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Processing

Analysis of the chemical constituents of *Thompson* atemoya seed oil

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Abstract-The objective of this study was to characterize the chemical composition in extracts of atemoya (*var*. Thompson) seed oil by spectrometric methods. The following extraction methods were performed: chemical extraction using hexane, mechanical extraction using a press, and partitioned extraction. The composition of each of the extracts was analysed by gas chromatography-mass spectrometry (GC-MS), and more than 100 compounds were identified. The major constituents of the hexane extraction were (Z)-hexadec-9-enal (49.42%) and triolein (23.28%), and the mechanically obtained extract contained elaidic acid (66.11%) and stearic acid (8.81%). In the partitioned extraction, the hydromethanolic fraction contained dihydroxyacetone (19.16%), 3-deoxy-d-mannoic lactone (16.34%), 5-hydroxymethylfurfural (10.77%), and 3-propanediol, 2-(hydroxymethyl)-2-nitro (9.89%); the hexane fraction contained gamma-sitosterol (31.73%), erucic acid (14.64%), stigmasterol (13.30%) and triolein (10.90%); the chloroform fraction contained gamma-sitosterol (22.11%), vaccenic acid (15.49%), triolein (14.65%) and stigmasterol (10.65%); and the ethyl acetate fraction contained (Z)-icos-9-enoic acid (31.28%), beta-sitosterol (16.29%), pentadecanoic acid (11.53%) and eicosanoic acid (8.01%).

Index terms: *Annona atemoya;* characterization; extraction; GC-MS.

Análise dos constituintes químicos do óleo de semente de atemoia *Thompson*

Resumo - O objetivo deste trabalho foi caracterizar a composição química do óleo de semente de atemoia *var*. Thompson através de métodos espectrométricos. Foi realizada extração química utilizando hexano, extração mecânica utilizando prensa e uma extração particionada. A composição de cada uma das extrações foi analisada por CG/EM, e mais de 100 compostos foram identificados. Os constituintes majoritários de cada extração foram hexano: (Z)-hexadec-9-enal (49,42%) e trioleína (23,28%); prensa: ácido elaídico (66,11%) e ácido esteárico (8,81%). Na extração particionada, têm-se as frações hidrometanólica: di-hidroxiacetona (19,16%), lactona 3-desoxi-d-mannóica (16,34%), 5-hidroximetilfurfural (10,77%), 3-propanodiol, 2-(hidroximetil)-2-nitro (9,89%); hexânica: gama-sitosterol (31,73%), ácido erúcico (14,64%), estigmasterol (13,30%) e trioleína (10,90%); clorofórmica: gama-sitosterol (22,11%), ácido vacênico (15,49%), trioleína (14,65%) e estigmasterol (10,65%); acetato de etila: ácido (Z)-icos-9-enoico (31,28%), beta-sitosterol (16,29%), ácido pentadecanoico (11,53%) e ácido icosanoico (8,01%).

Termos para indexação: *Annona atemoya*, caracterização, extração, CG/EM.

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Introduction

The family Annonaceae comprises a large number of genera and species, with approximately 2500 species, and occurs in all primary and secondary tropical forests worldwide (COUVREUR et al., 2019). Atemoya is an interspecific hybrid fruit resulting from artificial crossing between cherimoya (*Annona cherimola* Mill.) and sugar apple (*Annona squamosa* L.). The first recorded crosses occurred in 1908, and there are also natural hybrids (MORTON, 1987). The fruits are known to have a pleasant aroma and flavour (BARON et al., 2018) and have been gaining market share. The fame of *Annona* goes far beyond flavour. Since the 1980s, the acetogenins of Annonaceae have been extensively studied due to their highly valuable pharmacological properties, such as antitumour activity (CAVÉ et al., 1997; CHANG et al., 1999; DURET et al., 1997; DURET et al., 1998; HOYE and ZHUANG, 1988; KAZMAN et al., 2020; NESKE et al., 2020; RUPPRECHT et al., 1986; SOUZA et al., 2008; VILA-NOVA et al., 2011; ZAFRA-POLO et al., 1998).

Extracts from various parts of Annonaceae plants have been shown to have significant antibacterial, antifungal, anti-inflammatory, antioxidant, and cytotoxic properties (COSTA et al., 2017; MA et al., 2017; TAECHOWISAN et al., 2016; TAMFU et al., 2019). Regardless of the proportions and identity of the constituents in the total extract, they have the potential for biological activity (KRIMAT et al., 2017). The seeds are considered the plant component with the greatest diversity and richness of phytochemicals, including acetogenins, alkaloids and phenolic compounds (KAZMAN et al., 2020).

Different extraction methods give rise to different constituents. Hexane extracts constituents with low polarity, while mechanical extraction is considered an eco-friendly alternative with good yield (PETROPOULOS et al., 2018). Methanol allows the extraction of a greater number of compounds via the liquid-liquid partitioning process with solvents of increasing polarity (KRIM et al., 2020). The objective of this study was to determine the constituents of atemoya (*var* Thompson) seed oil obtained from different extraction methods by gas chromatography-mass spectrometry (GC-MS) analysis.

Experimental part

Seed preparation and extractions

Fresh fruits of atemoya variety Thompson from the voucher specimen #30,249 deposited in the Herbarium of ESAL at Federal University of Lavras (UFLA) (98 Kg, around 480 fruits) have been collected from 30 different 12-year-old plants. Those fruits have been purchased from a farmer in the city of Lavras/MG/Brazil (21° 14′ South, 44° 59′ West) during the 2016/2017 harvest. The seeds were removed, washed in deionized water, dried in a forced-air oven at a temperature of 60-65 °C for 7 days and placed in hermetically sealed pots for subsequent extraction.

