.	[81]
India	Maceration in Ac, EtOH, chloroform, MeOH and H_2O (15 days at 22 °C)	*Neisseria catarrhalis*, *S. mutans*, *S. salivarius*, *Streptococcus viridans*, *Bacillus megaterium*, and *P. aeruginosa*	Agar well diffusion assay	← Inhibition zones in Ac (15–29 mm), except for *N. catarrhalis* (20 mm in MeOH).	[82]
India	Maceration in MeOH (72 h). Fractionation with ethyl acetate	*S. aureus* and *S. mutans*	HRBC membrane stabilization method, disc and agar well diffusion assays	MeOH and ethyl acetate fraction ← protection (84–99%) to the inflammatory response. Inhibition zones (25–100 µg/mL) = 10.5 to 22	[83]

				mm by both methods. MICs = 0.48 (ethyl acetate) and 0.62 (MeOH) mg/mL.	
Taiwan	Maceration EtOH, Ac, H_2O (room temperature and 60 °C) (24 h)	Clone 9 rat liver cells	WST-1 and ALT assays	At >500 µg/mL cytotoxic effect of EtOH and Ac and 600 µg/mL for H_2O. At <200 µg/mL normal values were observed for H_2O and Ac, and EtOH (<500 µg/mL). At <100 µg/mL: Hepato-protective effect in EtOH and H_2O (full range).	

In vivo assays for digestive system related diseases.

Origin	**Extraction Method**	**Subject**	**Treatment**	**Main Results**	**Ref.**
India	Extraction with MeOH	Wistar rats	ASP, PL, and EtOH-induced ulcers	At 200 mg/kg: PL-induced ulcers: ↓ 64% ulcer formation (ui = 2.1), ↓ GV (5 to 2 mL), acid secretion (88 to 64 mEq/L/100 g), ← pH (2 to 5).Comparable to omeprazole; ASP (↓ 70.5%, ui = 2.5) and EtOH (↓ 70.4%, ui = 8.7)-induced systems.	[124]
Nigeria	Maceration in H_2O (24 h)	Albino rats	EtOH-induced ulcers	At 1000 mg/kg: ↓ MNL (9.4 to 2) ui (4.7 to 1).	[125]
Nigeria	Maceration with agitation in MeOH (24 h)	Wistar rats	EtOH-induced ulcers	At 1000 mg/kg: ↓ ui (17.7 to 6.3), ← protection (64.4%).	[126]
India	Maceration in EtOH:H_2O 90% (*v*/*v*) (72 h).	Wistar rats	PL and EtOH-induced ulcers	At 200 mg/kg: PL-induced: ↓ ulcer formation (77 to 84%), ui (5 to 1.3), GV (1.4 to 0.5 mL/100g), and acid secretion (28 to 23 mEq/L); ← pH (2.0 to 3.4). EtOH-induced: ↓ (63% to 79%, ui = 1.6 to 5.6), and gastric lesions (5.6–1.9).	[127]
South Africa	Maceration in H_2O (48 h)	Wistar rats and BALB/c mice	Castor oil-induced diarrhea and castor oil-induced enteropooling	At 400 mg/kg: ← 83.3% rat protection, ↓ 75% fluid accumulation in rats; ↓ 87.73% transit in rats and 77.2% in mice; ↓ 64.35% of contractions in mice.	[128]

