Phytochemical screening and cytotoxic activity of latex from *Manilkara zapota* (L.) P. Royen

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Abstract

Manilkara zapota fruiting species from tropical and tropical areas present latex as a product of special metabolism, with pharmacological properties. This study aimed to evaluate the qualitative phytochemical composition and toxicological activity on *Artemia salina* evaluated at different concentrations of the extract collected from the pedicel of the fruit of *M. zapota*. The latex was collected and immediately, the solubility in different polar and nonpolar solvents, the phytochemical prospection using different reagents and the toxicity assay on *A. salina* larvae in different concentrations of latex were performed. The latex showed solubility results only for water and ethanol. Several phytochemical classes were observed with positive results, especially for flavonoids, phenolics, steroids and saponins. The toxicological test on *A. salina* showed a lethal dose of 17.9 µg mL^{-1}, considered moderate. *Manilkara zapota* latex has shown great aptitude for new quantitative and evaluation studies for other biological models.

Keywords: *Manilkara* genus, *Artemia salina*, Special metabolites, Fruit trees, Biological activities

Resumo

Manilkara zapota espécie frutífera de áreas tropicais e neotropicais paresentam como produto do metabolismo especial o látex, com propriedades farmacológicas. Este estudo teve por objetivo avaliar a composição fitoquímica qualitativa e a atividade toxicológica sobre *Artemia salina* avaliado em diferentes concentrações do extrato coletado do pedicelo do fruto de *M. zapota*. O látex foi coletado e imediatamente, foi realizada a solubilidade em diferentes solventes polares e apolares, a prospecção fitoquímica utilizando diferentes reagentes e o ensaio de totoxicidade sobre larvas de *A. salina* em diferentes concentrações de látex. O látex apresentou resultado de solubilidade apenas para água e etanol. Foram observadas diversas classes fitoquímicas com resultados positivos em especial para flavonoides, fenólicos, esteroides e saponinas. O ensaio toxicológico sobre *A. salina* apresentou dose letal de 17.9 µg mL^{-1} considerada moderada. O látex de *Manilkara zapota* demonstrou grande aptidão para novos estudos quantitativos e de avaliação para outros modelos biológicos.

Palavras-chave: Gênero *Manilkara*, *Artemia salina*, Metabólitos especiais, Frutíferas, Atividades biológicas

Resumen

Las especies frutales de *Manilkara zapota* de áreas tropicales y neotropicales aparecen como un látex de metabolismo especial, con propiedades farmacológicas. Este estudio tuvo como objetivo evaluar la composición fitoquímica cualitativa y la actividad toxicológica sobre *Artemia salina* evaluada a diferentes concentraciones del extracto recolectado del pedicelo del fruto de *M. zapota*. Se recolectó el látex e inmediatamente se realizó la

solubilidad en diferentes solventes polares y no polares, la prospección fitoquímica con diferentes reactivos y el ensayo de toxicidad en larvas de *A. salina* en diferentes concentraciones de látex. El látex mostró resultados de solubilidad solo para agua y etanol. Se observaron varias clases de fitoquímicos con resultados positivos, especialmente para flavonoides, fenoles, esteroides y saponinas. La prueba toxicológica en *A. salina* mostró una dosis letal de 17,9 µg mL^{-1}, considerada moderada. El látex de *Manilkara zapota* mostró gran aptitud para nuevos estudios cuantitativos y evaluación para otros modelos biológicos.

Palabras clave: Género *Manilkara*, *Artemia salina*, Metabólitos especiales, Fructifero, Actividades biológicas

1. Introduction

Manilkara zapota (Figure 1) known as "sapotizeiro, sapoti, chiku or chicle" belongs to the genus *Manilkara* presenting ~79 species, this genus is circumscribed in the family Sapotaceae (Reyes-Gómez et al., 2018; Liu et al., 2019). It is an arboreal species that develops well in tropical climates, however, it can be cultivated all over the world in countries with tropical or neotropical climate (Thompson et al., 2015; Chunhakant; Chaicharoenpong, 2019).

It is an evergreen, glabrous tree, 8-15 m in height. It is cultivated throughout India, though it is native to Mexico, Central America and South America (Chanda; Nagani, 2010; Barbalho et al., 2015; Bangar et al., 2022). *M. zapota* is a fruit-bearing species, where its sweet and malty-tasting fruits are consumed in natura by both animals and humans. In addition, wood and latex can be used in woodworking and medicine (Uekane et al., 2017; Bashir, 2019).