Part of the atemoya whole seeds (150 g) was crushed, sieved (150 mesh) and placed in hexane reflux (500 mL) for 24 hours at 100 °C (KOUBAA et al., 2017). The solvent was removed by rotary evaporation, and the yield was calculated. The material obtained in this procedure was called ASO_H (atemoya seed oil, ASO).

A second portion of the seeds (150 g) was also ground and evaluated for grain size (150 mesh). Mechanical extraction with a bench screw press (Yoda - MQO001) was used to obtain the resulting material, which was called ASO_P. At the end of the process, the yield was calculated (MAGALHÃES et al., 2020).

The third portion of the seeds was subjected to partitioned extraction. A total of 180 g of dried, ground, and sieved seeds (150 mesh) was placed in 500 mL of methanol. The seeds were left in contact with the methanol for 3 days at a temperature of 25 ± 2 °C. After filtration, the residue was extracted two more times, as previously described. The solvent was removed by rotary evaporation at 50 °C and lyophilized, leaving 6.714 g of crude extract (COSTA et al., 2017).

The crude extract was resuspended in methanol (20 mL) + ultrapure water (20 mL) and vigorously stirred at room temperature (25 ± 2 °C), and after 45 minutes of decantation, it was centrifuged for 10 minutes at 3500 xg. The supernatant was collected and called the hydromethanolic fraction (ASO_{HM}). The precipitate was subjected to extraction with hexane (20 mL) with vigorous stirring at room temperature (25 ± 2 °C) and 15 minutes of decantation. This process was repeated 4 times, and centrifugation was performed for 10 minutes at 3500 xg. The supernatant was collected and called the hexane fraction (ASO_{HEX}). A total of 20 mL of chloroform was added to the precipitate, followed by vigorous stirring at room temperature (25 ± 2 °C) and 15 minutes of decantation. This process was repeated 4 times, and then centrifugation was performed for 10 minutes at 3500 xg. The supernatant was collected and called the chloroform fraction (ASO_{CHLO}). A total of 20 mL of ethyl acetate was added to the precipitate, followed by vigorous stirring at room temperature (25 ± 2 °C) and 15 minutes of decantation. This process was repeated 4 times, and at the end, there was no phase separation. The entire extract was collected and called the ethyl acetate fraction (ASO_{ACE}) (COSTA et al., 2017).

The elimination of all solvents was performed in an open amber flask protected from light in a desiccator until reaching constant weight. Finally, the samples were sent to the Analytical Centre of the Chemistry Institute of the University of Sao Paulo (USP) for analysis by GC-MS (QP2020 - Shimadzu). The analyses were performed using a capillary column (30 m x 0.25 mm x 0.25 µm, ZB-5HT, CarbolWAX). The injection conditions were 250 °C and 2.50 mL min^{-1} for ASO_H and ASO_P and 320 °C and 3.00 mL min^{-1} for ASO_{HM}, ASO_{HEX}, ASO_{CHLO} and ASO_{ACE}. The components of the ASO samples were identified through standard spectrum libraries for organic compounds, such as NIST107 and Wiley 229.

Results and discussion

The yield of each extraction is shown in Table 1, and the constituents identified in each extract by GC-MS and their biological activities are shown in Tables 2, 3, 4, 5, 6 and 7.

Table 1. ASO yield in each extraction

Extraction	Mass obtained (g)	Yield (%)
1st extraction ASO_H	23.881	15.92
2nd extraction ASO_P	22.562	14.97
3rd partitioned extraction	6.714	3.73
ASO_{HM}	2.173	32.37
ASO_{HEX}	0.466	6.94
ASO_{CHLO}	1.107	16.49
ASO_{ACE}	0.255	3.80

Table 1 shows the yield data for each extraction. As predicted, each extraction resulted in distinct constituents with diverse biological activities. These data are presented in detail for each constituent in Tables 2, 3, 4, 5, 6 and 7.

Each extraction presented 20 constituents, some of them with potential use for COVID-19 treatment. The four main constituents of the ASO_H extraction were:

i) Cis-9-hexadecenal (49.42%), a fatty aldehyde classified as agrochemical/attractant mainly used as a pheromone (TEAL et al., 1981) and also used as insecticide against mosquito larvae (GOYAL et al., 2019). Goyal et al has found much lower percentages in *Annona squamosa* essential oils seeds (11.82 and 6.78%, respectively to methanolic and hexanic extraction);

ii) Triolein (23.28%), a triglyceride formed by esterification of the three hydroxy groups of glycerol with oleic acid. Triolein is a component of Lorenzo's oil and used as adjuvant treatment for brain tumors (KIM et al., 2016);

iii) E,E,Z-1,3,12-nonadecatriene-5,14-diol (3.90%) with antidiabetic and antilipidemic properties (AHMADI et al., 2017);

iv) Gamma-sitosterol (3.36%), a member of the class of phytosterols that is a poriferast-5-ene carrying a beta-hydroxy substituent at position 3. A percentage of 17.40% has been found in the seed extract of *Annona squamosa* (BHARDWAJ et al., 2014). Gamma-sitosterol is a potentially useful candidate for COVID-19 (CHOWDHURY, 2020; LUO et al., 2020; MAURYA et al., 2020; NARKHEDE et al., 2020; WANG et al., 2020).