Nigeria	Soxhlet with EtOH:H_2O 70% (*v*/*v*)	Wistar rats	Castor oil-induced diarrhea	At 80 mg/kg: ↓ 53.03% transit in rats and ↓ 67.70% intestinal contractions.	[129]
Pakistan	Maceration with EtOH	BALB/c mice, rabbit jejunum	Castor oil-induced diarrhea, K^+-induced motility	At 1 g/kg: ← 81.1% mice protection; Spasmolytic effect (0.3–1 mg/mL) ↓ spontaneous contractions EC_{50} = 0.66 mg/mL in rabbits.	[130]
India	Decoction (1 h)	Wistar rats	CCl_4, PCM, and TAA-induced liver injury	At 500 mg/kg: CCl_4: ↓ ALT (384 to 17 U/L), AST (642 to 152 U/L), ALP (750 to 489 U/L), and bilirubin (1.6 to 0.3 mg/dL), ↓ control levels; PCM: ↓ ALT (384 to 87 U/L), AST (642 to 179 U/L), ALP (750 to 338 U/L), and bilirubin (1.6 to 0.6 mg/dL); TAA: ↓ ALT (337 to 32 U/L), AST (438 to 237 U/L), and ALP (770 to 479 U/L).	[131]
India	Soxhlet with EtOH	Wistar rats	PCM-induced liver injury	At 400 mg/kg: ↓ SGOT (475 to 370), SGPT (158 to 128), ALP (814 to 729), and bilirubin (0.7 to 0.6); ← total protein (5.15 to 5.6), albumin (2.6 to 3.1), and GLO (2.1 to 2.4). Histopathological observations: less diffuse granular degeneration and mild periportal lymphocytic infiltration.	[132]
India	Decoction (1 h)	Wistar rats	Acetaminophen-induced liver injury	At 500 mg/kg: ↓ AST (121 to 77 IU/L), ALT (80 to 57 IU/L), ALP (115 to 67 IU/L), and total bilirubin (4 to 2 mg/dL). Restored: total protein (5 to 7 g/dL), LPO (7 to 2 nmol/mg protein), GPx (13 to 19 µmol/mg protein), GSH (15 to 23 µmol/mg protein), CAT (14 to 24 µmol/mg protein), and SOD (48 to 63 µmol/mg protein). Histopathological observations: normal lobular structure.	[133]
Egypt	Maceration with agitation in EtOH:H_2O 70% (*v*/*v*) (24 h)	Albino rats	CCl_4-induced liver injury	At 500 mg/kg: ↓ ALT (94 to 55 U/mL), AST (199 to 82 U/mL), GGT (71 to 23 U/mL), lysosomal enzymes (50%), and LPO (7 to 3 nmol/mg protein); ← SOD (15 to 39 U/mg protein), CAT (5 to 15 µg/mg protein), GSH (6 to 8 µg/mg protein), GST (13 to 25 mM/min/mg protein), total protein (48 to 58 g/L), albumin (29 to 38 g/L), GLO (19 to 21 g/L).	[134]
Egypt	Decoction (1 h)	Wistar rats	PCM-induced liver injury	↓ AST (342 to 156 U/L), ALT (359 to 80 U/L), ALP (288 to 263 U/L), LDH (207 to 143 U/L), GGT (11 to 7 U/L), and total bilirubin (0.3 to 0.2 mg/dL).	[135]

				Restored SOD (13 to 24 U/g) and CAT (5 to 17 U/g).	

To test the effect of guava leaf extract, several randomized clinical trials have been conducted during the last two decades, although only two studies are available in the last decade. One of the studies consisted of evaluating the effect of guava leaf extract pills on primary dysmenorrhea disorder. For this, 197 women were divided into four groups, and each received a different dosage: 3 and 6 mg extract/day, 300 mg placebo/day and 1200 mg ibuprofen/day. The administration took place over five days during three consecutive cycles. The results demonstrated that 6 mg extract/day alleviated menstrual pain and could replace the use of medicaments like ibuprofen. In fact, guava leaves could be used as a broad-spectrum phyto-drug and not only as an anti-spasmodic agent [143]. Furthermore, Deguchi and Miyazaki[58] reviewed several works regarding the effect of the intake of a commercial guava leaf tea (Bansoureicha®, Yakult Honsha, Tokyo, Japan) on different pathologies of diabetes mellitus illness such as the influence on postprandial blood glucose, on insulin resistance and on hypertriglyceridemia and hypercholesterolemia. The authors concluded that the ingestion of guava leaf tea can ameliorate the symptoms of diabetes mellitus and that it could be used as an alimentotherapy.

