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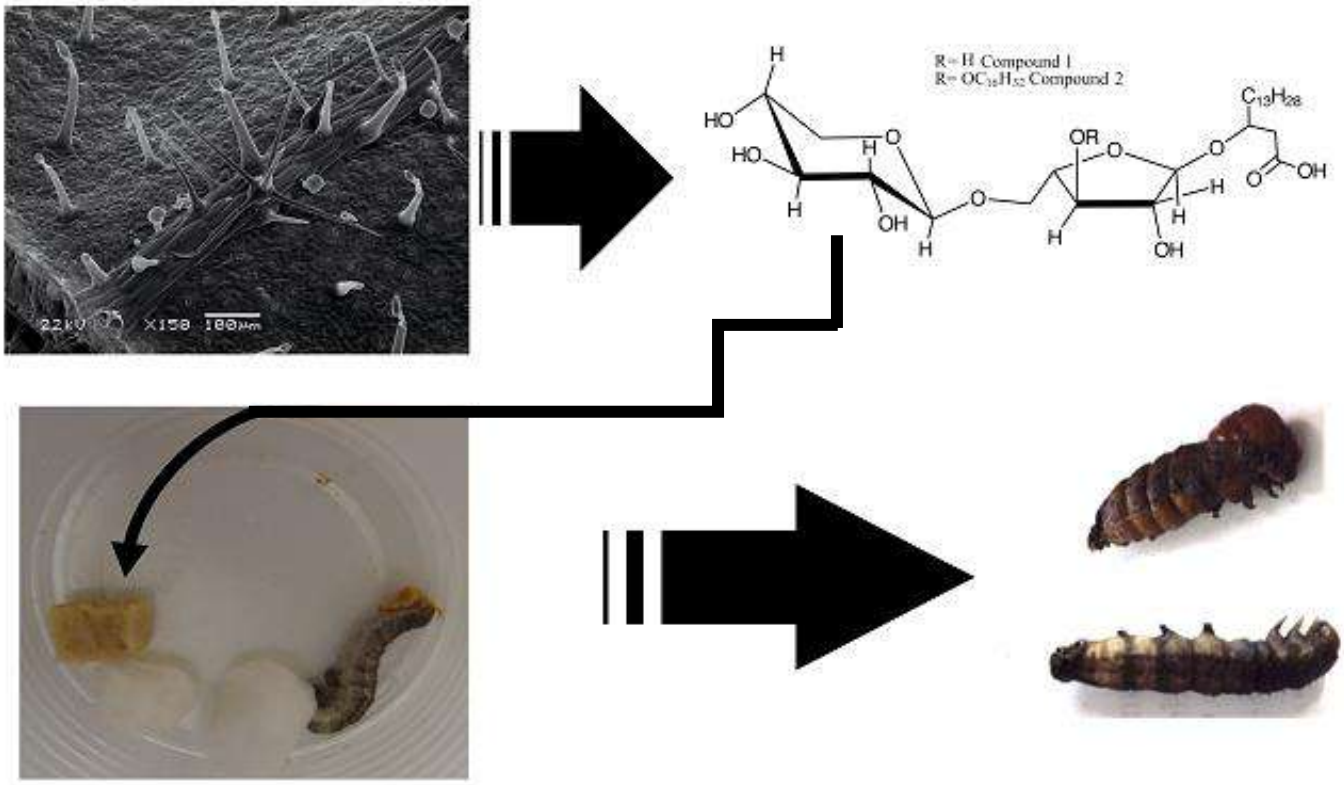
From the plant, isolation, purification and identification of compounds to bioactivity over *S. frugiperda*.



Campomanesia genus - a literature review of nonvolatile secondary metabolites, phytochemistry, popular use, biological activities and toxicology

Estrogenic mycotoxins in surface waters of the Rico Stream micro-basin, São Paulo, Brazil: occurrence and potential estrogenic contribution

Effects on the development of *Spodoptera frugiperda* feeding on diets spiked with *Solanum sisymbriifolium*

Study of the properties of lubricating oils obtained from biodiesel

Chemically modified cellulose as a potential oil adsorbent of contaminated marine ecosystems

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| Vol. 45 | n. 2 | 2020 |

Editorial

Despite the concerns spread by the Coronavirus, the Editor-in-Chief of Eclética Química Journal is pleased to announce the second issue of the year 2020. Readers of Eclética Química Journal will find in this issue a review article on the Campomanesia genus of the Myrtaceae family, characterized by citrus-flavored fruits, which reports the literature over the period 2005-2019. Parts of these plants have been used in many specific applications in food and pharmaceutical industries. This review discusses the literature embracing topics such as secondary metabolites and phytochemistry, popular use, biological activities, toxicology and concludes with future perspectives. The following paper deals with the obtention of a leaf dichloromethane extract that was purified according to a bioguide methodology, allowing to characterize two main compounds: furanoarabinoside (Compound 1) and its 3-O-palmitoyl derivative (Compound 2), which greatly influenced the survival and the development of S. frugiperda, when compared to an in vitro test control. Sequentially, the occurrence of zearalenone (ZEN) and five metabolites in the surface waters of northern São Paulo state (Brazil) are discussed and the associated potential estrogenic contribution to the aquatic environment is evaluated. The presence of estrogenic mycotoxins in the Brazilian river waters emphasizes the need to include these substances in future public policies concerning water quality. The next paper analyzes the properties of lubricant oils derived from biodiesel, before and after they went to the engine, and evaluates the dynamic viscosities of biodiesels in the engine. The results indicate that these biodiesels are candidates for commercial lubricants with advantages for the engine and the environment. The issue is closed with the description of a chemically modified cellulose potentially applied to oil-decontamination of marine ecosystems. The modified cellulose showed a satisfactory capacity to adsorb small amounts of viscous oils, like residual oil.

As a final note, the editor announced with great satisfaction at the beginning of this issue the databases in which the readers can find the articles published in Eclética Química Journal.

Assis Vicente Benedetti
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| Vol. 45 | n. 2 | 2020 |

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Summary
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| Vol. 45 | n. 2 | 2020 |

SUMMARY

Eclética Química Journal

Original review
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| Vol. 45 | n. 2 | 2020 |

Campomanesia genus – a literature review of nonvolatile secondary metabolites, phytochemistry, popular use, biological activities, and toxicology

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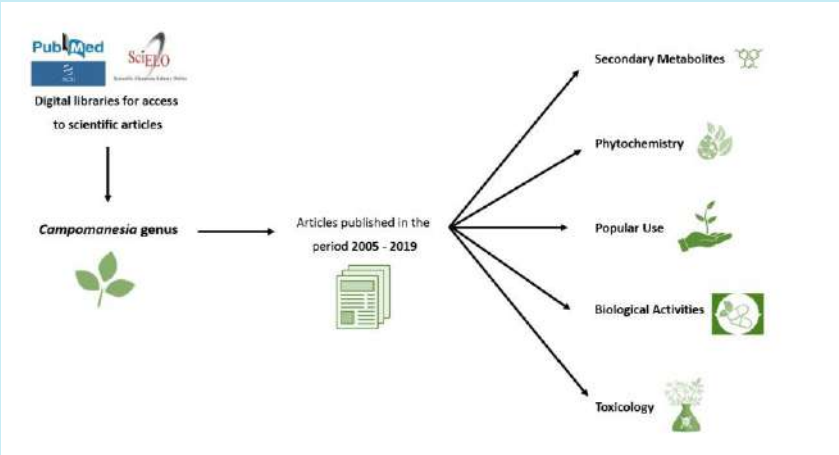
ABSTRACT: The genus *Campomanesia* belongs to the Myrtaceae family and has about 30 species. It is characterized by citrus-flavored fruits. Several articles describe the extensive use of its fruit, such as in the food industry, however, other parts of the plants are also used for food or pharmacological purposes such as the leaves, flowers, seeds, and roots. Analyzing works published on the genus in the period 2005-2019, we observed that the classes of main flavonoid compounds present are anthocyanins, chalcones, coumarins, tannins, and saponins. Species of this genus are also used as a medicine in the treatment of wounds, toothaches, fractures, and bruises. Therapeutic activities have also been detected for *Campomanesia*, such as antimicrobial, antiulcerogenic, antiprotozoal, anti-inflammatory, and antidiarrheal activity, antiproliferative and antioxidant potential, antiplatelet, antithrombotic, and fibrinolytic activities, as well as hypotensive effects. There are a small number of works demonstrating the low toxic potential of plant extracts. Thus, the *Campomanesia* genus presents pharmacological potential to be explored.



CONTENTS

1. Introduction

Plants can be used as sources of alternative substances, including therapeutic resources for human medicines[1]. Mesopotamian documents, dated 2600 BC, show the use of more than 1,000 production plants, including those that are now untreated, colds, parasitic, and inflammation[2]. When we analyze the medicines released between 1981 and 2014, 4% are produced from natural

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products, and 27% are synthetic compounds. However, considering the origin of the other products, 59.1% are semi-synthetic molecules, synthetic drugs with pharmacophoric groups, based on natural product structures, mimicking natural or botanical products[3].

The use of medicinal plants as an alternative or additional therapeutic resources has increased significantly[4]. Although synthetic drugs are usually the first choice of treatment for many diseases, these chemical drugs sometimes have undesirable effects and, consequently, the acceptance of alternative medicines has increased substantially[5]. Also, in some pathologies, resistance is developing to the drugs already used in treatment. These data demonstrate the importance of natural products in drug research, and the development of new medicines, justifying work in this area. The Scielo and PubMed databases were searched, during the period 2005-2019, and the keywords used were: *Campomanesia*, Myrtaceae, "*Campomanesia* and biological activities," "*Campomanesia* and popular use," "*Campomanesia* and toxicology."

The Myrtaceae family comprises 175 genera and 5,970 species, native to all continents of the southern hemisphere[6]. They are divided into shrubs and trees with sebaceous glands in the leaves, lower or semi-inferior ovaries, flowers, usually, with numerous stamens, internal phloem, and traces of xylem vessels[7,8]. The genus *Campomanesia* belongs to the Myrtaceae family and has about 30 species[9]. This genus is characterized by its citrus-flavored fruits[10]. However, although several articles describe the extensive use of the fruit, such as in the food industry, other parts of the plants are also used for food or pharmacological purposes, such as leaves, flowers, seeds, and roots.

The species include: *C. xanthocarpa*, *C. adamantium*, *C. corimbosa*, *C. cambessedean*, *C. pubescens*, *C. guazumifolia*, *C. reitziana*, and *C. lineatifolia*, among others.

2. Secondary metabolites and phytochemistry

A striking feature of the genus *Campomanesia* is the presence of the high content of phenolic compounds, mainly in *Campomanesia adamantium*. Analyzing works published in the period 2005-2019 for the genus, we observed that the main classes of flavonoid compounds present are anthocyanins, chalcones, coumarins, tannins, and saponins. These compounds have in common the biosynthetic pathway derived from phenylpropanoids that contribute to all aspects of plant responses to biotic and abiotic stimuli[11].

However, changes occur in the formation, concentration, and type of secondary metabolites in the various species, which may be influenced by the environmental stress, climate, and soil of each region. For example, fruits of *Campomanesia xanthocarpa* contain a high concentration of phenolic compounds, including chlorogenic acid and ascorbic acid[12] and the leaves contain a large number of saponins, tannins, and terpenes, besides the consistent presence of flavonoids and phenolic compounds, such as gallic acid, quercetin, and chlorogenic acid[13,14].

Campomanesia adamantium is one of the most commonly studied species. It has shown significant therapeutic potential, in addition to the presence of phenolic compounds such as gallic acid, catechins, ellagic acid, and flavonoids[15,16].

Fruits of *Campomanesia pubescens*, when ripe, have a high content of vitamin C and phenolic compounds[17], among them, flavanones and chalcones[18,19].

Few studies describe both the biological activities and compounds of *Campomanesia guazumifolia*. However, a survey by Catelan et al.[20] identified the presence of glycosylated flavonoids: quercetin pentose, quercetin deoxyhexoside, myricetin deoxyhexoside, and quinic acid. The fruits of the *C. reitziana* species contain dimethyl cardamonine as their main active compound[21].

Phytochemical studies with the *C. lineatifolia* species revealed the presence of flavonoids, tannins, catechin, quercitrin, and champanones A, B, and C[22]. A survey by Osorio et al. showed that the beta-tricetones compound contributes to the fruity odor[23].

Campomanesia leaves are rich in volatile oils, which were reviewed by Stefanello *et al.* in 2011, who described geraniol, α-pinene, limonene, linalool, spathulenol, and caryophyllene[24]. The essential oil from *C. guaviroba* was analyzed, and sixteen compounds were identified, of which the largest constituent was myrtenal[25].

3. Popular use

The Myrtaceae family presents a wide variety of fruits, which are consumed throughout the

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Brazilian territory and present characteristics of a considerable amount of acidity through ascorbic acid, minerals, fibers, and monoterpene hydrocarbons. Fruits are used to make liqueurs, juices, and sweets. However, the population uses other parts such as the leaves, seeds, and roots in the form of powders, gums, teas, juices, oils, or different types associated with medicinal use[10,26]. Native species from the Midwest region, such as *Campomanesia*, are used by indigenous peoples as food since they represent a source of vitamins and minerals[27]. However, species of this genus are also used as a medicine in the treatment of wounds, toothaches, fractures, and bruises[28].

From the advances in research directed to natural products, the use of medicinal plants has significantly increased, to provide alternative or additional forms of treatments used in the daily life of the population. *C. xanthocarpa* is an example of this species which is associated with popular and traditional use, as it demonstrates extensive medicinal use: fruit peel infusions for the treatment of productive cough and dysentery, leaf tea for reducing cholesterol and fighting urinary and uterine infections, and the peel can be used to treat cystitis, diarrhea, and hemorrhoids[13,28]. *C. pubescens* is used by the population of Mato Grosso (Brazilian State) as food (jelly, juices, and liqueurs) and also as a medicinal plant due to its purifying, antidiarrheal, and cholesterol-lowering action[29,30]. Infusions of *C. velutina* leaves and branches are popularly used to treat diarrhea and intestinal cramps[31].

4. Biological activities

One of the most commonly studied activities is *in vitro* antioxidant activity. DPPH is the most widely used method, probably because these are quick and cheap chemical tests[32]. This activity is related to the concentration of phenolic compounds present in samples. Antimicrobial activities against anti-inf, fungi, and protozoa are also widely studied[22,33,34].

The biological activities described are listed in Tab. 1, including anti-inflammatory, cytotoxic, antinociceptive, and protective activities of the gastric mucosa. These activities, in most cases, are related to flavonoid activities, which are effective in some cases[35].

C. xanthocarpa has been studied to confirm its popular use in treatment for hypercholesterolemic patients; this species was able to reduce blood levels of TC and LDL[13]. In addition to this effect, it has been shown that this species has important therapeutic activities such as antimicrobial[36], antiulcerogenic[37], antiprotozoal[34], anti-inflammatory[38], and antidiarrheal[39] as well as antioxidant potential[40], antiplatelet, antithrombotic, and fibrinolytic activities[41,42], and, more recently, hypotensive effects[14].

Campomanesia adamantium has the characteristic of being a small tree that produces edible fruits with beneficial effects on health, presenting activities such as antimicrobial[18,27,33,43], antidiarrheal[44], and antiproliferative[45,46]. In addition, its leaves and roots have anti-inflammatory and antinociceptive activities[47].

In the studies conducted with *C. guazumifolia*, the antioxidant activity, antimicrobial activity, and anti-inflammatory potential with low toxicity of leaf infusions of this species were evidenced[20]. Few biological activities are described for *C. reitziana* and *C. lineatifolia*. *C. reitziana* showed antinociceptive and gastroprotective potential, and *C. lineatifolia* demonstrated anti-inflammatory and gastroprotective effects[21,48,49].

5. Toxicology

Currently, plants are used as food, nutraceuticals, phytonutrients, medicinal plants, and herbal medicines, thus representing alternative therapies to existing treatments for various pathologies[28,50]. Despite the widespread use of plants as medicine and functional food, there is often no scientific evidence to support their pharmacological properties and toxic potential[51,52]. It is estimated that only three-quarters of the currently marketed natural products of plant origin have the safety information that enables their proper use[53]. Regarding *Campomanesia*, this scenario is no different. Although these species are widely used as food and medicine in folk medicine, studies on the toxicology of many species are still scarce[54].

A study on the acute and subacute toxicity of ethanolic extract of *C. guazumifolia* leaves, where adult and female rats received the extract orally at different concentrations for 14 and 28 days respectively, showed that the doses used did not produce significant physiological or pathological changes, or mortality, indicating that the LD50 is greater than 2000 mg kg-^{1} [54]. Leaf infusion of the same species was shown to have low toxicity in *in vivo* models of acute and subacute toxicity, with

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no observed clinical signs or changes in hematological, biochemical, or histological parameters, suggesting that the LD50 is above 5000 mg kg^{-1} [20].