The four main constituents of the ASO_P extraction were:

i) Elaidic acid (66.11%), the trans-isomer of oleic acid anion, and also a potential biomarker in biological age (ZHAO et al., 2020). Nwaehujor et al,, (2020) working with *Annona muricata* leaf found 8.61% of this compound (NWAEHUJOR et al., 2020);

ii) Stearic acid (8.81%), a saturated long-chain fatty acid with immunoregulatory and anti-inflammatory properties (CALDER, 1998). *Annona squamosa* pericarp oil has been shown to have 12.23% of this compound and anti-hepatoma effect (CHEN et al., 2020);

iii) Gadoleyl alcohol (6.67%) has no activities listed;

iv) Palmitic acid (3.76%), a long-chain fatty acid and a straight-chain saturated fatty acid. It is a conjugate acid of a hexadecanoate. It has antitumoral and anti-inflammatory properties (HARADA et al., 2002; JOSHI-BARVE et al., 2007). The palmitic acid can be a successful anti-COVID-19 agent (ELFIKY, 2020). The crude hexanic extract from aerial parts of atemoya demonstrated 0.82% of this compound, cytotoxic and antimicrobial activity (RABÊLO et al., 2021).

The four main constituents of the ASO_{HM} extraction were:

i) Dihydroxyacetone (19.16%), a ketotriose consisting of acetone bearing hydroxy substituents at positions 1 and 3. It is used in treatment of vitiligo, as antifungal, and photoprotector (FESQ et al., 2001; FUSARO and RICE, 2005; STOPIGLIA et al., 2011);

ii) 3-deoxy-d-mannoic lactone (16.34%) with antimicrobial activity (SHOBANA et al., 2009);

iii) 5-hydroxymethylfurfural (10.77%), a member of the class of furans. It has a role as an indicator of the product of the Maillard reaction, has antioxidant action, and is an antiproliferative (ZHAO et al., 2013). It can also have a potential use in improving hypoxemia in COVID-19 patients (WOYKE et al., 2020);

iv) 2-(hydroxymethyl)-2-nitropropane-1,3-diol (9.89 %), a propylene glycol used as microbicide and bacteriostat in disinfectants and industrial preservatives and as a disinfectant to control disease organisms in livestock and poultry areas on farms and equipment (AMPONIN et al., 2020; BEIER et al., 2008).

The four main constituents of the ASO_{HEX} extraction were:

i) Gamma-sitosterol (31.73%);

ii) Erucic acid (14.64%), a docosenoic acid having a cis- double bond at C-13 with antimicrobial, antitumor, and cardiac properties (ALTINOZ et al., 2018; SURESH et al., 2014);

iii) Stigmasterol (13.30%), a 3-beta-sterol and member of the phytosterols. It derives from a hydride of a stigmastane (ULBRICHT, 2016) and has anti-nociceptive action (WALKER et al., 2017). It can be a potentially useful candidate for COVID-19 (HUANG et al., 2020; KAR et al., 2020; LUO et al., 2020; ZHENJIE et al., 2020);

iv) Triolein (10.90 %).

The four main constituents of the ASO_{CHLO} extraction were:mma-sitosterol (22.11%);Vaccenic acid (15.49%), an unsaturated fatty acid with anti-inflammatory properties (SALES-CAMPOS et al., 2013); (14.65%); Stigmasterol (10.65%).

The four main constituents of the ASO_{ACE} extraction were:

i) Gadoleic acid (31.28%), an icosenoic acid having a cis- double bond at position 9 with antioxidant properties and cardiovascular disease protection (BAILÃO et al., 2015);

ii) Beta-sitosterol (16.29%), a member of the class of phytosterols that is stigmast-5-ene substituted by a beta-hydroxy group at position 3. It has antioxidant and antimicrobial properties, and induces apoptosis in MCF-7 cells (CHAI et al., 2008; HIDAYATHULLA et al., 2018). It is also a potentially useful candidate for COVID-19 (CHOWDHURY, 2020; LUO et al., 2020; MAURYA et al., 2020; WANG et al., 2020; ZHENJIE et al., 2020);

iii) Pentadecanoic acid (11.53%), a straight-chain saturated fatty acid containing fifteen-carbon atoms. It is a potential natural agrochemical and antifungal agent (DING et al., 2019; MANILAL et al., 2010);

iv) Eicosanoic acid (8.01%), a saturated long-chain fatty acid with a 20-carbon backbone with antioxidant and antiproliferative properties (HARUENKIT et al., 2010).

Among the parts of the atemoya (pulp, leaves and seeds), the seeds are the richest in phytochemicals, including anonaceous acetogenins, alkaloids and phenolic compounds (KAZMAN et al., 2020). In the ASO_P, ASO_{HEX}, ASO_{CHLO} and ASO_{ACE} extractions the compound octadecanoic acid has been observed. This fatty acid also has been found in the study by Wu et al (2005), conducted with oil extraction from atemoya seeds harvested in China in 1996 (WU et al., 2005).

In a study with volatile components of atemoya leaves, Campos et al (2019) reported a considerable amount of hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes, oxygenated sesquiterpenes and other classes (CAMPOS et al., 2019). Among these compounds, we emphasize the similar presence of copaene, germacrene D, beta-ylangen in the ASO_P extraction; and germacrene and n-decane in ASO_H. Thus, the essential oil of atemoya leaves proved to be rich in monoterpenes and sesquiterpenes (CAMPOS et al., 2014; KUMAR et al., 2021).

For being a hybrid species, Annona atemoya has phytochemical components common to Annona squamosa and Annona cherimola, regardless of the parts of the plant from which their constituents are isolated. (KAZMAN et al., 2020).

The fatty acid profile found in Annona squamosa seeds has shown to have some components that were also found in Atemoya seed oil extractions. n-hexadecanoic acid; ergost-5-en-3-ol; stigmasta-5,22-dien-3-ol; gamma-sitosterol and octadecanoic acid (ASO_P, ASO_{HEX}, ASO_{CHLO} and ASO_{ACE}) (ZAHID et al., 2018).