Figure 1. *Manilkara zapota* individual in fruiting period. Source: Authors, 2022.

Traditionally *M. zapota* has been used for several medicinal purposes. All parts of the plants are ascribed to carry medicinal properties and are used for a range of disease including diarrhea, cold, fever and ulcers (Bashir, 2019). Several studies have shown that this species has important phytomolecules (methyl 4-*O*-galloylchlorogenate, 4-*O*-galloychlorogenic acid, polyphenolics, methyl chlorogenate, dihydromyricetin, quercitrin, myricitrin, (+)-catechin, (-)-epicatechin, (+)-gallocatechin and gallic acid (Ma et al., 2003) involved in various types of biological activities that promote and potentially help maintain cells against free radicals (Ma et al., 2003; Chanda; Nagani, 2010; Pravin; Shashikant, 2019). Antioxidants may be defined as compounds that inhibit or delay the oxidation of other molecules (DNA, RNA, Lipoproteins) by inhibiting the initiation or propagation of oxidizing chain reactions.

Other activities such as antidiabetic and antilipidemic are reported for extracts of leaves and fruit pulp of *M. zapota* (Barbalho et al., 2015), antimicrobial (*Aspergillus flavus*, *Vasianfactum* sp., *Fusarium* sp., *Bacillus subtilis*, *Bacilus megaterium*, *Sarcina lutea*, *Escherichia coli* and *Salmonella typhi*, and cytotoxicity effect on *Artemia salina* (Osman et al., 2011), anti-tumor, anti-bacterial, anti-inflammatory and anti-diabetic (Bano; Ahmed, 2017), anti-arthritic (Singh et al., 2011), and antidiarrheal, anti-pyretic, hypoglycemic and hypocholesterolemic (Moura et al., 2019).

Among the products of special metabolism in various plants, latex is produced in different organs of *M. zapota* (Mahajan; Badgujar, 2008; Shinwari; Rao, 2020). Selvaraj & Pal (1984) described the sugars glucose, fructose and sucrose, in addition to starch and tannins, for latex extracted from the pedicel and mature fruit of *M. zapota*. Sibi et al. (2013) evaluated different latex-producing vegetables, where they observed that this compound obtained from *M. zapota* exhibited antifungal activity against *Aspergillus fumigatus*, *Aspegillus niger*, *Aspergillus terreus*, *Fusarium solani*, *Penicillium digitatum* and *Rhizopus arrhizus*. Among other activities *M. zapota* latex in the study by Kusuma et al. (2020), report hemostatic and fibrinogenolytic activity.

M. zapota latex still has great potential for study. Since, the variability of phytochemical groups found in this product presents great variation between the places where it was collected for studies. With this, it is still essential to strengthen the phytochemical bases of this latex, thus providing for observing which potential groups with pharmacological, agricultural or biotechnological actions present positive results in the most diverse studies in the world.

This study aimed to evaluate the preliminary phytochemistry of latex extracted from the pedicel of the fruit of *Manilkara zapota* L. in a plantation in the municipality of Rio Verde, Goiás State, Brasil.

2. Materials and Methods

The *M. zapota* latex (Figure 2) was collected Rio Verde, Goiás State, Brasil, in the month of July. The plant was identified by Msc. A. C. P. Menezes Filho, PhD Student, Department of Agrarian Science, Instituto Federal Goiano, Rio Verde, Goiás State, Brasil. The voucher specimen (HRV 137.091) for *M. zapota* plant is deposited in the herbarium of the Department of Biology, in Vegetable Systematic Laboratory, Instituto Federal Goiano, Rio Verde, Goiás State, Brasil. Latex draining from the pedicel was collected immediately after harvest and analysed.