A similar study of acute and sub-chronic toxicity using the aqueous extract of *C. velutina* leaves and branches demonstrated different changes in male and female Swiss mice. The extracts at doses of 600 and 1200 mg kg^{-1} showed signs of toxicity such as diarrhea, anemia, changes in the kidneys, brain, and heart, suggesting that the safest dose of the extracts is 300 mg kg^{-1} [31]. The ethanolic extract of *C. pubescens* leaves demonstrated cytotoxic and genotoxic effects through *Allium* strain bioassay, toxic effects were observed in the dividing cell and increased chromosomal alterations[20]. *In vivo* studies with *Wistar* and *Drosophila* melanogaster rats showed that the ethanolic extract obtained from fruits of *C. pubescens* under the experimental conditions used did not demonstrate significant genotoxic or clastogenic effects, indicating that fruit consumption is safe[54].

The aqueous extract and essential oil of *C. xanthocarpa* leaves were also evaluated using the *Allium* strain test, allowing the observation of a genotoxic effect of the samples: mitotic index reduction and chromosomal mutations[55]. The extract of the leaves of this same species, when submitted to acute toxicity test in vivo, showed no signs of toxicity[38]. Hydroalcoholic extract from *C. adamantium* fruits, when subjected to acute and subacute toxicity tests in *Wistar* rats, proved to be safe, as no clinical signs of toxicity were observed[56].

Preclinical toxicity studies of herbal medicines are recommended by international regulatory agencies. In Brazil, the National Health Surveillance Agency (ANVISA) is the body responsible for regulating these products and includes, among its various resolutions, a specific one for toxicity tests, aiming to ensure and evaluate the safety and quality of herbal medicines before they are used by the population[57]. However, toxicity tests with species of the genus *Campomanesia* are still scarce, requiring further studies in this area.

6. Conclusion and future perspectives

Several articles published in recent years demonstrate the importance of the genus *Campomanesia*, not only as food but also, mainly, for its pharmacological potential. In this sense, studies claim that different parts of the plants of this genus present promising results for various biological activities, ranging from antioxidant activity to antiproliferative activity, for example. The secondary metabolism of plants is responsible for the production of compounds that have these biological activities. In the case of the *Campomanesia* genus, phenolic acids and other groups of compounds such as flavonoids, anthocyanins, and tannins stand out. Through these studies, the potential of these species to treat different diseases becomes evident. However, for this genus to be used commercially, a lot of work is needed to produce a finished product. For herbal medicine, studies of the major compounds present, markers, and quantifications of these compounds should be performed. Besides, for the development of allopathic medications, active compounds must be isolated, and the structure identified, as well as analysis of the biological activities. Toxicity tests and possible mechanisms of action are also required. Given this, there is a vast territory to be explored, represented by the genus *Campomanesia*.

Eclética Química Journal, vol. 45, n. 2, 2020, 12-22
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Table 1. Description of the studied species, parts, biological activity found, and identified compounds from the *Campomanesia* genus.

Genus	Part of the plant and extract	Biological Activities	Compounds	Reference
C. adamantium	The ethanolic crude extract of leaves and ethyl acetate and butanol fractions	Antioxidant activity	Isoquercitrin and quercetin	[58]
C. adamantium	Ethyl acetate and aqueous extracts of leaves	Antinociceptive and anti-inflammatory activities	Myricitrin, myricetin, and quercetin	[47]
C. adamantium	Fruit and leaf extracts	Antiproliferative activity in the PC-3 cells	Cardamonin	[45]
C. adamantium	Hydroalcoholic extract of fruit peels	Anti-inflammatory, antihyperalgesic and antidepressant activities	Quercetin, myricetin, 5,7-dihydroxy-6 methylflavanone, 5,7 dihydroxy-8 methylflavanone and 2 '4′-dihydroxy6′-methoxycalcon and in the ethyl acetate fraction of 7-hydroxy-5-methoxy-6 -methylflavanone, 5,7-dihydroxy-6,8-dimethylflavanone, and 2 ', 4'-dihydroxy 3',5'-dimethyl-6'-methoxyicalcone	[56]
C. adamantium	Hydroalcoholic fruit extract	Hepatoprotective activity *in vitro*	Presence of flavonoids	[48]
C. adamantium	Ethanolic extracts of leaves, bark, and seeds	Antifungal Potential	-	[59]
C. adamantium	Aqueous root extract	Antioxidant and antihyperlipidemic	Gallic acid and ellagic acid	[60]
C. adamantium	Peel Extract	Antidiarrheal, cytotoxic, and anti-inflammatory activities	Phenolic compounds	[44]
C. adamantium	Aqueous extract of leaves and roots	Antileukemic activity	di-hexoside/quinic acid, ellagic acid O-pentoside, ellagic acid, O-methyl ellagic acid O-hexoside, ellagic acid O-deoxyhexoside, and O-methyl ellagic acid sulfate, gallic acid, ellagic acid O-hexoside, O-methyl ellagic acid O-deoxyhexoside, and O-dimethyl ellagic acid sulfate	[61]
C. adamantium	Dichloromethane extracts from pulp and fruit peel	Antiproliferative activity	7-hydroxy-5-methoxy6-C-methylflavanone, 5,7-dihydroxy6-C-methylflavanone, 5,7-dimethoxy6-C-methylflavanone, 5,7-dihydroxy-6, 8-C-methylflavanone, 4’,6’-dihydroxy-30-methyl20-methoxy-chalcone, Champanone C, 4’,6’-dihydroxy-30, 5’-dimethyl-2’ -methoxy -chalcone and Champanone D	[46]
C. adamantium	Methanolic bark extract	Potential antiplatelet effect	Phenolic Compounds, Total Flavonoids, Condensed Tannins	[62]
C. guazumifolia	Aqueous extract of the leaves	Anti-inflammatory potential with low toxicity	Quercetin pentose, quercetin deoxyhexoside, myricetin deoxyhexoside, and quinic acid.	[20]
C. lineatifolia	Methanolic seed extract	Antimicrobian activity	Champanone A, Champanone B, and Champanone C.	[22]
C. pubescens	Ethanolic Fruit Extract	Anxiolytic and antidepressant effects	1,2-hydroxy3′-methyl-4′, 6′-dimethoxychalcon and 2, 7-hydroxy5-methoxy-6-methylflavanone, 3, 5-hydroxy-7-methoxy-8-methylflavanone; 4, 2′, 4′-dihydroxy-3′, 5′-dimethyl6′-methoxychalcone; and 5, 2′, 4′-dihydroxy-5′-methyl-6′-methoxychalcona	[63]

Eclética Química Journal, vol. 45, n. 2, 2020, 12-22
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continuation of **Table 1.**				
C. pubescens	Fresh fruit ethanolic extract	Low acute and subchronic toxicity	2-hydroxy3′-methyl-4′, 6′-dimethoxychalcon; 7-hydroxy-5-methoxy-6-methylflavanone; 5-hydroxy-7-ethoxy-8-methylflavanone; 2′, 4′-dihydroxy3′, 5′-dimethyl-6′-methoxychalcone and 2′, 4′-dihydroxy-5′-methyl-6′-methoxychalcone.	[54]
C. pubescens	Ethanolic leaf extract	Antioxidant activity and suggested cytotoxic and genotoxic effects	7-hydroxy-6-methyl-5-methoxyflavanone, 5,7-dihydroxy-6-methylflavanone, 5,7-dihydroxy-8-methylflavanone, 2´,4´-dihydroxy-6´-methoxychalcone, 5,7-dihydroxy-6,8- dimethylflavanone, 2´,4´-dihydroxy-5´-methyl-6´-methoxychalcone and 2´,4´-dihydroxy-3´,5´-dimethyl-6´-methoxychalcone	[64]
C. pubescens	Ethanol extract of fruit pulp	Low toxicity without genotoxic or clastogenic effects.	High levels of flavonoids	[65]
C. velutina	Aqueous extract of leaves and branches	Acute and subchronic toxicity.	Presence of phenolic compounds including flavonoids and tannins	[31]
C. xanthocarpa	Aqueous extract of the leaves	Reduction in weight gain and blood glucose in animals	-	[66]
C. xanthocarpa	Hydroalcoholic extract of the leaves	Antiulcerogenic action and showed no acute toxic effects	Presence of flavonoids, saponins, and tannins	[37]
C. xanthocarpa	Aqueous extract of the leaves	Antiplatelet, antithrombotic, and fibrinolytic activity in mice and also in human blood and mice obtained antithrombotic activity.	Presence of saponins, tannins, and terpenes and a small presence of flavonoids	[41]
C. xanthocarpa	Fruits	Antioxidant activities	Phenolic compounds and ascorbic acid.	[40]
C. xanthocarpa	Aqueous extract	Anti-*Trichomonas vaginalis* activity	-	[34]
C. xanthocarpa	Extract of the leaves	Protective effect on the endothelium	-	[67]
C. xanthocarpa	Aqueous extract of fresh plant	Inhibit biofilm formation and bacterial growth.	-	[68]
C. xanthocarpa	Hydroalcoholic leaf extract	Anti-Inflammatory Activity without toxicity	2 ', 6'-dihydroxy-3'-methyl-4'-methoxychalcone and 2', 4'-dihydroxy-3 ', 5'-dimethyl-6'-methoxychalcone	[38]
C. xanthocarpa	Leaf extract	Antiplatelet Activity	-	[42]
C. xanthocarpa	Aqueous extract of the leaves	Hypotensive effect	Presence of gallic acid, chlorogenic aci, and quercetin, theobromine	[14]
C. xanthocarpa	Fruits	Antioxidant activity	Lutein, Zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, Lycopene, Thiamine, Riboflavin, Pantothenic Acid, Pyridoxine, Biotin, Vitamin C, Vitamin A	[69]
C. xanthocarpa	Aqueous extract of the leaves	Antioxidant activity, protective effect against DNA in blood cells and reduced LPO levels and increased SOD activity in kidney	Presence of quercetin, rutin, and ellagic, rosmarinic, caffeic, gallic, and chlorogenic acids in the extract.	[70]

Eclética Química Journal, vol. 45, n. 2, 2020, 12-22
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Estrogenic mycotoxins in surface waters of the Rico Stream microbasin, São Paulo, Brazil: occurrence and potential estrogenic contribution

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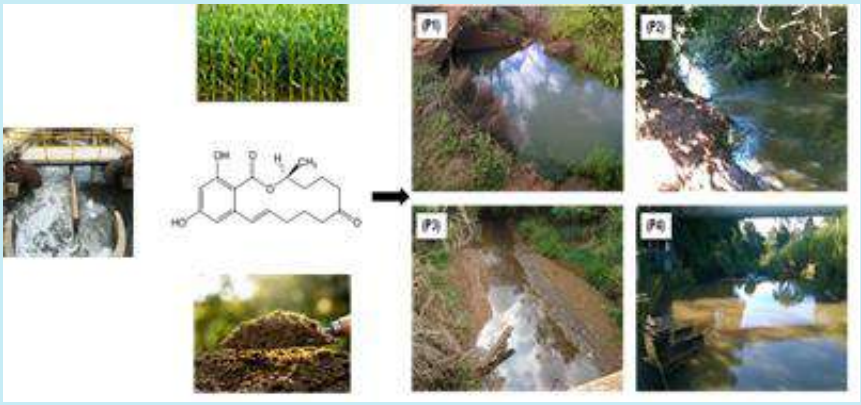
ABSTRACT: The aim of this study was to assess the occurrence of zearalenone (ZEN) and its metabolites zearalanone (ZAN), α-zearalenol (α-ZEL), β-zearalenol (β-ZEL) α-zearalanol (α-ZAL), and β-zearalanol (β-ZAL) in the surface waters of northern São Paulo state (Brazil) and to evaluate the associated potential estrogenic contribution to the aquatic environment. The determination of the estrogenic mycotoxins in water samples from the Rico Stream microbasin yielded levels of up to 59 ng L^{-1} and their corresponding calculated estrogenic equivalent (cEEQ) values were between < 0.03 and 1.4 ng L^{-1}, which are associated with negative effects on the reproduction and growth of some fish species. The physicochemical and microbiological parameters were evaluated to determine the water quality in the Rico stream region. This study revealed the first data about the presence of estrogenic mycotoxins in the Brazilian river waters and emphasizes the need to include these substances in future public policies concerning water quality, since these compounds are not yet legally regulated. From an environmental aspect, it is necessary to take into account the continuous introduction into surface water of microcontaminants associated with wastewater effluent, such as estrogenic mycotoxins.



1. Introduction

Among the hundreds of mycotoxin-producing fungi, the genus *Fusarium* is considered one of the most prevalents in terms of animal health implications and economic damage[1]. Resorcyclic acid lactones (RALs) are compounds that exhibit endocrine disruptive behavior and are produced by fungi of the genus *Fusarium*[2]. The representative mycotoxin of this class of RALs is zearalenone (ZEN), cited among the most common mycotoxins worldwide[3].

In Brazil, there have been few studies on the incidence of fusariotoxins. For example, the occurrence of ZEN was reported in maize[4], rice[5] and wheat[6] in the south and southeast Brazilian regions. Fusariotoxins are mostly produced under high humidity and at temperatures of approximately 20 to 26 °C. The Brazilian climate offers good conditions for these toxins to be present in grains used in the diets of animals and humans[7].

The incidence of ZEN and its metabolites has been reviewed extensively in food for humans and animals[8]. However, little is known about its impact or environmental distribution, with few studies having been carried out concerning these attributes[9]. Some publications have reported the occurrence of ZEN in surface water, groundwater

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and effluent from sewage treatment plants and industries, with concentrations of ZEN or its metabolites ranging from undetected levels to 4120 ng L^{-1} [10]. Zearalanone (ZAN) and β-zearalenol (β-ZEL) have rarely been detected or analyzed in aqueous environmental samples. ZEN and its metabolites can contribute to the overall estrogenic activity in the environment and therefore could pose a risk to wild fish in their natural habitat, as indicated by a zebrafish study in which there was a change in the reproductive capacity of animals exposed to low concentrations of estrogenic compounds (100 to 3200 ng L^{-1}) for 21 days[3].

Some studies conducted in Brazil have reported the occurrence of endocrine disruptors in surface waters, with monitoring results showing the periodic presence of these substances in natural waters, suggesting that different sources in addition to untreated sewage contribute to the input into water sources[11]. Possible aqueous environmental contamination routes of estrogenic mycotoxins include: drainage water from fields cultivated with infected plants, runoff from livestock facilities or manure applications to field crops and human excretions in urban wastewaters[12].

The purpose of this study was to examine for the first time the occurrence of estrogenic mycotoxins in Brazilian water samples, using dispersive liquid-liquid microextraction (DLLME) followed by liquid chromatography–tandem mass spectrometry and to identify possible sources of contamination, seasonal variations and potential contribution to total estrogenic activity in the southeast region of Brazil (São Paulo state).

2. Experimental

2.1 Standards and reagents

ZEN, α–ZEL, β–ZEL, α–ZAL, β–ZAL and ZAN standards were all obtained from Sigma Aldrich (St. Louis, MO, USA) with purities of at least 98%. The zearalenone–d6 (surrogate internal standard, ZEN–d6) was supplied from Toronto Research Chemicals (North York, ON, Canada). Acetonitrile (ACN) and methanol (MeOH), both HPLC grade were provided from J. T. Baker (Phillipsburg, NJ, USA). Bromocyclohexane was acquired from Alfa Aesar (MA, USA). Hydrochloric acid (37% m/v) and sodium hydroxide were purchased from J.T. Baker (Phillipsburg, NJ, USA) to adjust the pH of water samples. Deionized water was obtained using a Milli–Q purification system (Bedford, MA, USA).

2.2 Sampling area

The Rico Stream microbasin is localized in the northeast region of São Paulo state and consists of the municipalities Jaboticabal, Taquaritinga, Monte Alto, Guariba and Santa Ernestina (Fig. 1), occupying an area of approximately 563 km^2 at an altitude between 498 and 754 m.

The Rico Stream is the main source of water in the micro-basin, with a total of 59.2 km of watercourse length discharging into the Mogi Guaçu River. Because of its high representativeness in the region, Rico Stream stands out as the main supply of water for the city of Jaboticabal (population ca. 75,000). Anthropogenic impacts on the environment may occur both directly or indirectly, due to effluent discharges from domestic sewage treatment plants of the Monte Alto municipality and animal husbandry (poultry, swine and cattle)[13].

In accordance with the classification proposed by Köeppen, the region possesses a climate defined as Aw, characterized as tropical with summer rainfall and dry winters. The average temperature is 23 °C, and the total average annual rainfall is 1405 mm occurring mainly during the period from October to March, with the dry season extending from April to September[14].