Table 2. Bioactive components of ASO_H analysed by GC-MS.

Compound	Area (%)	Molecular Formula	Chemical Classification	Bioactivity
(Z)-hexadec-9-enal	49.42	$C_{16}H_{30}O$	Fatty aldehyde	Pheromone (TEAL et al., 1981)
2,3-bis[[(Z)-octadec-9-enoyl]oxy]propyl (Z)-octadec-9-enoate	23.28	$C_{57}H_{104}O_6$	Triacylglycerol	Adjuvant treatment for brain tumours (KIM et al., 2016)
(3E,12Z)-nonadeca-1,3,12-triene-5,14-diol	3.90	$C_{19}H_{34}O_2$	Not applicable	Antidiabetic and antilipidaemic (AHMADI et al., 2017)
(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5S)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	3.36	$C_{29}H_{50}O$	Gamma-sitosterol	Phytosterol (LIU et al., 2012) Potentially useful candidate for COVID-19 (CHOWDHURY, 2020; LUO et al., 2020; MAURYA et al., 2020; NARKHEDE et al., 2020; WANG et al., 2020)
Decane	2.09	$C_{10}H_{22}$	Decane	Marker/mechanism in erythema and sleep apnoea, obstructive (AOKI et al., 2017; MALLAMPATI et al., 2010)
1-methyl-3-methylidene-8-propan-2-yltricyclo[4.4.0.02,7]decane	1.82	$C_{15}H_{24}$	Sesquiterpene	Antibacterial (AJIBOYE et al., 2016) Sesquiterpene contributing to the flavour and aroma of wine (DUEHOLM et al., 2019)
(3S,8S,9S,10R,13R,14S,17R)-17-[(E,2R,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	1.65	$C_{29}H_{48}O$	Phytosterol	Phytosterol (ULBRICHT, 2016) and anti-nociceptive (WALKER et al., 2017) Potentially useful candidates for COVID-19 (HUANG et al., 2020; KAR et al., 2020; LUO et al., 2020; ZHENJIE et al., 2020)
Ethyl-octadec-9-enoate	1.58	$C_{20}H_{38}O_2$	Not applicable	Antitumoural, pheromone and biomarker of excessive alcohol chronic use (CASTILLO et al., 2012; GONZÁLEZ-ILLÁN et al., 2011; MANOSROI et al., 2012; POLITI et al., 2011)
[(E)-10,13,13-trimethyltetradec-11-enyl] acetate	1.47	$C_{19}H_{36}O_2$	Not applicable	No activities listed.
(5-formyl-5,9-dimethyl-14-tetracyclo [11.2.1.01,10.04,9] hexadecanyl)methyl acetate	1.31	$C_{22}H_{34}O_3$	Not applicable	No activities listed.

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Undecane	1.22	$C_{11}H_{24}$	Undecane	Pheromone (LENZ et al., 2013; YAMAGATA et al., 2005)
(3E,12Z)-nonadeca-1,3,12-triene-5,14-diol	1.15	$C_{19}H_{34}O_2$	Not applicable	No activities listed.
3-O-(2,2-dimethylpropyl)1-O-tridecyl propanedioate	0.90	$C_{21}H_{40}O_4$	Not applicable	No activities listed.
[(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-yl] acetate	0.90	$C_{31}H_{52}O_2$	Steroid ester	Antioxidant, antitumoural, anti-diarrhoeal, analgesic, anti-diabetic, antiproliferative, antibacterial and anti-inflammatory (EL-SHAZLY et al., 2012; ISLAM, 2020; OGUNLAKIN et al., 2020; STEFANI et al., 2019)
2-butyloctan-1-ol	0.85	$C_{12}H_{26}O$	Alkyl alcohol	Antioxidant, anti-inflammatory and pheromone (AHMED et al., 2017; TANG et al., 2016)
(3S)-17-(5,6-dimethylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	0.83	$C_{28}H_{48}O$	Phytosterol	Adjuvant in determination of sitosterolemia, synthesis of vitamins D (KOMBA et al., 2019; LEE et al., 2020)
(1S,6S,7S,8S)-1-methyl-3-methylidene-8-propan-2-yltricyclo[4.4.0.02,7]decane (1S,6S,7S,8S)-1-methyl-3-methylidene-8-propan-2-yltricyclo[4.4.0.02,7]decane	0.80	$C_{15}H_{24}$	Sesquiterpene	Pheromone and antimicrobial (FLATH et al., 1994; JELIAZKOVA et al., 2018)
(1Z,6Z,8S)-1-methyl-5-methylidene-8-propan-2-ylcyclodeca-1,6-diene	0.80	$C_{15}H_{24}$	Sesquiterpene	Anti-inflammatory, anti-oedematogenic, antiproliferative, antileishmanial, antibacterial, antifungal and antioxidant (OLIVEIRA-TINTINO et al., 2018; SIQUEIRA et al., 2015)
(2Z)-2,6-dimethylocta-2,7-diene-1,6-diol	0.80	$C_{10}H_{18}O_2$	Not applicable	No activities listed.

Table 3. Bioactive components of ASO_p analysed by GC-MS.