Díaz-de-Cerio E et al. (2017b). Health Effects of Psidium guajava L. Leaves: An Overview of the Last Decade. Int. J. Mol. Sci. 18(4), E897. PubMed, 2017 available at: https://www.ncbi.nlm.nih.gov/pubmed/28441777

“Leaves of Psidium guajava L. (guava) have been widely used in the popular way for prevention and treatment of various diseases. Thus, the objective of this study was to evaluate the inhibitory potential of leaves aqueous extract from three cultivars of P. guajava (Pedro Sato, Paluma and Século XXI) on α-amylase, α-glycosidase, lipase, and trypsin enzymes, in the presence or not of simulated gastric fluid and to determine the content of phenolic compounds by high performance liquid chromatography. All cultivars presented the same composition in phenolic compounds, but in different proportions. The compounds identified are gallic acid, epigallocatechin gallate, syringic acid, o-coumaric acid, resveratrol, quercetin, and catechin (which was the major compound in all the cultivars evaluated). In the absence of simulated gastric fluid, it was observed different inhibitions exercised by the leaves aqueous extracts from three cultivars of P. guajava on each enzyme. In presence of simulated gastric fluid, all cultivars showed increase in the inhibition of lipase and α-glycosidase, and decrease in inhibition of α-amylase and trypsin enzymes. These results indicate that P. guajava leaves aqueous extracts from all cultivars evaluated possess potential of use as an adjuvant in the treatment of obesity and other dyslipidemias.” As taken from Simão AA et al. 2017. An. Acad. Bras. Cienc. 89(3 Suppl), 2155-2165. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/28678960

“We analyzed guava fruits (Psidium guajava L. cv. Red Suprema) from Cuba to determine their chemical composition, total antioxidant capacity, as well as their protective effect against oxidative damage using an in vitro model of human dermal fibroblasts. The guava fruit is a natural source of bioactive compounds, such as polyphenols, vitamin C, folates and beta carotenes with proven health benefits. Human dermal fibroblasts were pre-incubated with different concentrations of guava crude extract and then subjected to oxidative stress using the AAPH stressor. The number of apoptotic and dead cells, as well as the markers of oxidative damage such as lipid and protein oxidation significantly decreased when cells were pre-incubated with guava crude extract and then exposed to the stressor. The activity of antioxidant enzymes also improved when cells were pre-incubated with guava crude extract in comparison to cells subjected to stress without prior pre-incubation with the guava

extract. The results obtained in this study highlight the health benefits of guava regarding oxidative stress, proving it to be an important source of bioactive compounds associated with important biological properties." As taken from Alvarez-Suarez JM et al. 2018. Plant Foods Hum. Nutr. 73(1), 18-24. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29455277

"Guava leaf (*Psidium guajava* L.) extracts are used in both traditional medicine and the pharmaceutical industry. The antioxidant compounds in *P. guajava* leaves can have positive effects including anti-inflammatory, anti-hyperglycemic, hepatoprotective, analgesic, anti-cancer effects, as well as protecting against cardiovascular diseases. In the present study, phenolic compounds and in vitro antioxidant capacity were measured in extracts obtained with polar and non-polar solvents from leaves of two varieties of guava, Calvillo Siglo XXI and Hidrozac. The quantity of total phenolics and total flavonoids were expressed as equivalents of gallic acid and quercetin, respectively. Hydroxyl radical, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and Oxygen Radical Absorbance Capacity using fluorescein (ORAC-FL) in vitro tests were used to assess the radical scavenging abilities of the extracts. The total phenolics were higher in the aqueous fraction of the variety Calvillo Siglo XXI, while in the Hidrozac variety total phenolics were higher in the acetone and chloroform fractions. Total flavonoids were higher in all fractions in the variety Calvillo Siglo XXI. Total phenolics showed a highly positive correlation for ORAC-FL, and a moderately positive correlation with hydroxyl radicals. Finally, total flavonoids showed a slightly positive correlation for ORAC-FL and hydroxyl radicals. Both varieties of guava leaf extract showed excellent antioxidant properties." As taken from Camarena-Tello JC et al. 2018. Antioxidants (Basel) 7(3), E34. PubMed, 2018 available at: https://www.ncbi.nlm.nih.gov/pubmed/29495514

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

Tobacco smoke condensates from cigarettes containing guava juice, concentrate and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of guava juice, concentrate. Table below provides tested level(s) and specific endpoint(s).