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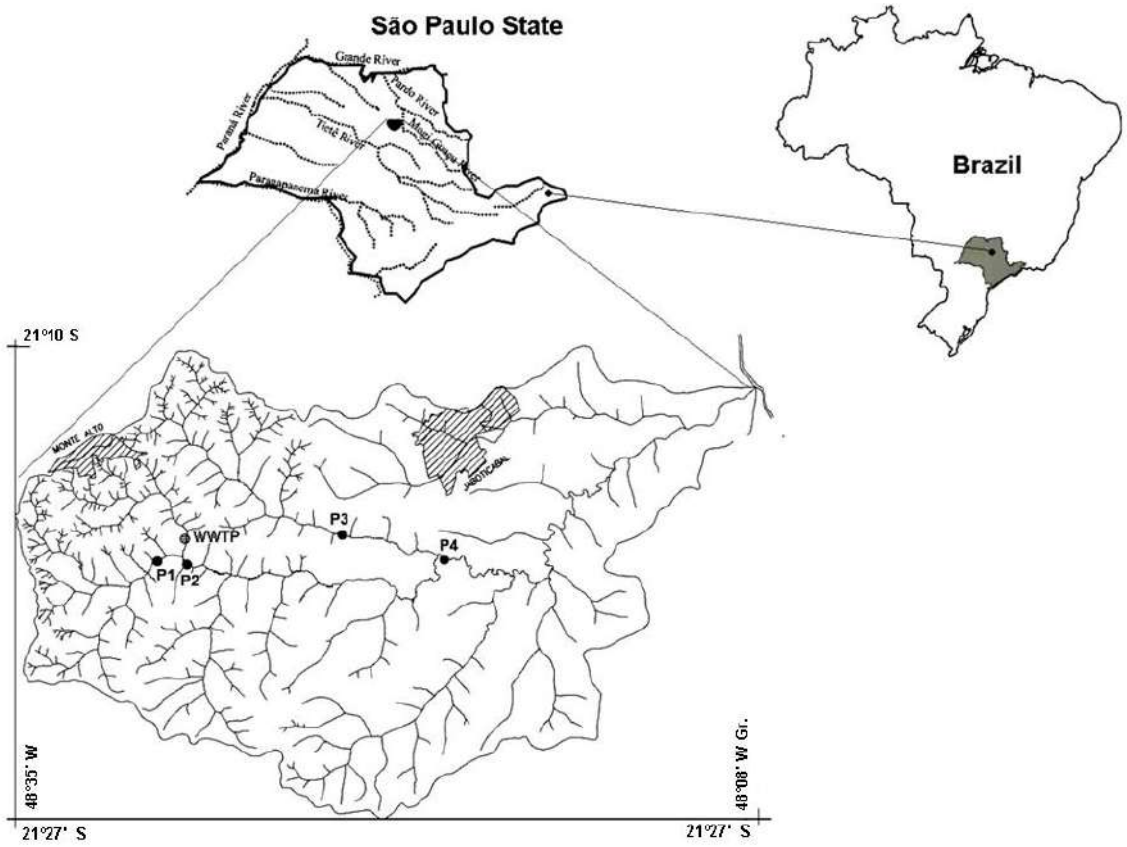

Figure 1. Map of the studied area showing the location of four sampling sites (Adapted from Pissarra *et al.*[15] and Takahashi *et al.*[16]).

2.3 Sample collection and pretreatment

The collection of water samples was performed from October 2014 to July 2015 (ten sampling campaigns) at four different sampling sites located in the Rico Stream, except one that was located in Tijuco Stream (stream tributary) between the municipalities of Monte Alto and Jaboticabal. References sites: P1 situated near the Rico Stream source with minor contamination impacts and upstream of wastewater treatment plant (WWTP) effluent discharge (21º18'37.26"S; 48º27'39.49"W); P2 situated at the downstream discharge point of the WWTP, which also represents the influence of input from agricultural industry and runoff from livestock facilities or fields receiving livestock manure applications (21º18'46.08"S; 48º26'56.33"W); P3 located in the Tijuco Stream, upstream of the public water supply and near the confluence with the Rico Stream (21º17'56.08"S; 48º22'31.69"W) and P4 located freshwater abstraction site for the public water supply to Jaboticabal city (21º18'36.07"S; 48º19'26.11"W).

The water samples were collected at 30 cm depth in 1000 mL amber glass bottles, previously rinsed thoroughly with ultrapure water. After collection, samples were transported to the laboratory under refrigeration at 4 °C (ice packs) and processed within 48 h. In the laboratory prior to extraction, the samples were filtered through a glass fiber filter with a pore size of 0.6 µm (Macherey-Nagel GF-3), acidified to pH 4 and spiked to 200 ng L^{-1} with an internal surrogate (ZEN-d_6). Samples were then immediately extracted and analyzed using the previously developed and validated dispersive liquid-liquid microextraction (DLLME) method and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)[17].

Eclética Química Journal, vol. 45, n. 2, 2020, 23-32
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2.4 Determination of physicochemical and microbiological parameters

A total of twelve physicochemical and microbiological parameters were used to assess water quality control and pollution. Parameters such as fecal coliforms, total organic carbon, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total phosphorous, total nitrogen, nitrate, nitrite, ammoniacal nitrogen, potassium, turbidity and total dissolved solids were determined using standard procedures described by the American Public Health Association[18].

2.5 Analytical Methodology

An aliquot of 10 mL of the water sample was added into a 12 mL conical glass centrifuge tube with screw cap. Thereafter, 100 µL of bromocyclohexane (extraction solvent) was added to the sample solution. The mixture was then vigorously shaken using a vortex mixer at 2500 rpm for 120 s. After centrifugation for 10 min at 3500 rpm, the organic sedimented phase was collected in a glass insert (300 µL) using a 100 µL Hamilton microsyringe and was evaporated in a N_2 flow. The residue was reconstituted in 80 µL of MeOH/0.1% formic acid in H_2O (50:50, v/v) and injected into the LC–ESI–MS/MS for analysis.

2.6 Instrumentation

The HPLC analyses were carried out on an Agilent 1200 series LC system coupled with a 3200 QTRAP mass spectrometer (Applied Biosystems/MDS Sciex Instruments) with an electrospray ionization source (ESI). The mass spectrometric parameters selected for experiments of full scan and ion fragmentation were as follow: ion spray voltage of −4 kV, curtain gas (nitrogen) at 15 psi, auxiliary gas at 40 psi, nebulizer gas at 50 psi, source temperature at 600 °C, interface heater and entrance potential of -10 V. Chromatographic separation of the estrogenic mycotoxins was achieved at 40 °C using a Gemini C_{18} column (150 × 4.6 mm, 5 µm; Phenomenex, Torrance, CA, USA) preceded by a Phenomenex C_{18} column guard (4 × 3 mm). The mobile phase consisted of 0.1% formic acid in water (48%, v/v), acetonitrile (25%, v/v) and methanol (27%, v/v), with a flow rate of 1.0 mL min^{-1} for 17 min and injection volume was set to 20 µL. Applied Biosystems Analyst 1.5 software was used for data acquisition.

2.7 Quality assurance/quality control

The calibration was performed by internal standardization method with matrix-matched blank samples. The calibration curve consists of eight different concentration values, in the range 8 to 680 ng L^{-1}. The analytes were extracted by DLLME prior to chromatographic analysis for each point of the calibration curve. The calibration curve was obtained from the ratio between the peak areas of analytes and the internal standard versus the analyte concentrations (ng L^{-1}). Every experimental point was analyzed in triplicate. The extraction efficiency (recovery percentage) of the analytes was evaluated by spiked with 200 ng L^{-1} of the internal surrogate (ZEN-d6) to all river water samples.

2.8 Estrogenicity

The estrogenic potential of the aqueous samples was calculated in equivalent activity compared to the reference substance (natural hormone 17-β-estradiol)[19]. The potential contribution of each estrogenic mycotoxin to the total estrogenic activity of the surface waters was calculated according to the Eq. 1:

$$cEEQ = concentration\ of\ estrogenic\ mycotoxin \times RP \quad (1)$$

where cEEQ is the calculated estrogenic equivalent (17-β-estradiol) of a specific estrogenic mycotoxin and RP is the relative potency of the mycotoxin in an individual bioassay.

3. Results and Discussion

3.1 Occurrence of estrogenic mycotoxins in environmental samples

The results obtained from the collected samples (during the period of October 2014 to July 2015) are shown in Tab. 1. Surrogate recovery values (ZEN-d6) ranged from 71 to 124% from the extraction method. These recovery values are

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suitable when dealing with complex environmental samples such as the river water analyzed in this study.

Table 1. Estrogenic mycotoxin concentrations (ng L^{-1}) at four sampling sites in the Rico Stream micro-basin.

Compounds	October/2014				November/2014			
	P1	P2	P3	P4	P1	P2	P3	P4
β-ZAL	n.d.[c]	n.d.	n.d.	n.d.	48	n.d.	n.d.	n.d.
β-ZEL	n.d.	<40[b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-ZAL	n.d.	n.d.	n.d.	n.d.	<20[b]	<20[b]	27	<20[b]
α-ZEL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ZAN	n.d.	n.d.	n.d.	n.d.	<8[b]	n.d.	n.d.	n.d.
ZEN-d_6[a]	112	108	107	106	107	96	76	91
ZEN	<8[b]	<8[b]	<8[b]	<8[b]	<8[b]	<8[b]	<8[b]	<8[b]
Compounds	**December/2014**				**January/2015**			
	P1	P2	P3	P4	P1	P2	P3	P4
β-ZAL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
β-ZEL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-ZAL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-ZEL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ZAN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ZEN-d_6[a]	89	80	91	93	112	81	74	77
ZEN	n.d.	n.d.	n.d.	n.d.	22	29	25	27
Compounds	**February/2015**				**March/2015**			
	P1	P2	P3	P4	P1	P2	P3	P4
β-ZAL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
β-ZEL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-ZAL	n.d.	n.d.	n.d.	n.d.	<20[b]	n.d.	n.d.	n.d.
α-ZEL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ZAN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ZEN-d_6[a]	118	105	97	97	73	71	115	100
ZEN	n.d.	n.d.	n.d.	n.d.	59	46	51	42
Compounds	**April/2015**				**May/2015**			
	P1	P2	P3	P4	P1	P2	P3	P4
β-ZAL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
β-ZEL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-ZAL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-ZEL	n.d.	n.d.	n.d.	n.d.	<20[b]	n.d.	n.d.	n.d.
ZAN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ZEN-d_6[a]	91	81	73	78	114	124	101	127
ZEN	n.d.	n.d.	n.d.	n.d.	18.8	n.d.	n.d.	n.d.
Compounds	**June/2015**				**July/2015**			
	P1	P2	P3	P4	P1	P2	P3	P4
β-ZAL	n.d.	n.d.	<40[b]	n.d.	n.d.	n.d.	n.d.	n.d.
β-ZEL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-ZAL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-ZEL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ZAN	n.d.	n.d.	19	n.d.	n.d.	n.d.	n.d.	n.d.
ZEN-d_6[a]	117	119	119	88	102	103	98	88
ZEN	14	12	11	<8[b]	n.d.	n.d.	n.d.	n.d.

[a]ZEN-d_6 - Recovery (%), spiked at 200 ng L^{-1} in triplicate
[b] limit of quantification value
[c] n.d. – not detected

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All analytes were detected at least once at each point over the sampling period. ZEN compound had a higher frequency of detection (52.5%), followed by α-ZAL (12.5%), β-ZAL (5.0%), ZAN (5.0%), α-ZEL (5.0%) and β-ZEL (2.5%). A total of 47.5% of the samples had concentrations above the limit of quantification (LOQ), ranging from 11 to 59 ng L^{-1}. Among the six analytes, ZEN was determined at all sampling points in five of the sampling campaigns performed. The highest levels were determined during the period of greatest rainfall. In addition, most ZEN metabolites were detected during the rainy season. The compounds β-ZAL, α-ZAL and ZAN were quantified only once, whereas α-ZEL and β-ZEL did not present in concentrations above the LOQ (Tab. 1). The limits of detection were for β-ZAL and β-ZEL of 20 ng L^{-1}, for α-ZAL and α-ZEL of 8 ng L^{-1} and for ZAN and ZEN of 4 ng L^{-1}.

The results obtained in this work are comparable to those obtained by other researchers. In previous studies, the detection frequencies for ZEN ranged from 0 to 30% and differed according to the seasons during sampling[9,20-22]. The highest concentrations of ZEN (up to 96 ng L^{-1}) were detected in small streams or channels near agricultural fields with crops contaminated by *Fusarium* sp.[9,20,23]. Low levels (up to 35 ng L^{-1}) were found in river and lake waters[18,22-25]. The metabolites β-ZAL and α-ZAL were investigated in two rivers contaminated by wastewater effluents[24]. The maximum concentration of both compounds was 3 ng L^{-1}. The concentrations of β-ZEL and α-ZEL metabolites were investigated in agricultural streams upstream and downstream of the WWTP in New York (USA). In agricultural fields, the concentrations of these compounds were generally below the limit of detection, but the highest detected concentrations reached tens or even hundreds of ng L^{-1}. In summary, the levels of estrogenic mycotoxins detected in rivers, streams and drainage channels occurred generally at levels of dozens of ng L^{-1}. However, much higher concentrations have occasionally been reported (single study), such as the detection of hundreds of ng L^{-1} for α-ZEL and β-ZEL in streams near agricultural areas or wastewater effluents in New York, USA[9].

The results obtained for physical, chemical and microbiological parameters at the sampling sites (Tab. 2) showed that variables such as fecal coliforms (December–July), BOD_5 (October and January), total phosphorus (October), nitrite concentration (December), and total ammoniacal nitrogen concentration (October) exceeded the values established by CONAMA for class 2 freshwater (Brazilian Environmental Council)[26].

According to the results of these parameters (Tab. 2), there was increase in the total phosphorus, potassium concentration, total dissolved solids and turbidity during the rainy season (November to March), which was characteristic of rainfall events and the corresponding transport of fine soil particles into the body of water. There was an increase in the concentration total nitrogen during the extensive dry season in October 2014. It is assumed that the high concentration of ammoniacal nitrogen (42 mg L^{-1} for P2 site) probably originated from runoff waters that carried residues from livestock animals or by the drainage of agricultural soils where animal excrement was used as fertilizer.

The fecal contamination indicator (fecal coliforms) exceeded the maximum limit of 1000 CFU 100 mL^{-1} [24] in samples from all months, with the exception of October and November 2014. These results indicated that Rico Stream is subject to a great anthropogenic influence, probably related to the use and soil occupation of this microbasin. The Rico Stream microbasin presents serious problems of environmental degradation, characterized by residential and rural areas with large deforestation events and lack of adequate management of animal husbandry waste, resulting in contamination of the whole drainage network.

The P2 site had the highest concentrations of eight of the hydrological variables (fecal coliforms, BOD, total phosphorous, nitrate, nitrite, potassium, total dissolved solids, ammoniacal nitrogen concentrations), including 14 values above CONAMA legislation. This sampling site is characterized by tributaries that drain from the urban area of the Monte Alto municipality and is located downstream of the effluent discharge of the wastewater treatment plant (WWTP) (site that receives the highest contribution among others).

Soil occupation and land use in the area of the Rico Stream consists of small farms with diversified production. According to field observations during the sampling period, horticulture, swine and several agricultural crops (especially sugarcane and peanuts) were present in the area. The presence of mechanized irrigation of agricultural crops during both the dry and rainy periods was also observed during the sampling period. It may be inferred that the presence of

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estrogenic mycotoxins in the Rico Stream was probably due to effluent discharge from the WWTP of Monte Alto municipality (detection in downstream of the WWTP – P2 site), runoff from livestock facilities or fields receiving livestock manure applications (detection in upstream of the WWTP – P1 site). Rico Stream contamination from fungi in agricultural crops is unlikely since maize (temporary tillage) is insignificant in the region.

Table 2. Data on physicochemical and microbiological parameters determined in Rico Stream micro-basin over the period of sampling.