Compound	Area (%)	Molecular Formula	Chemical Classification	Bioactivity
(E)-octadec-9-enoic acid	66.11	$C_{18}H_{34}O_2$	Long-chain fatty acid	Potential biomarker in biological age (ZHAO et al., 2020)
Octadecanoic acid	8.81	$C_{18}H_{36}O_2$	Straight chain fatty acid	Immunoregulatory and anti-inflammatory (CALDER, 1998)
(Z)-icos-9-en-1-ol	6.67	$C_{20}H_{40}O$	Not applicable	No activities listed.
Hexadecanoic acid	3.76	$C_{16}H_{32}O_2$	Straight chain fatty acid	Antitumoural, anti-inflammatory (HARADA et al., 2002; JOSHI-BARVE et al., 2007) Can be successful as anti-COVID-19 agent (ELFIKY, 2020)
(1S,6S,7S,8S)-1-methyl-3-methylidene-8-propan-2-yltricyclo[4.4.0.02,7]decane (1S,6S,7S,8S)-1-methyl-3-methylidene-8-propan-2-yltricyclo[4.4.0.02,7]decane	2.85	$C_{15}H_{24}$	Sesquiterpene	Antibacterial (AJIBOYE et al., 2016) Sesquiterpenes contribute to the flavour and aroma of wine (DUEHOLM et al., 2019)
(E)-octadec-9-en-1-ol	2.24	$C_{18}H_{36}O$	Fatty alcohol	No activities listed.
(1S,6S,7S,8S)-1-methyl-3-methylidene-8-propan-2-yltricyclo[4.4.0.02,7]decane (1S,6S,7S,8S)-1-methyl-3-methylidene-8-propan-2-yltricyclo[4.4.0.02,7]decane	1.31	$C_{15}H_{24}$	Sesquiterpene	Pheromone and antimicrobial (FLATH et al., 1994; JELIAZKOVA *et al.*, 2018)
(1R,4aR,4bS,7S,10aR)-7-ethenyl-1,4a,7-trimethyl-3,4,4b,5,6,8,10,10a-octahydro-2H-phenanthrene-1-carbaldehyde	1.24	$C_{20}H_{30}O$	Diterpenoid	No activities listed.
(3E,12Z)-nonadeca-1,3,12-triene-5,14-diol	1.02	$C_{19}H_{34}O_2$	Not applicable	No activities listed.
2-[(9Z,12Z)-octadeca-9,12-dienoxy]ethanol	0.97	$C_{20}H_{38}O_2$	Not applicable	No activities listed.
Heptacos-1-ene	0.92	$C_{27}H_{54}$	Fatty Acyl	Pheromone (EVISON et al., 2012)

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(1Z,6Z,8S)-1-methyl-5-methylidene-8-propan-2-ylcyclodeca-1,6-diene	0.83	$C_{15}H_{24}$	Sesquiterpene	Anti-inflammatory, anti-oedematogenic, antiproliferative, antileishmanial, antibacterial, antifungal and antioxidant (OLIVEIRA-TINTINO et al., 2018; SIQUEIRA et al., 2015)
5,5,9-trimethyl-14-methylidenetetracyclo[11.2.1.01,10.04,9]hexadecane	0.75	$C_{20}H_{32}$	Diterpene	Human metabolite and anti-allergic (CHEENPRACHA et al., 2006; LINGWOOD and SIMONS, 2010)
(S,1Z,6Z)-8-isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	0.61	$C_{15}H_{24}$	Sesquiterpene	Anti-inflammatory, anti-oedematogenic, antiproliferative, antileishmanial, antibacterial, antifungal and antioxidant (OLIVEIRA-TINTINO et al., 2018; SIQUEIRA et al., 2015)
(S,1Z,6Z)-8-isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	0.48	$C_{15}H_{24}$	Sesquiterpene	Anti-inflammatory, anti-oedematogenic, antiproliferative, antileishmanial, antibacterial, antifungal and antioxidant (OLIVEIRA-TINTINO et al., 2018; SIQUEIRA et al., 2015)
(S,1Z,6Z)-8-isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	0.38	$C_{15}H_{24}$	Sesquiterpene	Anti-inflammatory, anti-oedematogenic, antiproliferative, antileishmanial, antibacterial, antifungal and antioxidant (OLIVEIRA-TINTINO et al., 2018; SIQUEIRA et al., 2015)
(2E,4E)-deca-2,4-dienal	0.35	$C_{10}H_{16}O$	Aldehyde	No activities listed.
(1R,2S,6S,7S,8S)-1,3-dimethyl-8-propan-2-yltricyclo[4.4.0.02,7]dec-3-ene	0.29	$C_{15}H_{24}$	Sesquiterpene	Antioxidant, anti-inflammatory, and anticancer (KETHA et al., 2020)
(S,1Z,6Z)-8-isopropyl-1-methyl-5-methylenecy	0.20	$C_{15}H_{24}$	Sesquiterpene	Anti-inflammatory, anti-oedematogenic, antiproliferative, antileishmanial, antibacterial, antifungal and antioxidant (OLIVEIRA-TINTINO et al., 2018; SIQUEIRA et al., 2015)

Table 4. Bioactive components of ASO_{HM} analysed by GC-MS.