Endpoint	Tested level (ppm)	Reference
Smoke chemistry	1,500 (No CAS)	JTI KB Study Report(s)
In vitro genotoxicity	1,500 (No CAS)	JTI KB Study Report(s)
In vitro cytotoxicity	1,500 (No CAS)	JTI KB Study Report(s)
Inhalation study	1,500 (No CAS)	JTI KB Study Report(s)
Skin painting	1,500 (No CAS)	JTI KB Study Report(s)

9. Heated/vapor emissions toxicity

Aerosol from an electronic nicotine delivery system (ENDS) product that creates a vapor by heating an e-liquid; the vapor then passes through a capsule containing tobacco granules, containing Guava extract was tested in a battery of in vitro and/or in vivo test(s). Under the test conditions and within the sensitivity and specificity of the bioassay(s), no mutagenic, genotoxic or cytotoxic responses were observed when exposed to Aerosol Collected Matter (ACM) and/or aerosol Gas Vapor Phase (GVP) and no adverse findings from a 90-day in vivo repeat-dose inhalation toxicity study were observed after exposure to the aerosol even when exposure concentrations were the maximal amount that could be achieved with the specific product(s). These results are in contrast to those observed with combustible cigarette which showed mutagenic, genotoxic, cytotoxic and adverse effects upon exposure. The table below provides tested level(s) and specific endpoint(s):

Endpoint	**Tested level**	**Reference**
Aerosol chemistry	0.0003 mg/(tobacco portion; 310 mg)	Logic (2019)
In vitro genotoxicity	0.0003 mg/(tobacco portion; 310 mg)	Logic (2019)
In vitro cytotoxicity	0.0003 mg/(tobacco portion; 310 mg)	Logic (2019)

In vivo genotoxicity	0.0003 mg/(tobacco portion; 310 mg)	Logic (2019)
Inhalation study	0.0003 mg/(tobacco portion; 310 mg)	Logic (2019)

10. Ecotoxicity

10.1. Environmental fate

No data available to us at this time.

10.2. Aquatic toxicity

"In this research, we focused on the efficacy of aqueous and ethanol leaf extracts of Psidium guajava L. (guava) based experimental diets on the growth, immune, antioxidant and disease resistance of tilapia, Oreochromis mossambicus following challenge with Aeromonas hydrophila. The experimental diets were prepared by mixing powdered (1, 5 and 10 mg/g) aqueous and ethanol extract of guava leaf with commercial diet. The growth (FW, FCR and SGR), non-specific cellular immune (myeloperoxidase activity, reactive oxygen activity and reactive nitrogen activity) humoral immune (complement activity, antiprotease, alkaline phosphatase activity and lysozyme activity) and antioxidant enzyme responses (SOD, GPX, and CAT) were examined after 30 days of post-feeding. A significant enhancement in the biochemical and immunological parameters of fish were observed fed with experimental diets compared to control. The dietary supplementation of P. guajava leaf extract powder for 30 days significantly reduced the mortality and increased the disease resistance of O. mossambicus following challenge with A. hydrophila at 50 µl (1 × 107 cells ml-1) compared to control after post-infection. The results suggest that the guava leaf extract could be used as a promising feed additive in aquaculture." As taken from Gobi N et al. 2016. Fish Shellfish Immunol. 58, 572-583. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27702676

"This study examined the effects of an herbal extract composed of nine herbs i.e Aloe vera, Andrographis pariculata, Annona squamosa, Azadirachta indica, Citrus aurantifolia, Coriandrum sativum, Ocimum sanctum, Ollium cepa and Psidium guajava on growth, survival rate and immunoprotection against pathogenic Vibrio harveyi in the tiger shrimp Penaeus monodon. The petroleum ether, methanol and N-hexen extracts of different herbal plants were selected, processed and thoroughly mixed in equal proportions and added to

the shrimp diets at a concentration of 1.0, 2.5 and 5.0 mL kg-1. After 60 days of feeding, shrimps were challenged with V. harveyi bacteria (1 × 107 cells mL-1), which were isolated and propagated from the infected shrimps. The shrimps fed on diets with methanolic extraction of 2.5 mL kg-1 had significantly (P < 0.001) higher survival rate (76%), specific growth rate (4.26 ± 0.11%) and better food conversion ratio (1.5) than the other groups. This study indicates that addition of methanolic herbal extracts of 2.5 mL kg-1 can positively influence the immune response of tiger shrimp against V. harveyi infection." As taken from AftabUddin S et al. 2017. Fish Shellfish Immunol. 65, 52-58. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28365386