Month	Sampling sites	Fecal coliforms (CFU 100 mL^{-1})	Total organic carbon (mg L^{-1})	Chemical oxygen demand (mg L^{-1})	Biological oxygen demand (mg L^{-1})	Total phosphorus (mg L^{-1})	Nitrate (mg L^{-1})	Nitrite (mg L^{-1})	Total nitrogen (mg L^{-1})	Total ammoniacal nitrogen (mg L^{-1})	Total dissolved solids (mg L^{-1})	Potassium (mg L^{-1})	Turbidity (NTU)
October 2014	P1	850	10	24	5	0.098	1.0	<0.011	46	0.30	112	<1.109	11
	P2	65	72	180	81	1.820	3.4	0.621	46	42	343	<1.109	16
	P3	22	16	40	15	0.230	<1.0	<0.011	0.5	0.51	132	<1.109	7
	P4	40	21	52	30	0.176	5.5	0.040	6.0	0.50	159	<1.109	10
November 2014	P1	<1.0	9	23	5	0.123	6.3	<0.011	6.5	0.21	210	6.162	13
	P2	500	16	40	<5.0	0.420	7.2	<0.011	10	2.90	184	5.217	12
	P3	<1.0	22	55	<5.0	0.120	5.7	<0.011	5.9	0.20	168	6.801	12
	P4	70	8	20	<5.0	0.181	6.2	<0.011	6.4	0.25	198	5.209	13
December 2014	P1	2300	5	12	<5.0	0.055	0.33	0.024	0.5	0.16	48	2.482	14
	P2	2200	5	12	<5.0	0.707	0.78	1.301	2.4	0.26	83	4.091	16
	P3	3300	6	17	<5.0	0.059	0.75	0.032	0.9	0.13	48	2.719	15
	P4	3900	2	4	<5.0	0.072	2.6	0.026	2.7	0.11	47	3.073	15
January 2015	P1	600	14	37	15	<0.050	<1.0	0.020	0.4	0.12	94	1.593	4
	P2	20000	72	187	94	1.157	1.1	0.355	6.4	<0.05	111	3.892	7
	P3	5000	16	41	21	<0.050	<1.0	0.036	0.3	<0.05	138	1.946	46
	P4	6000	11	29	13	<0.050	1.6	0.032	2.3	0.25	132	2.034	58
February 2015	P1	95	<0.04	18	<5.0	<0.050	<1.0	0.013	0.2	<0.05	53	7.53	12
	P2	48	<0.04	18	<5.0	<0.050	<1.0	0.118	0.5	0.06	92	11.07	19
	P3	165	<0.04	22	<5.0	<0.050	<1.0	0.029	0.2	<0.05	54	7.699	14
	P4	1100	<0.04	11	<5.0	<0.050	3.8	0.071	4.0	0.14	54	9.994	15
March 2015	P1	61	8	19	<5.0	<0.050	<1.0	0.068	0.4	0.15	44	3.237	10
	P2	620	22	54	<5.0	0.196	<1.0	0.143	0.6	0.24	62	4.396	11
	P3	380	5	12	<5.0	<0.050	<1.0	0.069	0.6	0.36	40	3.886	56
	P4	3100	5	12	<5.0	0.037	1.3	0.100	2.0	0.34	40	3.611	37
April 2015	P1	140	<0.04	<4	<5.0	<0.050	1.0	<0.011	1.9	0.54	50	2.165	10
	P2	510	22	54	<5.0	0.237	12	0.108	14	1.82	90	4.569	10
	P3	1100	19	46	<5.0	<0.050	2.3	0.036	2.8	0.29	50	2.286	92
	P4	900	35	84	<5.0	<0.050	4.0	0.016	4.5	0.15	46	2.796	74
May 2015	P1	410	69	186	<5.0	<0.050	0.31	0.015	0.7	0.33	90	2.420	9
	P2	2000	136	367	<5.0	<0.050	9.0	0.019	10	0.44	152	3.230	11
	P3	4300	2	4	<5.0	<0.050	0.70	0.014	1.3	0.40	84	2.119	21
	P4	1200	40	108	<5.0	<0.050	2.3	0.011	2.5	0.09	84	2.276	19
June 2015	P1	1600	16	40	<5.0	<0.050	1.0	0.015	1.2	0.16	47	1.79	8
	P2	420	35	88	<5.0	<0.050	1.5	0.232	2.4	0.64	96	3.991	14
	P3	5000	22	56	<5.0	<0.050	0.8	0.020	0.9	0.07	42	1.892	14
	P4	960	21	52	<5.0	<0.050	3.4	0.047	3.4	<0.05	42	2.232	13
July 2015	P1	200	42	86	<5.0	<LQ	0.74	<LQ	0.7	<LQ	65	2.196	5.3
	P2	3600	57	93	<5.0	1.649	2.0	0.374	2.4	<LQ	106	3.671	9.7
	P3	2700	9	33	<5.0	<LQ	0.87	<LQ	0.9	<LQ	89	2.536	12.2
	P4	5200	12	29	<5.0	0.033	4.6	0.044	4.6	<LQ	76	2.474	9.26

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3.2 Estrogenicity

The calculated cEEQ values ranged from < 0.03 to 1.4 ng L^{-1} (mean value) (Tab. 3). The highest values of cEEQ were for α-ZAL, even though the maximum concentration calculated in the samples was lower than those obtained for ZEN, as α-ZAL showed the highest relative estrogenic potency among the quantified mycotoxins. The cEEQ values for the estrogenic mycotoxins in this study (Tab. 3) were higher than those reported in natural waters in Europe and USA (< 0.01to 0.63 ng L^{-1})[27], except for the study performed by Kolpin *et al.*[9], in which the highest value of cEEQ was calculated (New York, USA) for α-ZEL (< 8.8 to 388 ng L^{-1}).

In the specific case of ZEN, some adverse effects have been detected *in vivo* at concentrations comparable in this study. ZEN concentrations between 2 and 50 ng L^{-1} (lowest observed effect concentration - LOEC), activation of genes that regulate the growth and reproduction of fish (*Pimephales promelas*) has been reported[28]. The concentrations of ZEN reported in this work (Tab. 1) were higher or comparable to the LOEC from *in vivo* studies (fish)[28].

Table 3. Concentration range, cEEQ and relative estrogenic potency of the analytes in the Rico Stream microbasin.

Compounds	Concentration range (ng L^{-1})	cEEQ[a] (mean value) (ng L^{-1})	cEEQ range[b] (ng L^{-1})	RP[c]
ZEN	<4.0[d]–59	<0.03-0.47	<0.01-5.0	7.87×10^{-3}
ZAN	<4.0[d]–19	<0.07-0.33	<0.02-3.1	1.76×10^{-2}
α-ZAL	<8.0[d]–27	<0.42-1.4	<0.15-4.7	5.20×10^{-2}
β-ZAL	<20[d]–48	<0.43-1.0	<0.09-2.3	2.14×10^{-2}

[a] Mean cEEQs were calculated from individual mycotoxin concentrations multiplied by the geometric mean of relative estrogenic potencies (RPs) of analytes from different *in vitro* systems
[b] cEEQs ranges were calculated as the concentrations of mycotoxins multiplied by the minimum and maximum RPs of the analytes from different *in vitro* systems
[c] Geometric mean of the relative estrogenic potencies[27]
[d] LOD value

4. Conclusions

In this study, it was concluded that estrogenic mycotoxins are present along the Rico Stream microbasin and that the contribution routes of these compounds are the run-off from livestock farming practices, drainage of agricultural soils from animal manure or wastewater treatment plant effluent from Monte Alto city. Additionally, this is the first report of estrogenic mycotoxins in environmental waters in Brazil. All estrogenic mycotoxins were detected at some point during the study, and quantifiable concentrations (11 to 59 ng L^{-1}) were similar to those found in studies conducted in other countries that are influenced by agricultural areas, livestock around river waters, as well as waterbodies that receive WWTP effluents. Thus, our results can contribute to discussion of the improvement of the water and sewage treatment in Brazil, as well as to the development of global environmental legislation.

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Effects on the development of *Spodoptera frugiperda* feeding on diets spiked with *Solanum sisymbriifolium* extracts

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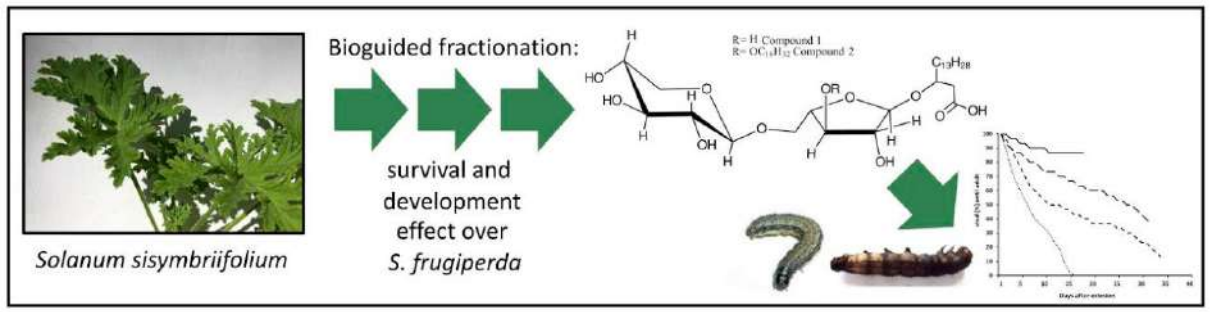

ABSTRACT: Aiming to evaluate the effects of *Solanum sisymbriifolium* extracts on the development of *Spodoptera frugiperda*, a leaf dichloromethane extract was obtained and subjected to further purification following a bioguided methodology. The structure of the two main compounds isolated from *S. sisymbriifolium* Type IV glandular trichomes have been completely elucidated by a combination of chemical and spectroscopic methods. They are glycosides of 3-(*R*)-hydroxypalmitic acid and xylo (β-1-5) furanoarabinoside (Compound 1) and its 3-O-palmitoyl derivative (Compound 2). These compounds greatly influenced the survival and the development of *S. frugiperda*, when compared to an *in vitro* test control. The extracts delayed the development, decreased survival and promote abnormalities in the immatures (larvae and pupae). They also showed increasing toxicity towards *S. frugiperda* in the purification following a bioguided fractionation. The pure compounds had the most deleterious activity, increasing the larval and pupal toxicity (100% mortality for Compound 2 at 2.50 mg mL^{-1} and 90% mortality for Compound 1 at 1.00 mg mL^{-1}).

1. Introduction

1.1. Solanum sisymbriifolium

Solanum sisymbriifolium L. is a spiky, perennial and invasive weed, native from the Rio de la Plata basin. By visual inspection, the shrub is almost free of herbivore insects. Interactions between plants and insects within an ecosystem are essentially physicochemical[1]. The insect is either attracted or repelled towards a plant, following complex and specific physicochemical cues[2]. *S. sisymbriifolium*'s epicuticle shows a very complex structural morphology, dense arrays of different kinds of hairs cover the abaxial and adaxial sides of the leaf[3]. Glandular trichomes are another physical/chemical defense line that contain preconceived compounds that are released when

the trichome is broken by some external stimulus. Because of this, the content of the trichomes is extracted with the epicuticular wax of the leaf[4]. However, it is possible to differentiate the origin of these compounds analyzing only the content of the glandular trichomes[5]. Epicuticular wax is removed from the surface of the leaves using non-polar solvents for a short period (normally 30 s) in order not to remove internal lipids[6,7]. Trichome compounds can be separated from waxes by freezing out an acetone solution of the epicuticular extract. Trichome chemistry is diverse and many bioactive metabolites have been isolated from these storage structures, such as terpenes, alkaloids, phenols, flavonoids and sugar esters. In most cases, the shape and morphology of trichomes is characteristic of the type of compounds they contain[8,9]. Visual inspection of *S. sisymbriifolium* leaf under the microscope, allowed the identification of type IV trichomes, which are the predominating ones over the abaxial side of the leaf. These trichomes are known for producing many bioactive compounds especially acyl sugars, compounds which play a key role within some of the chemically mediated interactions of many Solanaceae plants and their environment. Among the different bioactivities reported such as anti-hypertensive, analgesic, anti-diarrheic, antioxidant and antibacterial[10-14], some anti-insect properties of the compounds have been described in literature[15].

1.2. Spodoptera frugiperda

Spodoptera frugiperda is an endemic Lepidoptera all over the American continent that in the last decade has reached Africa, Europe and India and probably in some years will invade all the countries around the world[16]. It is considered as a key-pest and its larvae attack more than 350 plants belonging to 76 plant families, mainly Poaceae including cultivated plants as corn, rice, soybeans and cotton[17,18]. Rapid development and fertility allow *S. frugiperda* to have a high number of offspring (2.086×10^{29} individuals/female/year)[19,20]. Only in Brazil it is responsible for losses of over $ 40 million per year[21]. As these larvae attack a wide variety of plants is difficult to develop an effective control strategy[22]. Caterpillars are usually controlled with chemicals that can be harmful to the applicators and the environment and in several cases, remain in soil and plants causing biological unbalance and generating the appearance of resistant populations[23]. Moreover, these chemicals affect negatively the food safety.

In larval phase, insects choose appropriate food to obtain nutrients that favor their growing and development into sexually competitive adults. In field conditions *S. frugiperda* complete development cycle has a 30-day duration, with six instars, a larval phase of 14 days and pupae size of 14-18 mm with a duration period of 8-9 days when growing at 25 °C but when reared on artificial diets, the larval phase is shorter as well as the pupal phase[19].

In the present communication the chemical composition of the exudates from *S. sisymbriifolium* type IV trichomes and the bioactivity of the extracts isolated compounds towards *Spodopera frugiperda* is reported.

2. Experimental

2.1. General

GC was performed using an HP 6890 chromatograph – Mass Selective Detector 5973, Split Mode, T_{Inj}: 290 °C, $T_{interphase}$: 280 °C, T_{source}: 230 °C, $T_{quadrupole}$: 140 °C. Constant flow, Capillary HP-5 column, Carrier: Helium. Temperature program: T_i: 60 °C, 5 min, 5 °C min^{-1} until T_f: 270 °C, 5 min. The compound identification was done by comparison with the NIST-05 library.

NMR experiments (^1H and ^{13}C, mono and bi dimensional) were performed using a Bruker Avance 400 and 100 MHz respectively, using $CDCl_3$ as solvent and TMS as internal reference, using the standard sequences for the bidimensional experiments.

The MALDI-TOF/MS spectra were obtained using a Voyager DE-PRO instrument (Applied Biosystems), with 4-cyanohydroxycinnamic acid as matrix.

The absolute configuration of 3(*R*)-hydroxypalmitic acid was performed using a Krüss P8000 polarimeter (Krüss Optronic) equipped with software V3.0 using 5 cm cells.

All reagents were Sigma Aldrich analytical grade. The thin layer plates were Polygram Sil/UV254 0.25 mm Layer (Macherey-Nagel), and the mobile phase used was $CHCl_3$/MeOH (9:1). All extracts were concentrated under reduced pressure with temperatures below 60 °C.

The dyeing reagents used were:

1) 5% de $CuSO_4$ in 10% aqueous solution of H_3PO_4.

Eclética Química Journal, vol. 45, n. 2, 2020, 33-43
ISSN: 1678-4618
DOI: 10.26850/1678-4618eqj.v45.2.2020.p33-43

2) Sugar dyeing reagent: Diphenylamine:Aniline:Phosphoric Acid in Acetone.

2.2. Plant Material

S. sisymbriifolium samples were collected in Montevideo, Uruguay (-34.888761, -56.185015), they were identified and kept at the Jose Arechavaleta Herbarium in the Faculty of Chemistry, UdelaR, Uruguay (Voucher number 3520).

2.3. Extracts

The extraction method was performed following Rech-Cainelli *et al.* 2015[24], where 300 g of fresh leaves were immersed portion wise in 1000 mL of dichloromethane for 30 s in order to extract only epicuticular waxes compounds. The dichloromethane solutions were evaporated under reduced pressure to dryness (Dichloromethane extract). To yield the enriched fraction of sugar esters, 1 g of the previous extract was dissolved in acetone (100 mL) and the solution was cooled to -20 °C and kept overnight at this temperature. The resulting precipitate was discarded and the acetone extract was evaporated under reduced pressure to dryness (Dichlormethane/acetone extract).

2.4. Bioguided fractionation

The phytochemical study was done following a bio-guided fractionation, using the enriched fraction of sugar esters and the composition of the different fractions was evaluated by TLC. Open column chromatography was performed with the dichloromethane/acetone extract in order to isolate the two major sugar esters. 1 g of the extract was used into a 50 g of silica Gel (Baker 60-200 Mesh) column, and a total of 250 (10 mL) fractions were collected using an increasing polarity solvent mixture until the isolation of compounds 1 and 2.

The identification of Compounds 1 and 2 fatty acids was done following the methodology proposed by Heinzen *et al.* (1985)[25].

2.5. Absolute configuration of 3(R)-hydroxypalmitic acid

The methyl ester of the glycoside 2 (10 mg) was kept under stirring overnight at RT in 3 mL of 10% HCl in MeOH. A mixture of dichloromethane: water 1:1 (10 mL) was added and the phases partitioned through centrifugation. The polarized light deviation of the dichloromethane solution was measured and the {α} D calculated.

2.6. Evaluation of S. sisymbriifolium extracts bioactivity over S. frugiperda

The larvae were reared following the methodology described by Montezano *et al.* (2019)[19]. They were fed with artificial diet[26] and maintained under controlled conditions (25 ± 2 °C, 70 ± 10% RH and photoperiod of 14 h) at the Biotechnology Institute, Universidade de Caxias do Sul, Brazil.

The extracts were diluted in Tween-80 (5%) and mixed with the diet until total homogenization.

The bioassays were performed with 1 cm^3 diet blocks spiked with the test solution at 0.25, 1.00 and 2.50 mg mL^{-1} levels and a control group, in 50 mL plastic glasses. Neonate larvae were placed individually (each one was considered a repetition) in the plastic glasses for the assays.