Compound	Area (%)	Molecular Formula	Chemical Classification	Bioactivity
Dihydroxyacetone	19.16	$C_3H_6O_3$	Monosaccharide	Treatment of vitiligo, antifungal, photoprotector (FESQ et al., 2001; FUSARO and RICE, 2005; STOPIGLIA et al., 2011)
3,5-dihydroxy-6-(hydroxymethyl)oxan-2-one	16.34	$C_6H_{10}O_5$	Not applicable	Antimicrobial activity (SHOBANA et al., 2009)
5-hydroxymethylfurfural	10.77	$C_6H_6O_3$	Aldehyde	Antioxidant, antiproliferative (ZHAO et al., 2013) Potentially used to improve hypoxemia in COVID-19 patients (WOYKE et al.,2020)
2-(hydroxymethyl)-2-nitropropane-1,3-diol	9.89	$C_4H_9NO_5$	Propylene Glycol	Antimicrobial (AMPONIN et al., 2020; BEIER et al., 2008)
4-hydroxy-3-methylbutan-2-one	6.68	$C_5H_{10}O_2$	Beta-hydroxy ketone	Hypoglycaemic, antiviral, anti-inflammatory, antioxidant, and anti-apoptotic (AVETISYAN et al., 1982; HAFEZ et al., 2020)
6-oxoheptanoic acid	5.37	$C_7H_{12}O_3$	Oxo fatty acid	No activities listed.
4-hydroxyoxolan-2-one	4.19	$C_4H_6O_3$	Lactone	C4 chiral synthon for the synthesis of chiral pharmaceuticals and nutraceuticals such as HIV inhibitors, synthetic statins, and L-carnitine (LEE; PARK, 2009)
3-hydroxyoxolan-2-one	3.37	$C_4H_6O_3$	Lactone	Antibacterial (MURUGAN et al., 2013)
1-(2-hydroxypropoxy)propan-2-ol	3.25	$C_6H_{14}O_3$	Solvent	Larvicidal (SHENG et al., 2020)
1-(2-methyltetrazol-5-yl)ethenyl acetate	3.20	$C_6H_8N_4O_2$	Solvent	No activities listed.
5,5-dimethyl-1,3-oxazolidine-2,4-dione	2.98	$C_5H_7NO_3$	Oxazole	Pancreatic stone dissolution and anticonvulsant (NODA et al., 1984; OKAMOTO et al., 1977)
2,3-dihydroxypropyl acetate	2.97	$C_5H_{10}O_4$	Glyceride	Potential mosquito repellent (GADDAGUTI et al., 2016)
3-methylideneoxolane-2,5-dione	2.65	$C_5H_4O_3$	Transferase	*In vitro* cytotoxic activity against human breast tumours (POUDEL et al., 2015)
3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one	1.70	$C_6H_8O_4$	Pyrone	Antioxidant (MOZAFARI et al., 2018)
Pent-4-enoic acid	1.62	$C_5H_8O_2$	Fatty Acids, Unsaturated	Marker/mechanism in fatty liver and Reye syndrome (GHONGHADZE et al., 2017; TANG et al., 1995)
Furan-2-ylmethanol	1.60	$C_5H_6O_2$	Furan	Marker/mechanism in adenoma, dermatitis, allergic contact, dermatitis, irritant, kidney neoplasms and respiratory hypersensitivity (FRANKO et al., 2012; SPALDING et al., 2000)
Furan-2-carbaldehyde	1.32	$C_5H_4O_2$	Furaldehyde	Agrochemical (BADAWY, 2008)
5-oxo-2-propylhexanal	1.11	$C_9H_{16}O_2$	Not applicable	No activities listed.
2,5-dimethyloxolane-3,4-dione	0.97	$C_6H_8O_3$	Not applicable	No activities listed.

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Table 5. Bioactive components of ASO_{HEX} analysed by GC-MS.

Compound	Area (%)	Molecular Formula	Chemical Classification	Bioactivity
(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5S)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	31.73	$C_{29}H_{50}O$	Gamma-sitosterol	Phytosterol (LIU; CHEN; SHI; WANG et al., 2012) Potentially useful candidate for COVID-19 (CHOWDHURY, 2020; LUO et al., 2020; MAURYA et al., 2020; NARKHEDE et al., 2020; WANG et al., 2020)
(Z)-docos-13-enoic acid	14.64	$C_{22}H_{42}O_2$	Fatty Acid, Unsaturated	Antimicrobial, antitumour and cardiac (ALTINOZ et al., 2018; SURESH et al., 2014)
(3S,8S,9S,10R,13R,14S,17R)-17-[(E,2R,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	13.30	$C_{29}H_{48}O$	Phytosterol	Phytosterol (ULBRICHT, 2016) and anti-nociceptive(WALKER et al., 2017) Potentially useful candidate for COVID-19 (HUANG et al., 2020; KAR et al., 2020; LUO et al., 2020; ZHENJIE et al., 2020)
2,3-bis[[(Z)-octadec-9-enoyl]oxy]propyl (Z)-octadec-9-enoate	10.90	$C_{57}H_{104}O_6$	Fat unsaturated	Adjuvant treatment for brain tumours (KIM et al., 2016)
(3S)-17-(5,6-dimethylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	6.01	$C_{28}H_{48}O$	Phytosterol	Adjuvant in determination of sitosterolemia, synthesis of vitamin D (KOMBA et al., 2019; LEE et al., 2020)
Hexadecanoic acid	5.12	$C_{16}H_{32}O_2$	Straight chain fatty acid	Antitumour, anti-inflammatory (HARADA et al., 2002; JOSHI-BARVE et al., 2007) Can be successful as an anti-COVID-19 agent (ELFIKY, 2020)
(11E,13Z)-octadeca-1,11,13-triene	3.35	$C_{18}H_{32}$	Not applicable	Antitumour (SWANTARA et al., 2019)
2-[12-(oxiran-2-yl)dodecyl]oxirane	2.70	$C_{16}H_{30}O_2$	Not applicable	No activities listed.
Icosanoic acid	2.33	$C_{20}H_{40}O_2$	Fatty Acid	Antioxidant and antiproliferative (HARUENKIT et al., 2010)
1,3-dihydroxypropan-2-yl hexadecanoate	2.03	$C_{19}H_{38}O_4$	Glyceride	Marker/mechanism in diabetes mellitus type 2 (PICCOLO et al., 2016)
Methyl 8-(2-hexylcyclopropyl)octanoate	1.08	$C_{18}H_{34}O_2$	Not applicable	No activities listed.
5-(6-bromo-2-hydroxy-2,5,5,8a-tetramethyl-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl)-3-methylidenepentane-1,2-diol	0.98	$C_{20}H_{35}BrO_3$	Not applicable	No activities listed.