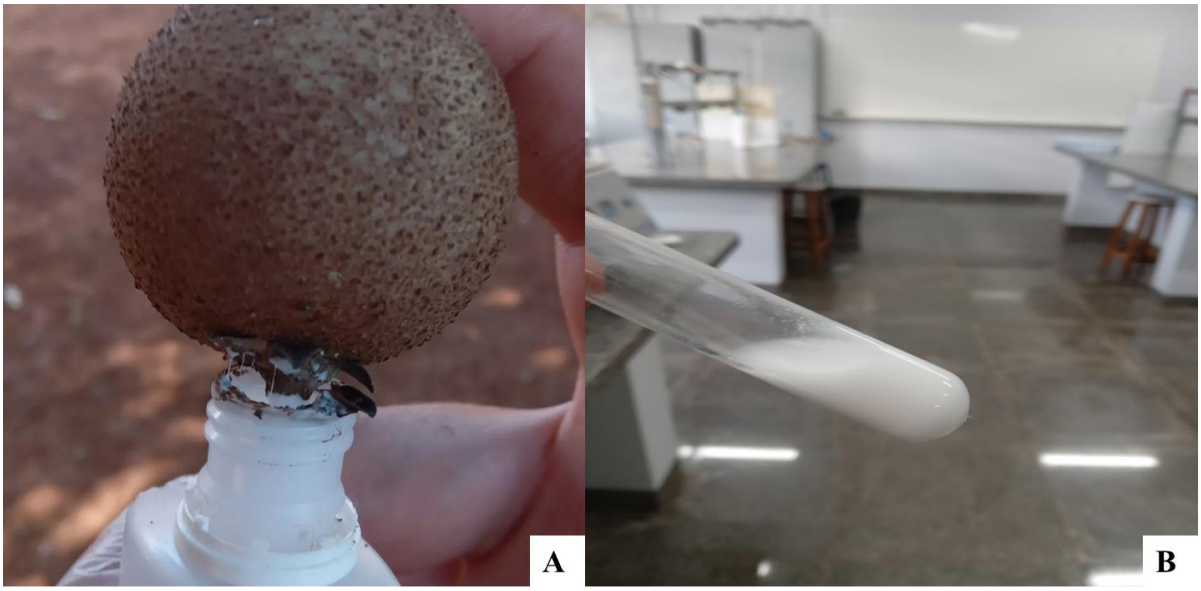

Figure 2. Latex collection in the pedicel of the *Manilkara zapota* fruit (**A**), and in (**B**) latex. Source: Authors, 2022.

The solubility assay followed as described by Gallardo-Vásquez et al. (2019), where 500 µL of solvent (water, ethanol, methanol, butanol, ethyl acetate, chloroform, *n*-hexane, acetone, xylene, benzene, ethyl ether and petroleum ether) and 500 µL of in natura latex were added to tubes. of *M. zapota*. Direct visual analysis method was used.

The phytochemical tests to detect the presence of heterosides, phenols, saponins(foamy and hemolytic), tannins (blue (hydrolyzable or gallic) and green (condensed or catechic) coloration), reducingsugars, no-reducing sugars, resins, amino acids, purines, aromatic and aliphaticcompounds, polysaccharides, carbohydrates, carboxylic acids, oxylates,flavonoids, steroids, triterpenes, coumarines, quinones, organic acids, alkaloids and depsides & depsidones were performed following the method described by Matos (1997), Madike et al. (2017), Mehdi et al. (2019) and Menezes Filho et al. (2022). These tests were based on the visual observations of color modification or precipitate formation after the addition of specific reagents (-) negative or (+) present (León et al., 2018).

The toxicity test on *A. salina* was performed as described by Meyer et al. (1982). The test was performed at different latex concentrations and performed in triplicate. Saline water and Tween 80 were used as a negative control, and an aqueous solution of potassium dichromate as a positive control. After 24 h, the surviving larvae of *A. salina* were counted at different concentrations.

The lethal concentration (LC_{50}) calculation was obtained by linear regression, being considered significant when $LC_{50} < 1000$ µg mL^{-1}. The statistical program used was the Microsoft Office 365 package (Paid version). The photoprotective assay was performed with a scan in the ultraviolet A, B and C regions (UVA, UVB and UVC) between 250 and 400 nm as described by Violante et al. (2009).

3. Results and Discussion

M. zapota latex exhibited in our findings the ability to dissolve only in water and ethanol (Table 1 and Figure 3). Similar results were observed by Gallardo-Vásquez et al. (2019) evaluating the latex of *Jatropha curcas* belonging to f. Euphorbiaceae.

Table 1. Solubility test of *Manilkara zapota* latex in different solvents.

Solvents	**Latex**
Water	+
Ethanol	+
Methanol	-
Ethyl acetate	-
Chloroform	-
n-Hexane	-
Acetone	-
Xylene	-
Ethyl ether	-
Petroleum ether	-
Dichloromethane	-
Toluene	-

Note: P. A grade polar and non-polar solvents were used. The water used was ultra-distilled. Source: Authors, 2022.

Figure 3 shows the solubility results of the different polar and non-polar solvents.

Figure 3. Solubility test in different solvents on *Manilkara zapota* latex. **Note:** From left to right, water, ethanol, methanol, acetone, hexane, ethyl ether, petroleum ether, chloroform, ethyl acetate, dichloromethane, toluene and xylene. Source: Authors, 2022.