Two bioassays were performed: the first one used 700 newborn larvae (100 replicates for each condition) to evaluate the effect of the three levels of extract 1 (E1 at 0.25, E1 at 1.00 and E1 at 2.50 mg mL^{-1}) and the three levels of extract 2 (E2 at 0.25, E2 at 1.00 and E2 at 2.50 mg mL^{-1}), besides the control group.

The second bioassay used 210 newborn larvae (30 replicates for each condition) to evaluate the effect of the three levels of Compound 1 (C1 0.25, C1 1.00 and C1 2.50 mg mL^{-1}) and three levels of Compound 2 (C2 0.25, C2 1.00 and C2 2.50 mg mL^{-1}), besides the control group.

All the replicates were observed individually until the moth emergence (adult), registering the daily survival and adult malformations.

2.7. Statistical analysis

The average survival time curves (larvae to adult) were elaborated using Kaplan-Meier estimator and comparisons between survival curves were made using Log Rank test with R

Eclética Química Journal, vol. 45, n. 2, 2020, 33-43
ISSN: 1678-4618
DOI: 10.26850/1678-4618eqj.v45.2.2020.p33-43

version 3.5.1[27]. The curves were compared between each extract and each compound.

3. Results and Discussion

The addition of *S. sisymbriifolium* dichloromethane extract (E1) and dichloromethane/acetone extract (sugar esters enriched extract) E2 at 0.25, E2 at 1.00 and E2 at 2.50 mg mL^{-1} concentrations to the diet affected the development of *S. frugiperda*, effect that can be verified by the survival curves (Fig. 1).

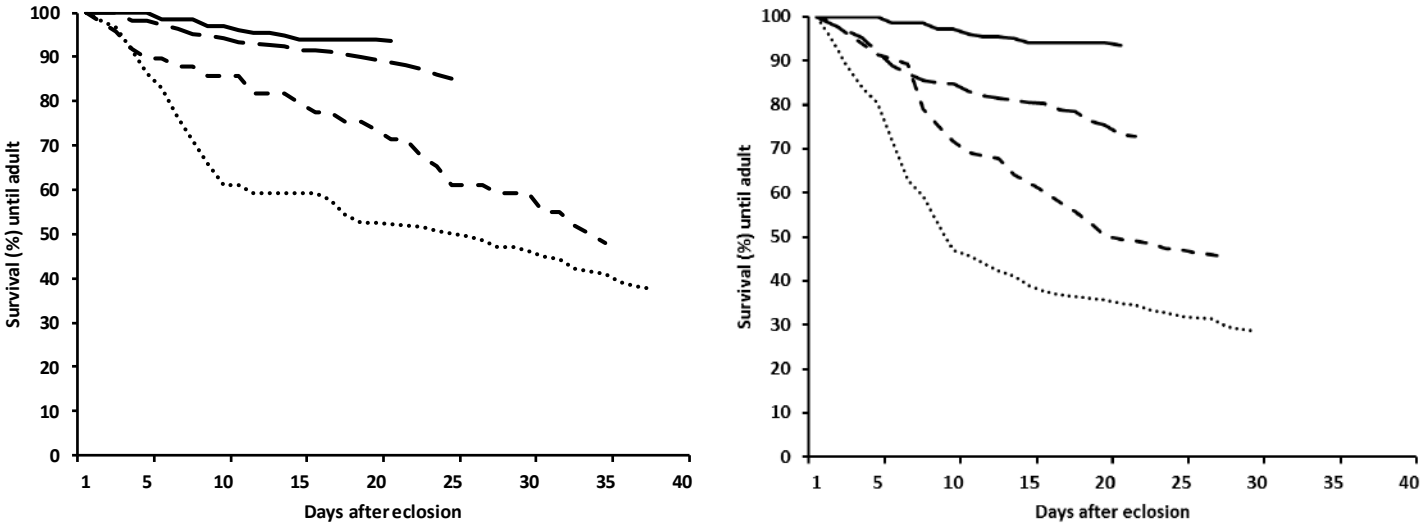

Figure 1. Survival curves of immature *Spodoptera frugiperda* (larvae, prepupae, and pupae) from larvae reared on artificial diet spiked with dichloromethane extract **E1** (a) and **E2** (b). Continuous line control (without extract); dashed line (large dashes) extract at 0.25 mg mL^{-1}; dashed line (short dashes) extract at 1.00 mg mL^{-1} and dotted line extract at 2.50 mg mL^{-1}. Note that the curves stopped at the percentage of adults emerged (Log Rank test, χ 2 = 110-118, df =3, $p < 0.001$) see Table 2.

The larvae fed on artificial diet with dichloromethane extract (E1) showed a survival percentage of 85% (0.25 mg mL^{-1}), 48% (1.00 mg mL^{-1}) and 38% (2.50 mg mL^{-1}) (Fig. 1a), in a dose dependent mortality effect (Fig. 1).

The larvae fed on artificial diet with dichloromethane / acetone extract (E2) showed greater effects on survival of *S. frugiperda* with ratios of 72% (0.25 mg mL^{-1}), 45% (1.00 mg mL^{-1}) and 29% (2.50 mg mL^{-1}) (Fig. 1b), also with a dose dependent mortality effect (Fig. 1). Because E2 showed a greater toxic overall effect and this extract was further fractionated.

It was assumed that the decreased insect survival could be related to compounds from type IV glandular trichomes which were isolated with the leaf wax and subsequently purified in the dichloromethane/acetone extract. These types of compounds are acyl sugars, with known bioactivity against insects and fungi[5,28-30].

Two major acyl sugar derivatives were isolated after silica gel column chromatography, visualized in TLC plates with the Sugar dyeing reagent (R_f = 0.3 and 0.25).

The structure of both isolated compounds was totally elucidated by NMR and GC as described in experimental section. In previous works, Cesio *et al*. (2006)[7] reported a rough description of the structure of acyl sugar methyl esters, not the naturally occurring compound. The fatty acid composition was determined by GC/MS of the methyl esters derivatives. The whole structure was proposed using the GC alditol analysis combined with the HMBC NMR experiment. The structure of the occurring natural product, the non-esterified compounds, was deduced after a thoroughly study of the MALDI–TOF/MS spectrum, and additional bidimensional NMR experiments.

Basic hydrolysis of Compound 2 yielded only palmitic acid. On the other hand, its exhaustive acid hydrolysis yielded two pentose residues, and a

Eclética Química Journal, vol. 45, n. 2, 2020, 33-43
ISSN: 1678-4618
DOI: 10.26850/1678-4618eqj.v45.2.2020.p33-43

levorotatory β-hydroxy fatty acid, identified as 3-(*R*) hydroxypalmitic acid after GC-MS analysis and the comparison to literature values of the optical properties of 3-hydroxy acids. The pentoses were identified through alditol analysis as arabinose and xylose in a 1:1 relationship. These findings suggested a compound having the structure of a glycoside of an arabinoxylan linked to the hydroxypalmitic acid and esterified with palmitic acid, Further confirmation was obtained through MALDI-TOF/MS analysis of the methyl ester of the glycoside, which detected a neat m/z=797.5392 $[M + Na]^+$ peak, and smaller one at m/z=775.5574 $[M + H]^+$. The final structure determination of Compound 1 was achieved through NMR experiments. A complex pattern of ^{13}C and ^{1}H NMR signals of a disaccharide spectra was obtained for Compound 2. A detailed analysis of ^{13}C spectra of Compound 1 yielded two carboxyl carbons at δ=177 ppm and a signal at δ=107.8 ppm, characteristic of an arabinofuranoside conformation. The other anomeric carbon was detected at δ=104.2 ppm, consistent with a carbohydrate in the pyranose conformation. The signals corresponding to 9 C-O resonances appeared between δ=60 and δ=80 ppm. Finally, two signals at δ=14 and δ=17 ppm that correspond to the methyl groups of the fatty acid chains that substituted the arabinofuranose carbohydrate framework were also detected. The ^{1}H NMR spectra of Compound 1 showed a region of methylenic protons between δ=1 and δ=2 ppm, a multiplet at δ=2.8 ppm which are consistent with the alpha protons of a β-hydroxyacid and another multiplet centered at δ=2.2 ppm corresponding to the protons alfa to the acyl residue attached to the dissacharide. The sugar protons appeared between δ=3.2 and δ=5.2 ppm. Bidimensional NMR experiments allowed the full characterization of the molecule. The HSQC-TOCSY experiment showed four spin systems. The two carbohydrate residues were clearly differentiated. The two anomeric carbons were detected at δ=107.8 ppm and δ=104.2 ppm in the ^{13}C NMR spectrum, the monosaccharide units with anomeric protons at δ=5.2 and 4.45 ppm (J=5.4) corresponded to two pentoses with their methylene carbons at δ=68.7 and δ=65.8 ppm, respectively, and were identified as α-L-furanoarabinose and β-D-xylose. The third spin system belonged to the fatty acid residue attached to the disaccharide. Whereas the fourth spin system corresponded to a β-hydroxy carboxylic acid system δ=4.11, 2.8 ppm aligned through the ^{13}C signal at (δ=74.1). COSY ^{1}H-^{1}H connectivity allowed the unambiguous assignment of all protons and therefore the identification of the structure of each monosaccharide (Tab. 1). The HMBC experiment gave the intramolecular connectivity that allowed the final structure elucidation of Compound 1. Cross peaks between *araf* C-1 (δ=107.8) H-3 of hydroxypalmitic (δ=4.11) and C-3 (δ=74.1) of hydroxypalmitic and the anomeric signal of *araf* (5.15); between C-1 (δ=177) of palmitic acid and *araf* H-3 (δ=4.11) showed the substitution of the arabinose residue. A third group of cross peaks were detected between the C-5 of *araf* (δ=68.7), and H-1 xyl (δ=4.20); the geminal protons H-5 *araf* (δ=4.07; 3.80) and the C-1 (δ=104.2) of the terminal xylose unit. They were indicative of a glycosidic linkage between the anomeric carbon of the xylose residue and the C-5 *araf*- geminal protons, showing the xylo-β-1-5-ara*f*- structure of Compound 1[31]. Based on the above discussed data, we propose that the Compound 2 structure is the 3-*araf*- palmitate of. [3-(*R*)-hydroxypalmitic acid] (*araf*-1)→xylo (β1-5) furanoarabinoside, (Fig. 2).

The structure of Compound 2 was deduced comparing the spectral data and chemical properties of Compound 1. The acid hydrolysis of Compound 2 gave the same carbohydrate and hydroxy acid residues as for Compound 1 but no palmitic acid was detected after basic hydrolysis. MALDI-TOF/MS spectrum showed a peak at m/z=559.3095 $[M +Na]^+$ which is consistent to a disaccharide of xylose and arabinofuranose with a 3-(*R*) hydroxypalmitic acid. ^{13}C NMR spectrum showed only the signal at δ=14 ppm from one methyl group and only one signal at δ=176 ppm was detected. The signal at δ=4.2 ppm had shifted to δ=3.6 ppm, indicating that Compound 2 is the unesterified glycoside of Compound 1. Bidimensional NMR experiments (HSQC-TOCSY, ^{1}H-^{1}H COSY, HMBC) were consistent and confirmed Compound 1 as [3-(*R*)-hydroxypalmitic acid] (*araf*-1) and xylo (β1-5) arabinofuranoside (Fig. 2).

Eclética Química Journal, vol. 45, n. 2, 2020, 33-43
ISSN: 1678-4618
DOI: 10.26850/1678-4618eqj.v45.2.2020.p33-43

Table 1. ^{1}H and ^{13}C relevant assignments of the glycosidic template of Compound 1 and 2 using 1D and 2D NMR experiments with a 100 MHz field in $CDCl_3$.

Compound 1				
Carbon	**xylose**		**arabinose *(f)***	
	H	**C**	**H**	**C**
1	4.45 (J = 2.7 Hz)	104.2	5.06 (J = 5.4 Hz)	107.8
2	3.45 (J = 3.5, 2.7 Hz)	76.8	3.93 (J = 7.1, 5.4 Hz)	76.2
3	3.35 (J = 3.5 Hz)	73.7	3.99 (J = 7.1, 4.4 Hz)	76.4
4	3.62 (J = 3.5, 2.9, 2.7 Hz)	69.9	3.98 (J = 4.7, 4.4 Hz)	83.2
5	3.23 (J = 13.3, 2.9 Hz)	65.8	4.02 (J = 4.7 Hz)	68.7
	3.92 (J = 13.3, 2.7 Hz)		3.75 (J = 4.7 Hz)	

Compound 2				
Carbon	**xylose**		**arabinose (f)**	
	H	**C**	**H**	**C**
1	4.45 (J = 2.7 Hz)	104.2	5.15 (J = 5.4 Hz)	107.8
2	3.45 (J = 3.5, 2.7 Hz)	76.8	4.05 (J = 7.1, 5.4 Hz)	80.4
3	3.35 (J = 3.5 Hz)	73.7	4.90 (J = 7.1, 4.4 Hz)	80.2
4	3.62 (J = 3.5, 2.9, 2.7 Hz)	69.9	4.15 (J = 4.4, 4.3 Hz)	83.1
5	3.23 (J = 13.3, 2.9 Hz)	65.8	4.07 (J = 4.3 Hz)	68.7
	3.92 (J = 13.3, 2.7 Hz)		3.80 (J = 4.3 Hz)	

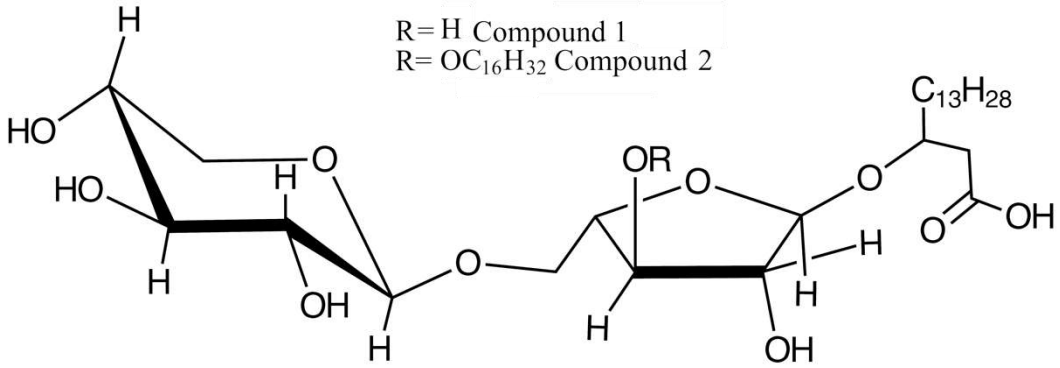

Figure 2. Molecular structures of Compounds 1 and 2.

Summarizing we postulate that the structures of the acyl sugars from *S. sisymbriifolium* are:

Compound 1: xylo (β-1-5) furanoarabinoside (α-1-3)3-(*R*)-hydroxypalmitic acid $C_{26}H_{48}O_{11}$. MALDI-TOF/MS $[M+Na]^+$ = 559.3095. Calculated 559.3094, and for **Compound 2**: 3-O *araf*- Palmitic acid ester of xylo (β-1-5) furanoarabinoside (α-1-33-(*R*)-hydroxypalmitic acid $C_{42}H_{78}O_{12}$ $[M+Na]^+$ = 797.5392 Calculated 797.5391.

Furthermore, the biological activity of the two isolated compounds was studied in bioassays conducted on artificial diet spiked with them. The results are shown in Fig. 3. Tables 2, 3 and 4 show the results of the Log Rank test with the c^2 values for the different situations at different confidence levels. This test is the preferred one to compare if

Eclética Química Journal, vol. 45, n. 2, 2020, 33-43
ISSN: 1678-4618
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the survival of populations when exposed to different stressors are independent events or not. All the compounds and extracts caused significantly higher mortality than the control during the bioassays. Furthermore, the toxicity of the pure compounds was also significantly higher than that caused by the crude extracts (see Tab. 4).