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(1S,4R,5R)-4-methyl-1-propan-2-ylbicyclo[3.1.0]hexan-3-one	0.96	$C_{10}H_{16}O$	Bicyclic Monoterpene	Antifungal (BAI et al., 2020)
N-(2-hydroxyethyl)octanamide	0.83	$C_{10}H_{21}NO_2$	Ethanolamine	Larvicidal (CHANDRASEKARAN et al., 2018)
2-(dimethylamino)ethyl 2-methylprop-2-enoate	0.79	$C_8H_{15}NO_2$	Methacrylate	Used for drug delivery (XU et al., 2006)
Pentadecanal	0.76	$C_{15}H_{30}O$	Aldehyde	Antioxidant and cytotoxicity (SHAROPOV et al., 2020)
1,3-dihydroxypropan-2-yl hexadecanoate	0,71	$C_{19}H_{38}O_4$	Glyceride	Marker/mechanism in diabetes mellitus type 2 (PICCOLO et al., 2016)
1-hexadecyl-2,3-dihydro-1H-indene	0.68	$C_{25}H_{42}$	Not applicable	No activities listed.
6-ethyldecan-3-yloxy(trimethyl)silane	0.62	$C_{15}H_{34}OSi$	Not applicable	No activities listed.

Table 6. Bioactive components of ASO_{CHLO} analysed by GC-MS.

Compound	Area (%)	Molecular Formula	Chemical Classification	Bioactivity
(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5S)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	22.11	$C_{29}H_{50}O$	Gamma-sitosterol	Phytosterol (LIU; CHEN; SHI; WANG et al., 2012) Potentially useful candidate for COVID-19 (CHOWDHURY, 2020; LUO et al., 2020; MAURYA et al., 2020; NARKHEDE et al., 2020; WANG et al., 2020)
(Z)-octadec-11-enoic acid	15.49	$C_{18}H_{34}O_2$	Fatty Acid, Unsaturated	Anti-inflammatory (SALES-CAMPOS et al., 2013)
2,3-bis[[(Z)-octadec-9-enoyl]oxy]propyl (Z)-octadec-9-enoate	14.65	$C_{57}H_{104}O_6$	Fats unsaturated	Adjuvant treatment for brain tumours (KIM et al., 2016)
(3S,8S,9S,10R,13R,14S,17R)-17-[(E,2R,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	10.65	$C_{29}H_{48}O$	Phytosterol	Phytosterol (ULBRICHT, 2016) and anti-nociceptive(WALKER et al., 2017) Potentially useful candidate for COVID-19 (HUANG et al., 2020; KAR et al., 2020; LUO et al., 2020; ZHENJIE et al., 2020)
(3S)-17-(5,6-dimethylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	5.57	$C_{28}H_{48}O$	Phytosterol	Adjuvant in determination of sitosterolemia, synthesis of vitamin D (KOMBA et al., 2019; LEE et al., 2020)
Dodecyl 3-(3-dodecoxy-3-oxopropyl) sulfanylpropanoate	4.56	$C_{30}H_{58}O_4S$	Propionate	Antioxidant (CHIEN; BOSS, 1972)

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Hexadecanoic acid	4.23	$C_{16}H_{32}O_2$	Straight chain fatty acid	Antitumour, anti-inflammatory (HARADA et al., 2002; JOSHI-BARVE et al., 2007) Can be successful as an anti-COVID-19 agent (ELFIKY, 2020)
1,3-dihydroxypropan-2-yl hexadecanoate	3.48	$C_{19}H_{38}O_4$	Glyceride	Marker/mechanism in diabetes mellitus type 2 (PICCOLO et al., 2016)
(9Z,12Z)-octadeca-9,12-dienoic acid	3.34	$C_{18}H_{32}O_2$	Fatty Acids, Omega-6	Therapeutic use: Barth syndrome, dermatitis, fatty liver, hepatitis C, hypercholesterolemia, hyperlipidaemia, hypertriglyceridemia, lung neoplasms, neoplasm invasiveness and metastasis and anti-inflammatory (CHEN et al., 2013; JANG et al., 2008; KOTHAPALLI et al., 2020; MALHOTRA et al.,2014; SHEU et al., 2002; TORIYAMA-BABA et al., 2001; YANO et al., 2007) Binding pocket in the locked structure of SARS-CoV-2 spike protein (TOELZER et al., 2020)
Octadecanoic acid	3.14	$C_{18}H_{36}O_2$	Straight chain fatty acid	Immunoregulatory and anti-inflammatory (CALDER, 1998)
(9Z,12Z)-octadeca-9,12-dienoyl chloride	3.02	$C_{18}H_{31}ClO$	Not applicable	No activities listed.
6-ethyldecan-3-yloxy(trimethyl)silane	1.62	$C_{15}H_{34}OSi$	Not applicable	No activities listed.
1,3-dihydroxypropan-2-yl pentadecanoate	1.57	$C_{18}H_{36}O_4$	Glyceride	Potentially used in the detection of colorectal cancer (GOEDERT et al., 2014; MONLEÓN et al., 2009; PHUA et al., 2014; RITCHIE et al., 2010; SINHA et al., 2016; WEIR et al., 2013)
Octadecanamide	1.21	$C_{18}H_{37}NO$	Amide	Potentially used in the detection of colorectal cancer (GOEDERT et al., 2014; MONLEÓN et al., 2009; PHUA et al., 2014; RITCHIE et al., 2010; SINHA et al., 2016; WEIR et al., 2013)
N-(2-hydroxyethyl)octanamide	1.20	$C_{10}H_{21}NO_2$	Ethanolamine	Larvicidal (CHANDRASEKARAN et al., 2018)
5,5-bis(heptylsulfanyl)pentane-1,2,3-triol	1.03	$C_{19}H_{40}O_3S_2$	Not applicable	No activities listed.
(3-hexadecanoyloxy-2-trimethylsilyloxypropyl) hexadecanoate	0.86	$C_{38}H_{76}O_5Si$	Not applicable	No activities listed.
3,5-di-*tert*-butylphenol	0.82	$C_{14}H_{22}O$	Phenol	No activities listed.
(Z)-docos-13-enamide	0.77	$C_{22}H_{43}NO$	Fatty Acids, Unsaturated	Antidepressant and anxiolytic-like behavioural effect (LI et al., 2017)

Table 7. Bioactive components of ASO_{ACE} analysed by GC-MS.