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

“The emerging resistance of Plasmodium species to currently available anti-malarials remains a public health concern, hence the need for new effective, safe and affordable drugs. Natural products remain a reliable source of drugs. Nefang is a polyherbal anti-malarial of the Cameroonian folklore medicine with demonstrated in vitro antiplasmodial and antioxidant activities. It is composed of Mangifera indica (bark and leaf), Psidium guajava, Carica papaya, Cymbopogon citratus, Citrus sinensis, Ocimum gratissimum (leaves). This study aimed at investigating the suppressive, prophylactic and curative activities of Nefang in Plasmodium infected rodent models. ….. BALB/c mice and Wistar rats were inoculated with Plasmodium chabaudi chabaudi and Plasmodium berghei, respectively, and treated with Nefang, the Mangifera indica bark/Psidium guajava combination and a Psidium guajava leaf aqueous extracts (75, 150, 300 and 600 mgkg-1 bwt). ….. The prophylactic and curative (Rane's Test) activity of Nefang was also evaluated by determining the parasitaemia, survival time, body weight and temperature in pre-treated rodents. ….. Percent suppressions of parasitaemia at 600 mgkg-1 bwt were as follows (P. berghei/P. chabaudi): Nefang - 82.9/86.3, Mangifera indica bark/Psidium guajava leaf combination extract - 79.5/81.2 and Psidium guajava leaf - 58.9/67.4. ….. These results indicate that Nefang has excellent in vivo anti-malarial activities against P. berghei and P. chabaudi, upholding earlier in vitro antiplasmodial activities against multi-drug resistant P. falciparum parasites as well as its traditional use. Hence, Nefang represents a promising source of new anti-malarial agents.” As taken from Arrey Tarkang P et al. 2014. Malar. J. 13(1), 456. PubMed, 2014 available at: http://www.ncbi.nlm.nih.gov/pubmed/25421605

"ETHNOPHARMACOLOGICAL RELEVANCE: Psidium guajava and Tagetes erecta have been used traditionally to treat gastrointestinal parasites, but their active metabolites and mechanisms of action remain largely unknown. AIM OF THE STUDY: To evaluate the anthelmintic potential of Psidium guajava and Tagetes erecta extracts on Levamisole-sensitive and Levamisole-resistant strains of the model nematode Caenorhabditis elegans. MATERIALS AND METHODS: Aqueous extracts of Psidium guajava (PGE) and Tagetes erecta (TEE) were assayed on locomotion and egg-laying behaviors of the wild-type (N2) and Levamisole-resistant (CB193) strains of Caenorhabditis elegans. RESULTS: Both extracts paralyzed wild-type and Levamisole-resistant nematodes in a dose-dependent manner. In wild-type worms, TEE 25mg/mL induced a 75% paralysis after 8h of treatment and PGE 25mg/mL induced a 100% paralysis after 4h of treatment. PGE exerted a similar paralyzing effect on N2 wild-type and CB193 Levamisole-resistant worms, while TEE only partially paralyzed CB193 worms. TEE 25mg/mL decreased N2 egg-laying by 65% with respect to the untreated control, while PGE did it by 40%. CONCLUSIONS: Psidium guajava leaves and Tagetes erecta flower-heads possess hydrosoluble compounds that block the motility of Caenorhabditis elegans by a mechanism different to that of the anthelmintic drug Levamisole. Effects are also observable on oviposition, which was diminished in the wild-type worms. The strong anthelmintic effects in crude extracts of these plants warrants future work to identify their active compounds and to elucidate their molecular mechanisms of action." As taken from Piña-Vázquez DM et al. 2017. J. Ethnopharmacol. 202, 92-96. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28286043

10.5. All other relevant types of ecotoxicity

No data available to us at this time.

11. References for conventional products

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12. Other information

No data available to us at this time.

13. Last audited

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