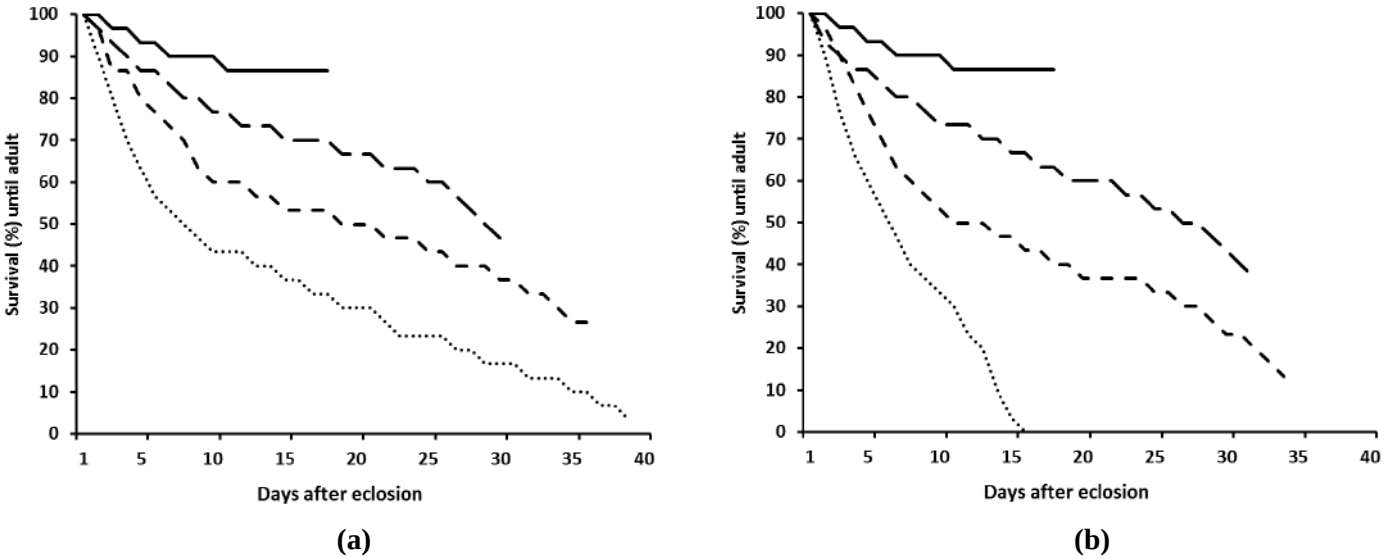

Figure 3. Survival curves of immature *Spodoptera frugiperda* (larvae, prepupae, and pupae) from larvae reared on artificial diet spiked with Compounds **1** (a) and **2** (b). Continuous line control (without extract); dashed line (large dashes) 0.25 mg mL^{-1}; dashed line (short dashes) 1.00 mg mL^{-1} and dotted line 2.50 mg mL^{-1}. Note that the curves stopped at the percentage of adults emerged (Log Rank test, χ 2 =38-51, df =3, p < 0.001) see Tab. 3.

Table 2. Chi-square and p-values from Log Rank test for comparison between survival curves of *Spodoptera frugiperda* (larvae, prepupae, and pupae) from larvae reared on artificial diet spiked with dichloromethane extract E1 and E2.

	DF	χ^2 Extract 1	p	χ^2 Extract 2	p
General comparison among all curves	3	110.1	<0.001	118.0	<0.001
Control × 0.25 mg mL^{-1}	1	5.5	=0.020	18.9	<0.001
Control × 1.00 mg mL^{-1}	1	54.2	<0.001	60.8	<0.001
Control × 2.50 mg mL^{-1}	1	75.9	<0.001	98.4	<0.001
	DF	**χ^2 Extract 1**	**p**	**χ^2 Extract 2**	**p**
0.25 mg mL^{-1} × 1.00 mg mL^{-1}	1	29.4	<0.001	16.1	<0.001
0.25 mg mL^{-1} × 2.50 mg mL^{-1}	1	49.1	<0.001	43.3	<0.001
	DF	**χ^2 Extract 1**	**p**	**χ^2 Extract 2**	**p**
1.00 mg mL^{-1} × 2.50 mg mL^{-1}	1	3.8	=0.050	7.7	=0.006

Eclética Química Journal, vol. 45, n. 2, 2020, 33-43
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Table 3. Chi-square and p-values from Log Rank test for comparison between survival curves of *Spodoptera frugiperda* (larvae, prepupae, and pupae) from larvae reared on artificial diet spiked with Compounds 1 and 2.

	DF	χ^2 Compound 1	p	χ^2 Compound 2	p
General comparison among all curves	3	38.2	<0.001	58.9	<0.001
Control × 0.25 mg mL^{-1}	1	9.3	=0.002	12.7	<0.001
Control × 1.00 mg mL^{-1}	1	20.9	<0.001	26.1	<0.001
Control × 2.50 mg mL^{-1}	1	36.7	<0.001	48.4	<0.001
	DF	**χ^2 Compound 1**	**p**	**χ^2 Compound 2**	**p**
0.25 mg mL^{-1} × 1.00 mg mL^{-1}	1	2.7	=0.100	2.7	=0.100
0.25 mg mL^{-1} × 2.50 mg mL^{-1}	1	10.6	=0.001	21.2	<0.001
	DF	**χ^2 Compound 1**	**p**	**χ^2 Compound 2**	**p**
1.00 mg mL^{-1} × 2.50 mg mL^{-1}	1	2.4	=0.100	9.1	=0.003

Table 4. Chi-square and p-values from Log Rank test for comparison between survival curves of *Spodoptera frugiperda* (larvae, prepupae, and pupae) from larvae reared on artificial diet spiked with extracts 1 and 2 and Compounds 1 and 2 at each concentration.

	DF	χ^2 Extract 1 × Extract 2	p
0.25 mg mL^{-1}	1	4.7	=0.030
1.00 mg mL^{-1}	1	0.8	<0.400
2.50 mg mL^{-1}	1	2.1	<0.100
	DF	**χ^2 Compound 1 × Compound 2**	**p**
0.25 mg mL^{-1}	1	0.4	=0.500
1.00 mg mL^{-1}	1	0.5	=0.500
2.50 mg mL^{-1}	1	4.7	=0.030

It was observed that compounds 1 and 2 at concentrations of 0.25, 1.00 and 2.50 mg mL^{-1} affected the development of *S. frugiperda*. The observed mortality was concentration-dependent for compound 1 and 2 (Fig. 3). After 40 days of evaluation, Compound 1 did not reach 100% mortality with the higher evaluated concentration. But Compound 2 achieved 100% mortality evaluated in the same condition after 15 days of experiment.

Moreover, the overall number of insects reaching the adult phase were 46.29% (0.25 mg mL^{-1}), 15.27% (1.00 mg mL^{-1}) and 3.46% (2.50 mg mL^{-1}) for Compound 1 (Fig. 3).

When Compound 2 was evaluated, the survival observed percentages were 36.72% (0.25 mg mL^{-1}), 11.11% (1.00 mg/mL) and zero (2.50 mg mL^{-1}) (Fig. 3).

S. frugiperda feeding behavior as a polyphagous insect requires large amounts of food for their development to reach the adult stage. There is no evidence of sugar esters acute toxicity to insects, and the intake may be a prerequisite to detect the deleterious effects of these compounds[32].

As observed in other studies evaluating effects of plant extracts on *S. frugiperda* development[33-35], in all treatments, beyond mortality, were observed abnormalities in immatures that which qualitatively demonstrate the effects of extracts and compounds on the development of *S. frugiperda*.

The isolated compounds, evaluated at the same concentration levels, were more toxic than the crude extracts assayed and caused noticeable abnormalities in the metamorphosis phase. The abnormalities that resulted in the interruption of the metamorphosis process have similar characteristics as those already described for lefenuron, which acts as an insect's growth regulator[36].

Eclética Química Journal, vol. 45, n. 2, 2020, 33-43
ISSN: 1678-4618
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4. Conclusions

The two evaluated extracts increase the immature mortality of *S. frugiperda*. This effect could be related to the individual acyl sugars tested.

The dichloromethane / acetone extract (sugar ester enriched extract) E2, highly affected the larval survival rather than the pupal.

The response was dose dependent and the major compounds (1 and 2) from this extract were isolated and their structures elucidated.

When the pure compounds were added to the diet, a significant effect was observed in the metamorphosis phase, as many of the exposed caterpillars failed to accomplish the transformation to reach the pupal stage. See Fig. 4a, 4b, 4c. With Compound 1, the rates of mortality of the insects failing to survive the larval to pupal phase transition were 27% (1.00 mg mL^{-1}) and 53% (2.50 mg mL^{-1}). The mortality of the insects that could not complete their metamorphosis was 17% (0.25 mg mL^{-1}) and 50% (mg mL^{-1}), both compounds showed important effects over the larval stage. See Fig. 4d and 4e.

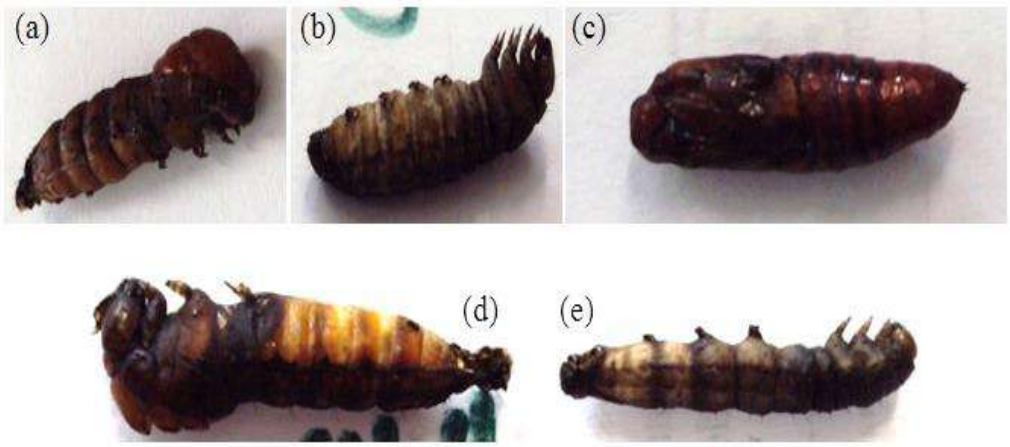

Figure 4. Abnormalities found in *S. frugiperda* larval stage when fed with artificial diet spiked with Compound 2 (a, b & c) and with Compound 1 (d & e).

Compounds 1 and 2 showed high toxicity levels over the *S. frugiperda* larval phase with almost 100% mortality for Compound 1 at 2.50 mg mL^{-1} and 90% mortality for Compound 2 at 1.00 mg mL^{-1}.

These results show that the acyl sugars isolated from *S. sisymbriifolium* trichomes are a new class of bioactive compounds that affect the development of the fall armyworm *S. frugiperda*. Due to their simple chemical structures, they could be interesting lead compounds for the synthesis of new agents for pest control working as biopesticides contributing with the sustainability of different ecosystems.

5. Acknowledgments

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Study of the properties of lubricating oils obtained from biodiesel

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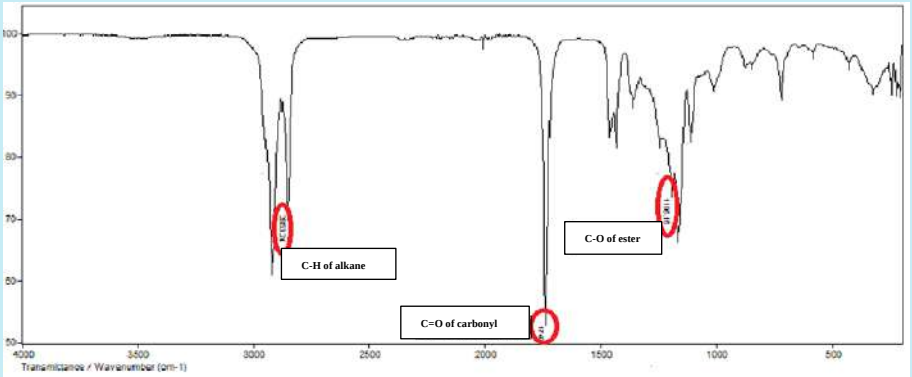
ABSTRACT: In this work, nine different biodiesels were obtained by transesterification reaction with three types of vegetable oils and three variant alcohols. The objective was to analyze them physicochemically before and after they went to the engine, to know if they had the necessary properties to act as lubricants. Subsequently, the same biodiesels were analyzed in the engine in order to observe the behavior of their dynamic viscosities, to evaluate if they were similar or superior to commercial lubricants. It was possible to note that all biodiesel produced are within the National Agency for Petroleum, Natural Gas and Biofuels (ANP) legislation and presented as potential lubricants, due to the kinematic viscosity behavior when compared before and after the engine. These results made them possible replacements for commercial lubricants, besides having greater advantages for the engine and the environment. However, although coconut oil-derived biodiesel has good yields, it is not promising because, at temperatures below 25 °C tends to solidify, causing short, medium- and long-term engine parts wear.



The obtained biodiesels proved to be potential lubricants and could replace the commercial lubricants of the 4-stroke engines.

1. Introduction

In the last 10 years, the world's energy consumption grew by 2.5%. Oil has been steadily falling, but it is still considered the largest primary source of used energy, although the energy consumption from renewable sources is growing. China has been reducing its energy consumption over the years, but it is still considered the most energy consuming country in the world, importing about 58% of oil. Following on the list of the world's largest energy consumers, there are the United States, India, Germany and Brazil. Brazil is expected to grow 2.2% by the year 2040, with emphasis on renewable energies (4.5%)[1].

It has now become clear that the world's perceptions about renewable energy have changed a lot and are now seen as an outlet to address some needs such as improving energy security, reducing impacts on health and the environment, mitigating greenhouse gas emissions, improving opportunities in the area of education, creation of new labor poles and poverty reduction[2].

Biodiesel appears as an alternative, since its main application is fuels, because it is free of aromatic compounds and sulfur, an important characteristic in the environmental sector; besides being more economically viable for stimulating agricultural production, reducing importation of oil from other countries. It can be obtained through a mixture of different esters resulting from the

esterification reaction of fatty acids or transesterification of glycerides[3].

Several raw materials are used in the production of biodiesel, such as vegetable oils, animal fats and used frying oil. According to the National Petroleum Agency[4], biodiesel produced in Brazil comes from soybean oil (76.4%), animal fat (19.8%), cotton oil (2.2%) and other vegetable oils (1.6%).

In addition to using biodiesel as fuel, it can also be used as lubricant. When it acts as a lubricant, it performs much better than commercial lubricants, due to its high lubricating power, higher viscosity and less wear on the engine parts[5].

For the development of a lubricant, specified physicochemical properties must be obtained, such as viscosity, resistance to corrosion, total acidity index, pour point, chemiluminescence, among others. In the case of biolubricant esters which are derived from organic esters, hydrolytic, oxidative and thermal stabilities must be obtained[6].

The lubricating oil used in the engine must meet all lubrication needs in the different tribological pairs found in the engine[7]. Thus, it can be stated that the ignition quality of the fuel, the types of hydrocarbons present in the fuel and the presence of impurities or additives in it affect the performance of the engine lubricating oil.

In this study, the synthesis of biodiesel from vegetable oils was proposed, as well as the analyses of their physicochemical properties to act as lubricants, evaluating the kinematic viscosity before and after the sample in the engine to verify if the performance of biodiesel as a lubricant is similar to or higher than commercial lubricants.

2. Experimental

2.1. Production of biodiesel

For the transesterification reactions, the methodology described by Geris *et al.*[8] was used, which initially consisted of the preparation of an intermediate through the mixture of potassium hydroxide and varying alcohols, such as methanol, ethanol and isopropyl alcohol, under heating at 45 °C and constant stirring until complete dissolution of the solid. Later, the intermediate was mixed with the oils, which were also varied in the reaction as: being coconut oil, castor oil and rice oil. They were commercially purchased, and the reaction were carried out at 45 °C for 10 min. Afterwards, the biodiesel was elaborated, pouring the previous mixture and separating it through a separation funnel, isolating the biodiesel and the glycerin, and the excesses of base and alcohol. The biodiesel was treated with distilled water at 70 °C, followed by aqueous solution of hydrochloric acid 0.5% v/v until it reached neutral pH. Finally, moisture was withdrawn with anhydrous magnesium sulfate under constant stirring for 15 min.

Thus, nine combinations of reactions were performed: coconut oil and methyl alcohol, castor oil and methyl alcohol, rice oil and methyl alcohol, coconut oil and ethyl alcohol, castor oil and ethyl alcohol, rice and ethyl alcohol, coconut oil and isopropyl alcohol, castor oil and isopropyl alcohol and rice oil and isopropyl alcohol.

2.2. Biodiesel yield

Regarding the mass yield of biodiesel produced through the basic transesterification process, the quantity of biodiesel obtained from the ratio of 1:6 with the highest amount of oleic acid was accounted for. Yield was evaluated from the alcohol and vegetable oil used in the reaction.

All reactions were performed in triplicate, as approximately 500 mL were required for further use in the engine.

2.3. Validation of the lubrication property of the lubricants in the internal combustion engine by the kinematic viscosity analysis

The samples were assayed in a seven-stroke gasoline four-stroke engine, Manual Start - NMG70, at Univiçosa Physics Laboratory. Initially, it was necessary to use 500 mL of commercial fuel to start the engine. Subsequently, each sample was for 3 nonconsecutive h in the motor.

For the engine to run, 500 mL of each synthesized biodiesel were used, according to routes previously described in the methodology. It is important to note that when below this amount, the motor does not run to rotate the sample.

2.4. Physicochemical characterization of biodiesel

All the biodiesel obtained were characterized by pH, specific mass, total acidity index, moisture content, kinematic viscosity, plus metal detection tests and infrared. All analyzes were performed in

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triplicate, except for the viscosity that was performed five times.