Compound	Area (%)	Molecular Formula	Chemical Classification	Bioactivity
(Z)-icos-9-enoic acid	31.28	$C_{20}H_{38}O_2$	Fatty acid derivative	Antioxidant and cardiovascular disease protection (BAILÃO et al., 2015)
(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	16.29	$C_{29}H_{50}O$	Phytosterol	Antioxidant, antimicrobial and induces apoptosis in MCF-7 cells (CHAI et al., 2008; HIDAYATHULLA et al., 2018) Potentially useful candidate for COVID-19 (CHOWDHURY, 2020; LUO et al., 2020; MAURYA et al., 2020; WANG et al., 2020; ZHENJIE et al., 2020)
Pentadecanoic acid	11.53	$C_{15}H_{30}O_2$	Fatty Acid	Potential natural agrochemical and antifungal (DING et al., 2019; MANILAL et al., 2010)
Eicosanoic acid	8.01	$C_{20}H_{40}O_2$	Fatty Acid	Antioxidant and antiproliferative (HARUENKIT et al., 2010)
(3S,8S,9S,10R,13R,14S,17R)-17-[(E,2R,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	6.41	$C_{29}H_{48}O$	Phytosterol	Phytosterol (ULBRICHT, 2016) and anti-nociceptive (WALKER et al., 2017) Potentially useful candidate for COVID-19 (HUANG et al., 2020; KAR et al., 2020; LUO et al., 2020; ZHENJIE et al., 2020)
(3S)-17-(5,6-dimethylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	3.13	$C_{28}H_{48}O$	Phytosterol	Adjuvant in determination of sitosterolemia, synthesis of vitamin D (KOMBA et al., 2019; LEE et al., 2020)
(1E,4E)-1,5-diphenylpenta-1,4-dien-3-one	2.86	$C_{17}H_{14}O$	Pentanone	Antioxidant (NASEER et al., 2015)
(9Z)-octadeca-9,17-dienal	2.63	$C_{18}H_{32}O$	Not applicable	Potential selective MAO-A inhibitor (depression, anxiety, and cognitive impairments in Alzheimer's and Parkinson's diseases) (MARGRET et al., 2015)
(Z)-hexadec-7-enal	2.35	$C_{16}H_{30}O$	Fatty aldehyde	Pheromone (TEAL et al., 1981)
Dioctyl benzene-1,2-dicarboxylate	1.97	$C_{24}H_{38}O_4$	Phthalic Acid	Antitumour (ANGELINI et al., 2011; HABIB; KARIM, 2012; MARUYAMA et al., 1994)
[2-[2-[(E)-3-phenylprop-2-enoyl]oxyphenyl]phenyl] (E)-3-phenylprop-2-enoate	1.88	$C_{30}H_{22}O_4$	Not applicable	No activities listed.
[(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-yl] carbonochloridate	1.78	$C_{28}H_{45}ClO_2$	Not applicable	Used in long circulating liposomes (DEODHAR et al., 2020)

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(9Z,12Z)-octadeca-9,12-dienoyl chloride	1.49	$C_{18}H_{31}ClO$	Not applicable	No activities listed.
(1E,4E)-1,5-diphenylpenta-1,4-dien-3-one	1.45	$C_{17}H_{14}O$	Pentanone	Antioxidant (NASEER et al., 2015)
(Z)-octadec-9-enamide	1.43	$C_{18}H_{35}NO$	Food Additives	Therapeutic use: amnesia and seizures (HEO et al., 2003; WU et al., 2003)
4-[[(E)-2-methoxyhexadec-4-enoxy]methyl]-2,2-dimethyl-1,3-dioxolane	1.27	$C_{23}H_{44}O_4$	Not applicable	No activities listed.
1-acetyloxyethyl acetate	1.25	$C_6H_{10}O_4$	Resins, synthetic	No activities listed.
2-hydroxyethyl acetate	1.15	$C_4H_8O_3$	Ethylene Glycol	No activities listed.
Bicyclo[3.3.1]nonan-2-ol	0.99	$C_9H_{16}O$	Not applicable	No activities listed.

Conclusion

In summary, this work presented an interesting analysis of the chemical composition of different extractions of atemoya seed oil (*var*: Thompson). Among the one hundred and fourteen compounds identified, 20 had antioxidant activity, 17 had anti-inflammatory activity, 10 had antibacterial activity, 9 had antifungal activity, 7 had antitumor activity, 4 had auxiliary activity in the treatment of cancer, 3 had larvicidal activity and 2 showed antidiabetic activity. Furthermore, 12 are potential adjuvants in coping with COVID-19. In addition, some compounds are pheromones, have auxiliary effects in neglected diseases, and act as biological biomarkers, among other important activities. The results have demonstrated that each method of extraction provides compounds with specific biological potential. Thus, these results give important information to aid in the selection of extraction methods when considering the desired constituents.

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