Our findings showed the positive presence of numerous phytochemical classes (Table 2). The presence of heterosides, reducing sugars, resins, carbohydrates, coumarins, quinones, depsides & depsidones, proteins, phlobotannins, cardiac glycosides and double ofefins in the latex of *M. zapota* were not observed.

Osman et al. (2011) found the presence of terpenoids, glycosides and flavonoids in the extracts (ethyl acetate) solvents (*n*-hexane/petroleum ether and *n*-hexane/ethylacetate) of leaves and stem bark of *M. zapota*. Different solvents demonstrated the presence of phytogroups in *M. zapota* in the latex study by Sibi et al. (2013). The researchers observed the presence for the aqueous extract (alkaloids, glycosides, saponins and terpenes), petroleum ether (flavonoids, glycosides, saponins ans terpenes), chloroform (terpenes), ethyl acetate (glycosides, saponins and terpenes), acetone (alkaloids, flavonoids, glycosides, terpenes), and methanol (alkaloids, glycosides and terpenes).

Different latex-producing plant families exhibit a complex phytochemical composition. In *Himatanthus drasticus* latex, showed a positive presence for alkaloids, tannins, saponins, triterpenes and coumarins, these phytomolecules have a special biological effect such as antimicrobial, anti-inflammatory, antiviral, cytotoxic and antioxidant (Melro et al., 2020), vasodilator, cell renewal and growth, antitumor, analgesic, antipyretic, insecticide, antimalarial, antitussive, hypoanalgesic and myorelaxant (David et al., 2019). Similar results were obtained by Nascimento et al. (2018) where they found condensed tannins, catechins, flavones, flavanols, xanthones, flavanols and flavanones for the fraction of acetoethyl latex (*H. drasticus*) extract.

In the study by David et al. (2019) the researchers found for the latexes of *Brosimum parinarioides* and *Parahancornia amapa* the presence of the following phytochemical groups (alkaloids and derivatives of coumarins) and (organic acids, alkaloids, anthraquinones, depside & depsidones, and purines), respectively. Lopes et al. (2020) they observed many phytochemical classes present in *Symphonia globulifera* latex such as

saponins, reducing sugars, polysaccharides, phenolics, tannins, flavanones, flavanonols, catechins, xanthones, anthraquinones, depsides & depsidons and coumarins. Gallardo-Vásquez et al. (2019) verified the presence of flavonoids, tannins, phenolic alkaloids, carbohydrates, steroids and triterpenes in the latex of *J. curcas*. Silva et al. (2018) verified the presence of the main phytochemical groups also in the latex of *Euphorbia milli* and *Euphorbia tirucalli* (alkaloids, terpenes and saponins). Our findings show some similarity with other plant latex in different botanical families.

Table 2. Phytochemical screening of *Manilkara zapota* latex.

Compounds	**Results**
Heterosides	-
Foamy saponins	+
Phenols	+
Hemolytic saponins	+
Tannins	Green
Reducing sugars	-
No-reducing sugars	+
Resins	-
Amino acids	+
Purines	+
Aromatic & aliphatic compounds	Red
Polysaccharides	-
Carbohydrates	-
Carboxylic acids	+
Oxylates	+
Flavonoids	+
Steroids	+
Triterpenes	+
Coumarins	-
Quinones	-
Organic acids	+
Alkaloids	+
Depsides & depsidones	-
Proteins	-
Phlobatannins	-
Cardiac glycosides	-
Double olefins	-

Note: (-) negative assay. (+) positive assay. Green: condensed or catechic. Red: aromatic compounds. Source: Authors, 2022.

The larval mortality rate in the *M. zapota* aqueous latex toxicity assay ranged from 65 to 100%. The dose required to kill 50% of the larvae (LC_{50}) was calculated at 17.9 µg mL^{-1}, showing significant activity with LC_{50} < 1000 µg mL^{-1}. Several studies evaluating a large amount of latex extracted from plants show a high capacity of toxicity on this biological model.

Brito et al. (2010) evaluated the latex of *Calotropis procera* (Asclepidaceae) where they observed that the ethyl acetate fractions varied between 50-99% and methanolic between 10-90% in the mortality of *A. salina*. The LC_{50} obtained for this species was 16.5 and 10 µg mL^{-1} respectively, being < 1000 µg mL^{-1} (high toxicity). The *n*-hexane and water fractions showed LC_{50} > 1000 µg mL^{-1}.