2.4.1. Appearance and color

In order to characterize the biodiesel in relation to aspect and color, a preliminary analysis was carried out looking for the presence of visual impurities, such as suspended materials, sediments or any turbidity in the sample, which may be due to the presence of water. In the absence of any of these contaminants, biodiesel was classified as clear and free of impurities, according to NBR 14954 [9].

2.4.2. pH

The pH was measured with a pHmeter, which can present values ranging from 0 to 14, indicating acidity, neutrality or basicity of the medium. For biodiesel, the pH must be 7, that is neutral[10].

2.4.3. Specific mass

To determine the specific mass, a 25 mL glass pycnometer was used. First the empty pycnometer was weighed and written down to its mass. Then it was filled with distilled water, weighed and its mass was noted. Subsequently, the clean and dry pycnometer was filled with biodiesel, being again weighed with its mass noted, according to NBR 7148/14065 [11]. The specific mass was calculated with Eq. 1:

$$\rho = \frac{m}{v} \quad (1)^{11}$$

2.4.4. Total acidity index

To calculate the acid number, it was necessary to measure a mass of approximately 10 g of biodiesel in a 125 mL Erlenmeyer flask. A mixture of ethyl alcohol and ethyl ether in the ratio of 2:1 by volume was prepared. Subsequently, 25 mL of this mixture was added to the Erlenmeyer flask along with 3 drops of phenolphthalein. Titration of the solution was then carried out with 0.02 mol L^{-1} sodium hydroxide, and the blank was titrated, that is, 25 mL of the mixture of ethyl ether and ethyl alcohol together with 3 drops of phenolphthalein without the presence of biodiesel, according to NBR 14448 [12]. The calculation of the acid number is performed by Eq. 2:

$$Ia = \frac{(V_{spent} - V_{blank\ reaction}) x f x 5.61}{m_{oil}} \quad (2)^{12}$$

2.4.5. Moisture content

To determine the moisture content of the biodiesel, a greenhouse, in which a mass of approximately 20 g of biodiesel was placed in the crucible free of moisture, was used and then weighed.

The sample was then placed in an oven, with temperature at 100 °C for 24 h. After this time, the sample was cooled in a desiccator and the sample was weighed again until the value remained constant, thus obtaining the moisture content by the difference between the initial and final masses, through Eq. 3, according to ASTMD 6304 [13].

$$\text{Moisture content} = P_i - P_f \quad (3)^{13}$$

P_i is the initial weight of the crucible with biodiesel and P_f is the final weight of the crucible with biodiesel.

2.4.6. Kinematic viscosity

The kinematic viscosity was determined with the aid of a pipette, adding 15 mL of sample to the viscometer through the ventilation tube, and waited 5-10 min to equilibrate the liquid temperature to 25 °C. With the aid of the pipettor fitted to the capillary tube, the fluid was suctioned up to above the upper measurement mark, according to NBR 10441[4]. The kinematic viscosity can be calculated by Eq. 4:

$$\nu = K \times t \quad (4)^{11}$$

Where ν is the kinematic viscosity of the sample (in $mm^2\ s^{-1}$), K is the viscometer constant calculated for a known fluid (in $mm^2\ s^{-2}$) and t is the average of the time the sample took to move from the measurement mark top to bottom.

2.4.8. Qualitative test for metal detection

For the qualitative test of metal detection, electromagnetic magnets were used. The magnets were approximated to the biodiesel samples. In case of attraction, it indicates the presence of metals and if nothing happens, absence[8].

2.4.9. Infrared

Eclética Química Journal, vol. 45, n. 2, 2020, 44-53
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The spectra were collected in the Perkin-Elmer FT-IR 1000 spectrophotometer in the range of 4000 to 200 cm^{-1} in the Department of Chemistry – UFV and were made in ATR.

3. Results and Discussion

3.1. Characterization of biodiesel

The biodiesel characterization was carried out from the results of some parameters, through which the quality of the biodiesel produced can be evaluated. The quality has a direct relation with the operation and the life of an engine, so it was possible to ascertain the quality of the biodiesel produced.

According to *National Agency* for *Petroleum*, Natural Gas and Biofuels regulation 25/2014 [4], in order to the product obtained to be considered a biodiesel, it must comply with some rules such as: be clear and free of impurities; have neutral pH; have density, total acidity index, moisture content and kinematic viscosity within the established limits.

Tables 1, 2 and 3 present the methodologies used in each parameter, the results obtained by the analysis of coconut, castor and rice biodiesel, respectively, following the limits established by the Brazilian legislation[4].

Table 1. Results obtained for acidity index tests (mg KOH g^{-1}), relative density (kg m^{-3}), moisture content (mg kg^{-1}), followed by the Brazilian legislation[4].

Parameters	Methodology adopted	BCOM	BCOE	BCOI	NPA Resolution, 2004
Aspect	NBR 14954	Clean and free of impurities	Clean and free of impurities	Clean and free of impurities	Clean and free of impurities
Specific mass	NBR 7148/14065	868.432 ± 0.002	867.012 ± 0.005	872.476 ± 0.003	850 to 900
Specific relative mass	-	0.835 ± 0.002	0.794 ± 0.005	0.780 ± 0.003	-
Acidity Index level	NBR 14448	0.090 ± 0.008	0.059 ± 0.008	0.042 ± 0.007	<0.5
Moisture content	ASTMD 6304	197.000 ± 0.003	189.09 ± 0.02	190.73 ± 0.03	<200.0

BCOM - Biodiesel coconut oil with methanol; **BCOE** - Biodiesel coconut oil with ethanol; **BCOI** - Biodiesel coconut oil with isopropanol; **BCAOM** - Biodiesel castor oil with methanol; **BCAOE** - Biodiesel castor oil with ethanol; **BCAOI** - Biodiesel castor oil with isopropanol; **BROM** - Biodiesel rice oil with methanol; **BROE** - Biodiesel rice oil with ethanol; **BROI** - Biodiesel rice oil with isopropanol.

For biodiesel produced from methanol, all are classified as clear and free of impurity, as specified by Brazilian legislation.[4], since they have no impurities or turbidity, so biodiesel is within the cited specification.

The specific mass values obtained at 20 °C for all biodiesel are within the limits of 850 to 900 kg m^{-3} established by NBR 7148/14065[4]. The values were within the range required by the Brazilian legislation[4], which indicates high purity of the products obtained.

Considering that Resolution nº 255, dated November 15, 2003, indicates that the maximum limit of acidity index is 0.50 mg KOH g^{-1} of oil, so the values observed in Tab. 1 are within the allowed values, which is a good result, since an acidity index above the allowed one leads to the aging of the lubricant and it wears the parts of the motor in the long term.

For the analysis of the moisture content, Eq. 3 was used, according to methodology. It can be observed that all values obtained for biodiesel from methanol, in Tab. 1, were within the limit of 0.5 mg kg^{-1} required by the Brazilian legislation[4], which is important since the water content above the allowance impairs the property of the lubricant and oxidizes the engine parts over time.

The relative specific mass is given by the specific mass of the biodiesel in relation to the specific mass of the water, both obtained experimentally. Even though a limit is not specified[4], as the specific masses were within the limit, consequently, the relative specific masses are too.

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Table 2. Results obtained for acidity index tests (mg KOH g^{-1}), relative density (kg m^{-3}), moisture content (mg kg^{-1}), followed by the Brazilian legislation[4].

Parameters	Methodology adopted	BCAOM	BCAOE	BCAOI	NPA Resolution, 2004
Aspect	NBR 14954	Clean and free of impurities	Clean and free of impurities	Clean and free of impurities	Clean and free of impurities
Specific mass	NBR 7148/14065	869.352 ± 0.002	898.540 ± 0.003	861.676 ± 0.002	850 a 900
Specific relative mass	-	0.853 ± 0.002	0.833 ± 0.003	0.628 ± 0.002	-
Acidity Index level	NBR 14448	0.045 ± 0.006	0.20 ± 0.02	0.070 ± 0.005	<0.5
Moisture content	ASTMD 6304	189.08 ± 0.02	190.76 ± 0.02	189.09 ± 0.02	<200.0

BCAOM - Biodiesel castor oil with methanol; **BCAOE** - Biodiesel castor oil with ethanol; **BCAOI** - Biodiesel castor oil with isopropanol.

In Tab. 2, it can be observed that all the biodiesels obtained through the castor oil were also clear and free of impurities.

The specific masses were within the range of 850 to 900 kg m^{-3}, indicating high purity of these products obtained by the transesterification reaction. In addition, the specific mass of a biodiesel is directly linked to the molecular structure of its molecules. The longer the carbon chain of the ester, the greater its specific mass, but the value will decrease the higher the numbers of unsaturated bonds present in the molecule. The presence of impurities may also influence the specific mass. As observed, ethanol has a higher carbon chain than methanol, so its specific mass is higher. In contrast, isopropanol has branching; consequently, there is a decrease in its specific mass, as stated by Lace *et al.*[14].

The acidity index was also within the Brazilian legislation[4] limit, being less than 0.5 mg KOH g^{-1}, with very low standard deviations, which corresponds to the accuracy of the triplicate analyzes.

Regarding the moisture content, the values obtained were lower than 200 mg kg^{-1}, indicating that all biodiesel obtained from castor oil are within the limits of the Brazilian legislation[4].

Table 3. Results obtained for acidity index tests (mg KOH g^{-1}), relative density (kg m^{-3}), moisture content (mg kg^{-1}), followed by standard legislation[4].

Parameters	Methodology adopted	BROM	BROE	BROI	NPA Resolution 2004
Aspect	NBR 14954	Clean and free of impurities	Clean and free of impurities	Clean and free of impurities	Clean and free of impurities
Specific mass	NBR 7148/14065	871.412 ± 0.014	876.768 ± 0.018	866.012 ± 0.021	850 to 900
Specific relative mass	-	0.83 ± 0.03	0.84 ± 0.04	0.82 ± 0.02	-
Acidity Index level	NBR 14448	0.034 ± 0.003	0.059 ± 0.002	0.037 ± 0.005	<0.5
Moisture content	ASTMD 6304	178.11 ± 0.03	178.92 ± 0.03	198.52 ± 0.03	<200.0

BROM - Biodiesel rice oil with methanol; **BROE** - Biodiesel rice oil with ethanol; **BROI** - Biodiesel rice oil with isopropanol.

Through Tab. 3, it was possible to observe that all the biodiesels obtained with isopropanol were clear and free of impurities.

For the specific mass, the values were within the limit of 850 to 900 kg m^{-3} according to the legislation[4], indicating the high purity of these obtained biodiesels. In the same way, the ethanol has greater carbonic chain than the methanol, therefore, greater specific mass; while the isopropanol has branching, then it has a lower specific mass, according to Lace *et al.*[14].

Eclética Química Journal, vol. 45, n. 2, 2020, 44-53
ISSN: 1678-4618
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The acidity index was also within the limit of 0.5 mg KOH g^{-1}, presenting low values, which is a good sign since it does not indicate wear of the engine parts.

Regarding the moisture content, the values were within the limit of 200 mg kg^{-1} of the Brazilian legislation[4]. It is very important that the analyzes of moisture content are within the limit described by the National Agency for Petroleum, Natural Gas and Biofuels, since, according to Lace *et al.*[14], water promotes the hydrolysis of biodiesel resulting in free fatty acids, contributing to the proliferation of microorganisms, equipment corrosion and deposition of sediments. In addition, the presence of water contributes to the increase in acidity, which is not desired.

3.2. Biodiesel yield

The yield calculation was useful to verify which vegetable oil and alcohol had the best yield in obtaining biodiesel, that is, how much was converted from reagent (vegetable oil) to product (biodiesel).

Tables 4, 5 and 6 below indicate these yields:

Table 4. Yield for biodiesel derived from coconut oil.

% biodiesel	BCOM	BCOI	BCOE
Reaction 1	60	60	58
Reaction 2	75	66	63
Reaction 3	89	70	55

Some studies by Nascimento, Vasconcelos and Azevedo[15] show a technique of microwave optimization, leading to a yield of approximately 100%.

The biodiesel obtained by means of coconut oil with isopropanol presented values higher than expected, although there are no comparisons with the literature. However, according to Geris *et al.*[8], isopropanol is not as viable for transesterification reaction due to its higher carbon chain and greater branching when compared to the other alcohols used.

Regarding biodiesel derived from coconut oil with isopropanol, the values were within the expected range, as described by Shimada *et al.*[16], from 45 to 80%, a little lower than methanol due to its higher carbon chain, less toxic than it.

Geris *et al.*[8] showed that biodiesel obtained from methanol yields between 58 and 89%, due to the lower carbon chain that facilitates the transesterification reaction; followed by ethyl alcohol in yields between 55 and 75%, being even less toxic. The higher the carbon chains and the ramifications, the lower the yields of biodiesel due to the greater difficulty of reacting. In relation to coconut oil, for Dias, Ferraz e Almeida[17] the yields with methanol are around 60 to 90%, while with ethanol, for Shimada *et al.*[16], they vary between 45 and 80%.

Table 5. Yield values for biodiesel derived from castor oil.

% biodiesel	BCAOM	BCAOI	BCAOE
Reaction 1	65	45	56
Reaction 2	87	47	51
Reaction 3	93	74	64

Table 5 shows that the biodiesel obtained through castor oil with methanol presented good yields, according to the range of 60 to 96% specified by Dias, Ferraz e Almeida[17].

For biodiesel derived from castor oil with isopropanol, it is believed that the values were low as expected by the chemical explanation regarding the amount of carbonic chain and branching, as explained by Geris *et al.*[8].

The biodiesel obtained by means of castor oil with ethanol presented yield between the limit expected by Dias, Ferraz e Almeida[17], from 40 to 75%. As shown in Tab. 4, yields of biodiesel obtained from coconut oil by methanol were considered as expected, since according to Dias, Ferraz e Almeida[17], a yield of 60 to 90% for biodiesel from coconut oil from methanol.

Table 6. Yield values for biodiesel derived from rice oil.

% biodiesel	BROM	BROI	BROE
Reaction 1	83	46	54
Reaction 2	71	53	66
Reaction 3	81	64	96

Table 6 shows that biodiesel obtained from rice oil with methanol presented high values, as expected by Charoenchaitrakool and Thienmethangkoon[18], from 70 to 95%.

The biodiesel obtained through the rice oil with isopropanol presented low yields, but already expected according to the chemical explanation of the carbon chain, as suggested by Geris *et al.*[8].

For the biodiesel obtained by means of the rice oil with ethanol, the yields were within the expected, with highlight to the 3rd reaction with

Eclética Química Journal, vol. 45, n. 2, 2020, 44-53
ISSN: 1678-4618
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yield of 96%, higher than expected by the limit of 50 to 80% of Charoenchaitrakool and Thienmethangkoon[18].

In relation to the performance of biodiesel in the engine, the yield does not directly influence. However, good yields are feasible because of the amount of 500 mL needed for the lubricant to run on the engine. A low yield means having to perform a greater amount of reaction to achieve what is required for the internal combustion engine, making the process more expensive and exhausting.

3.3. Validation of the lubrication property of the lubricants in the internal combustion engine by the kinematic viscosity analysis

It was observed that the engine maintained the correct operating temperature, since a laser thermometer was used to carry out the temperature measurements during operation, so that there was no overheating of the engine components. Also, no smoke or anomaly was observed during or after the tests.

Kinematic viscosity analyzes are listed in Tab. 7, 8 and 9, both before and after each sample for 3 h. The analyses were performed in quintuplicate and the results were given by the mean.

Analyzes before the engine will be called BE and after engine AE.

Table 7. Kinematic viscosity results in biodiesel derived from coconut oil.

Kinematic viscosity (mm^2s^{-1})	BOCM (BE)	BOCI (BE)	BOCE (BE)	BOCM (AE)	BOCI (AE)	BOCE (AE)
Average	2.600 ± 0.002	5.980 ± 0.004	6.000 ± 0.007	6.400 ± 0.004	-	6.500 ± 0.008

In Tab. 7, it was possible to observe that the biodiesel obtained through coconut oil before the engine showed satisfactory results, within the range of 3.0 to 6.0 $mm^2\ s^{-1}$ of the NBR[4]. Only the biodiesel obtained from coconut oil with methanol showed below-expected value, which is detrimental since lubricant that has low viscosity may not sufficiently protect engine parts, increasing parts wear, causing more friction and oxidizing faster, as explained by Farias *et al.*[5]. The viscosity values after the engine increased, but not excessively, which was expected due to the oxidation of the biodiesel in the engine.

Table 8. Result of kinematic viscosity in biodiesel derived from castor oil.