Although as previously reported in this and other studies the action of toxicity, David et al. (2019) did not find a lethal dose for latex from *B. parinariodides* and *P. amapa* on assay with *A. salina*. Thus, the in natura ingestion of both latexes does not negatively influence a health risk. The mortality rate on *A. salina* at a concentration of 1.000 µg mL^{-1}, results of 13.13% and 10.0%, respectively for *B. parinariodides* and *P. amapa*, considered extremely low.

Dahms (1996), Morrison (2002) and Silva et al. (2013) in studies, present about the importance in the development of products with sun protection capacity from the natural raw material coming from native or exotic plants. Consumers have a certain demand in search of products with natural principles in daily use. However, it is necessary to evaluate the real activity of extracts, oils, latex and other products from vegetables, so that it can promote real protection.

According to Menezes Filho et al. (2021) and Orlanda & Vale (2015), the various phytochemical groups such as phenolics and flavonoids have high antioxidant and photoprotective potential, preventing premature skin aging and various types of cancers such as melanoma. *M. zapota* latex has a high photoprotection capacity with maximum intensity at 273 nm, presenting a broad and intense band in the UVA band (Figure 4).

According to Rosa et al. (2008) and Orlanda & Vale (2015), UVA has greater relevance in terms of photoactivity where there is a high induction in the breakdown of cyclobutanepyridine dimers found in epidermal DNA. Natural products that express photoprotection activity can compete with synthetic sunscreen solutions. Violante et al. (2009) corroborates our statement, and also adds that the photoprotection test by UV-*Vis* spectrophotometry has high reliability and agility in obtaining reliable results in technical and scientific research.

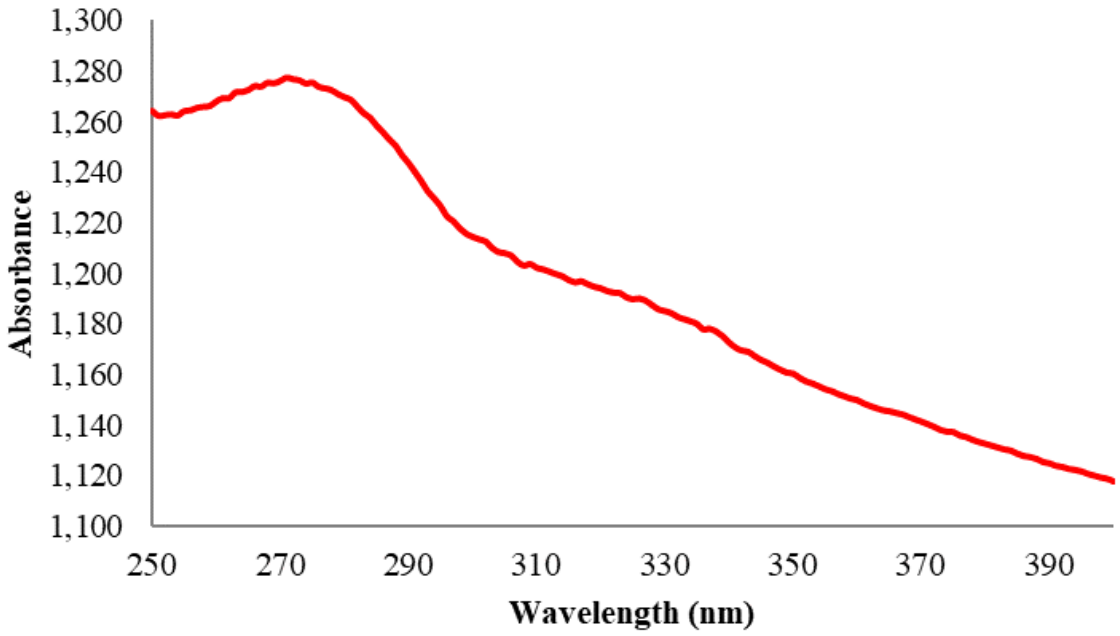

Figure 4. Assay of photoprotection activity at critical wavelength by UV-*Vis* spectrophotometry. Source: Authors, 2022.

4. Conclusions

Manilkara zapota latex exhibited a wide variety of phytogroups in the qualitative assay and moderate toxicity activity over the *Artemia salina* assay. Further studies should be carried out isolating these groups of

phytomolecules, thus knowing the quantitative profile of this product of the special metabolism of *M. zapota*.

Furthermore, one should focus on new biological activities to determine the possible positive effects on this natural product that can be applied in the pharmaceutical, agricultural and biotechnology industries.

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