Kinematic viscosity (mm^2s^{-1})	BCAOM (BE)	BCAOI (BE)	BCAOE (BE)	BCAOM (AE)	BCAOI (AE)	BCAOE (AE)
Average	6.01 ± 0.01	5.80 ± 0.01	6.200 ± 0.003	11.10 ± 0.01	-	22.60 ± 0.02

From Tab. 8, it was possible to observe that the biodiesel obtained through castor oil was within the limits of standard legislation[4], except for biodiesel from castor oil with ethanol, which presented a value above the limit of 6.0 $mm^2\ s^{-1}$. A viscosity above the limit is detrimental because some parts of the engine do not receive the necessary flux to form the lubricating film, which can result in more wear, accelerating the oxidation and shortening the life of the equipment, as explained by Farias *et al.*[5]. Regarding the biodiesel analyzed after the engine, the kinematic viscosity values increased, but not excessively, as expected.

Table 9. Kinematic viscosity analysis in biodiesel derived from rice oil.

Kinematic viscosity ($mm^2\ s^{-1}$)	BROM (BE)	BROI (BE)	BROE (BE)	BROM (AE)	BROI (AE)	BROE (AE)
Average	6.10 ± 0.01	3.60 ± 0.01	8.90 ± 0.02	5.14 ± 0.01	10.12 ± 0.01	-

From Tab. 9, it was possible to observe that the biodiesel derived from rice oil were within the limits of the Brazilian legislation[4], except for the rice oil with ethanol that presented divergent values, well above the expected, being considered a lubricant able to wear the parts of the engine and cause oxidation in a shorter time. After the engine, the results are also as expected, with kinematic viscosity increasing.

Biodiesel from coconut oil with isopropanol, castor oil with isopropanol, and rice with ethanol were not analyzed in the engine because of their

Eclética Química Journal, vol. 45, n. 2, 2020, 44-53
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low reaction yields, making it impossible to produce more due to the quantity of reagents available.

3.4. Qualitative test for the detection of metals

The metal detection test analyses are listed in Tabs. 10, 11 and 12 below. They were performed in triplicate and the results were given by the average.

Table 10. Analysis of the presence of metals in biodiesel derived from coconut oil.

Presence or absence of metals	BOCM	BOCI	BOCE
Average	Absent	Absent	Absent

Table 11. Analysis of the presence of metals in biodiesel derived from castor oil.

Presence or absence of metals	BORM	BORI	BORE
Average	Absent	Absent	Absent

Table 12. Analysis of the presence of metals in biodiesel derived from rice oil.

Presence or absence of metals	BOAM	BOAI	BOAE
Average	Absent	Absent	Absent

The results obtained in Tabs. 10, 11 and 12 were satisfactory since the presence of metals could impair engine performance[5]. It is important to point out that this is a qualitative analysis. To be sure, it is necessary to carry out more restricted analyses, such as atomic absorption.

3.5. Infrared

The major bands for biodiesel are given in Tab. 13.

Table 13. The main infrared bands for the biodiesel obtained.

Compounds	νC-H$_{alif.}$ (cm^{-1})	νC=O (cm^{-1})	νC-O (cm^{-1})
BOCM	2853	1740	1495
BOCI	2853	1664	1395
BOCE	2853	-	1435
BORM	2854	1685	1451
BORI	2853	-	1453
BORE	2853	1654	1451
BOAM	2853	-	1454
BOAI	2854	1667	1460
BOAE	2853	1668	1462

From the analysis of the infrared spectra, it was possible to observe in all biodiesel the presence of bands characteristic of carbonyls (C=O), aliphatic C-H and C-O ester. According to Barbosa[19], a band in the range of 1650 to 1750 cm^{-1} indicates axial deformation of the carbonyl C=O bond. In addition, the presence of bands between 2853 and 2854 cm^{-1} indicates an aliphatic C-H band; and in the range of 1390 to 1500 cm^{-1} correspond to ester C-O.

In this case, the characteristic bands of aliphatic C-H and C=O of carbonyl correspond to the starting vegetable oil, whereas the band referring to the ester C-O bond indicates the formation of the desired product, biodiesel. In this way, the infrared appears as another way of confirming that the product (biodiesel) was obtained through the starting vegetable oil. In the absence of the ester C-O band, there would be no conversion of triacylglyceride to ester, having only the starting vegetable oil, not biodiesel.

This can be observed in Fig. 1. The other spectra are not shown, since the three characteristic bands, which are the bands to emphasize in the work, are the same for all biodiesels, only occurring displacements due to the alcohol of departure of the reaction.

Eclética Química Journal, vol. 45, n. 2, 2020, 44-53
ISSN: 1678-4618
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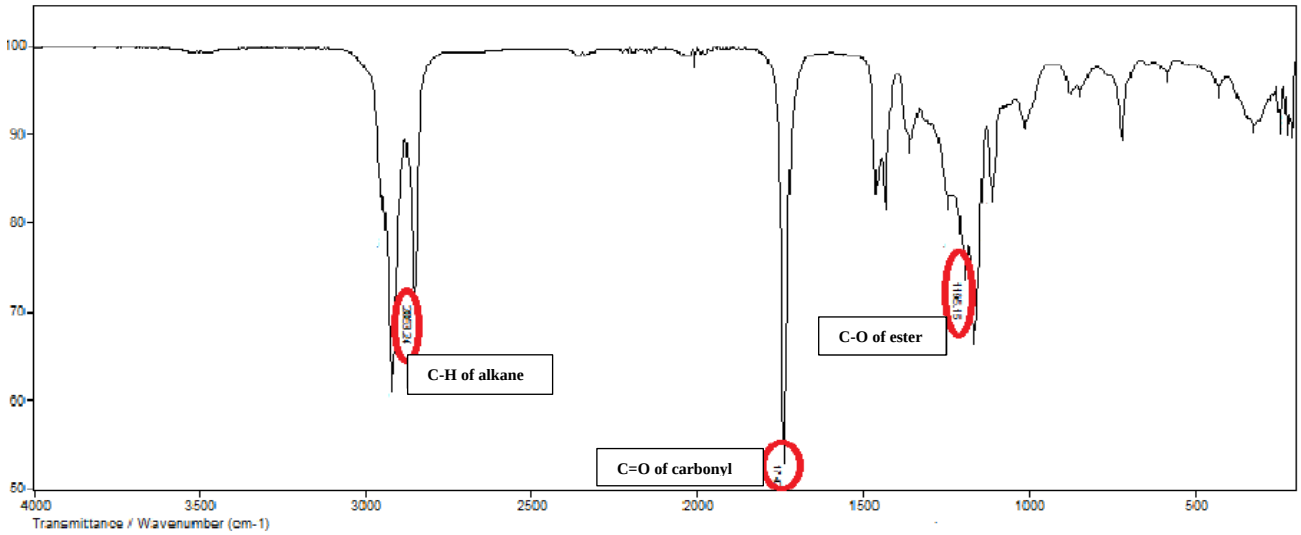

Figure 1. BOCM Infrared spectrum

4. Conclusions

Nine esters (biodiesels) were synthesized from transesterification reactions, varying the vegetable oils and the starting alcohols. These biodiesels were characterized by physicochemical analyses in order to confirm their formation and the characteristic properties of biodiesel, meeting the requirements of the National Agency for Petroleum, Natural Gas and Biofuels.

The biodiesel obtained also had its kinematic viscosity analyzed before and after the engine, demonstrating a similar profile to commercial engine lubricants, being good substituents.

Although biodiesel produced from coconut oil has good reaction yields, it is not intended to act as lubricants in the engine as it tends to solidify at temperatures below 25 °C and can cause engine parts to wear with particle adhesion in suspension.

Biodiesel derived from isopropyl alcohol is not economically viable to produce lubricants because of the poor performance to obtain it, and many reactions are necessary to obtain the necessary lubricant for the engine.

However, it was observed that the majority of biodiesel obtained have promising results to act as substituents of commercial lubricants, which fill a gap in the scientific literature.

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Eclética Química Journal, vol. 45, n. 2, 2020, 44-53
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Chemically modified cellulose as a potential oil adsorbent of contaminated marine ecosystems

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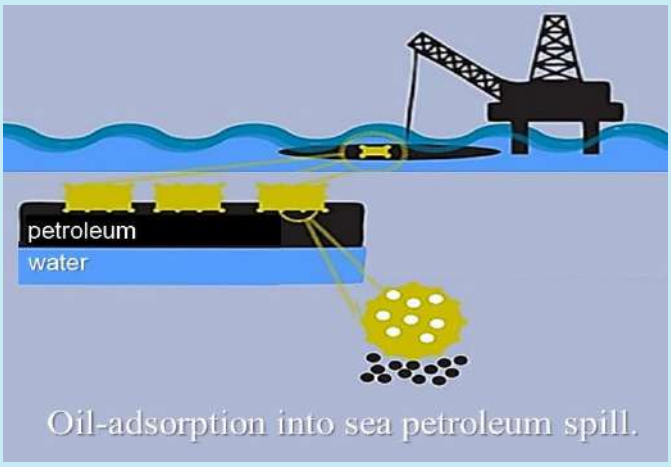
ABSTRACT: Petroleum exploration, as well as environmental impacts descendant of aquatic contaminations involving sea oil spills, foments the development of new technologies of marine ecosystems protection. Acknowledged like the most abundant polymer available today worldwide, cellulose is a linear 1.4-β-glucan, composed of D-anhydroglucopyranose units, linked together by β-(l→4)-glycosidic bonds. In this study, a chemical modification route of the cellulose polymer was accomplished using glycidyl methacrylate and stearin to test its oily adsorption capacity of soybean, diesel, and residual oils. Scanning Electron Microscopy (SEM) and Fourier-Transform Infrared (FT-IR) Analyses were applied to the characterization of the morphological and functional structure of microcrystalline cellulose. The results obtained proved the reach of an average hydrophobicity grade of 78.3±0.9% and a mass gain of MG = 2.89%, suggesting the possible insertion of hydrophobic groups onto the cellulose molecule and corroborating the hypothesis of successful grafting of glycidyl methacrylate and stearin onto the polymer. The oily adsorption tests showed a satisfactory capacity of the modified cellulose to adsorb small amounts of viscous oils, like residual oil.



1. Introduction

Water resources scarcity associated with the occurrence of accidental contaminations of oil and their derivatives spills, mainly caused by petroleum exploration in the 21st century, are themes which merit investigation and substantiate the continuous research efforts to promote the study and development of new technics or technological improvements in favor of the marine ecosystems environmental protection.

In Brazil, one of the most serious accidents involving the petroleum spill occurred in 2011, in the Campos Basin, where 588 thousand liters of oil were spilled into the sea. On the international scene, the Deepwater Horizon platform's accident, considered by many experts like the worst environmental disaster, occurred in the Gulf Coast and resulted in a sea oil spill of 780 million liters[1-3]. Every year, 600 thousand tons of oil are spilled into the sea. Petroleum and their products' spills are considered environmental catastrophes that affect the entire marine ecosystem. Fish die from asphyxiation when they come in contact with hydrocarbons and birds suffer from intoxication. Moreover, these tragedies affect coastal communities that survive from fishing, among other deleterious effects for the marine ecosystems[4-6].

Marine water resources are one of the great world riches. In Brazil, the environmental protection to marine waters is entangled on the Federal Constitution, the Civil Code, and environmental crimes laws, which deals of sea oil contamination, regarding preventive, control and inspection measures[7]. Current Brazilian legislation

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is more punitive than preventive and only came into effect after the impacts caused by environmental accidents that occurred over time with the petroleum exploitation, such as 265/2000 Resolution of the Conselho Nacional do Meio Ambiente, created after the oil spill in Guanabara Bay in 2000[4,8].

Selection of the most appropriate method to contain oil and their derivatives' spills is of crucial importance to minimize the environmental impacts to marine ecosystems. Among the used techniques are controlled combustion, containment barriers' use, skimmers technology, chemical dispersants or absorbent and adsorbent materials' application. Controlled burning is one of the most efficient practices when it comes to removing oil from the sea. However, the method is ecologically unfeasible because, in addition to the release of carbon dioxide, the waste from the combustion of petroleum is highly viscous. If they reach greater depths to the ocean floor, their damage may include environmental damage to water quality and aquatic species. The use of containment barriers and skimmers technology are examples of methods employed that avoid spreading oil beyond the spill origin. It consists of concentrating and directing the oil slick to the region where it is collected, preferably away from the coastal regions. skimmers work as a filtering membrane that collects and separates the oil and water mixture with subsequent oil recovery[5,8,9].

Another technique that can be used to assist the removal of the oils is the chemical dispersants and absorbent materials use. Dispersants consist of the application of chemicals (surfactants and solvents) that aid in the fragmentation of hydrocarbon chains into smaller molecules. This process facilitates the formation of droplets which are then consumed by naturally occurring bacteria in the aquatic environment. However, this is a process that does not provide oil recovery. The absorbents are indicated to collect all type of oil and derivatives. They can be found in various forms and absorb up to 25 times their own weight[5].

Adsorption process has been widely used in recent years in the treatment of industrial effluents contaminated by oils, heavy metals, dyes, and other pollutants. It is characterized by the ability of a specific component (adsorbed), dispersed or dissolved in a mixture, to adhere to the surface of a substance, called adsorbent. There are two types of adsorption. Physical adsorption also called physisorption and chemical adsorption called chemisorption. The first occurs when the adsorbent and adsorbate realize inter-molecular interactions of the Van der Waals type. Due to the low strength of these interactions, the process is reversible. However, the chemisorption is an irreversible process, once the adsorbed substance makes chemical bonds with the adsorbent to atomic levels[10-13].

Currently, several research lines have been developed with an emphasis on the study of new technologies aiming the biodegradable raw materials use and obtaining of products with different properties for various application potentials. Cellulose, a linear 1.4-β-glucan, is the most abundant polymer available today worldwide. It is a linear syndiotactic homopolymer composed of D-anhydroglucopyranose units, which are linked together by β-(1→4)-glycosidic bonds. Taking the dimer cellobiose as the basic unit, cellulose can be considered as an isotactic polymer of cellobiose (Fig. 1)[14]. Cellulose structure is formed by hydrogen bonds between its hydroxyl groups. Like a result of the supramolecular structure, cellulose presents highly ordered regions, crystalline regions, intermediated by less ordered regions, called amorphous regions. Due to lower stability, amorphous regions are more accessible to attack by reagents and enzymes[15].

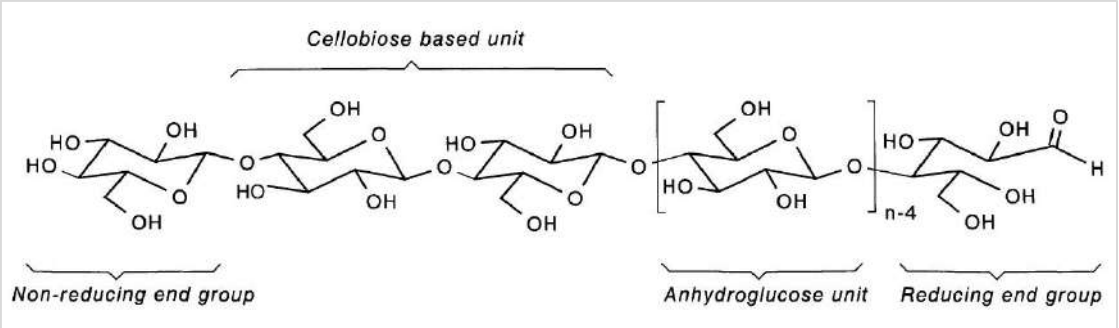

Figure 1. Molecular structure of cellulose[14].

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In microbiology, bacterial cellulose has been applied in the sponges' form in the mining and refining area to collect oil leaks and toxins absorption[16]. In medicine, cellulose has been applied in the development of new hybrid fibers that may have anti-inflammatory, bactericidal, antifungal and cicatrizing properties[17]. In the field of nanotechnology, the nano-fibrillated cellulose obtained by the disintegration of the polymer stands out to have singular physical and mechanical properties, such as high density and high resistance to traction and bursting[12].

Scientists have developed research in the organic synthesis area, studying different chemical modification routes of cellulose molecule with focus on increasing its adsorption capacity and development of new properties and functionalities[18-26]. Among them, Brum *et al.*[18] synthesized cellulose acetate from bean straw using ethanoyl ethanoate as well as pyridine and 1-bromo-2.5-pyrrolidinedione as catalysts. The researchers succeeded in the chemical modification, achieving a hydrophobicity percentage of 93.5% for cellulose acetylated with pyridine and 98.7% for acetylated cellulose with 1-bromo-2.5-pyrrolidinedione. Macedo[21] proposed the hydrophobization of wood pulp fibers of *Pinus elliotti* in a study of the influence of oil sorption using triethoxyvinylsilane and ethanoic acid as reagents. The authors obtained physical sorption of more than twenty grams of oil per each gram of modified cellulose. Oliveira[23] studied the development of oil adsorbent materials by chemical modification of residues from coffee processing consisting of a high content of fibers (cellulose, hemicelluloses, and lignin) with ethanoyl ethanoate and 1-bromo-2.5-pyrrolidinedione, reaching adsorption of circa two grams of oil per gram of modified material. Zimmermann[24] functionalized cellulose using the 2-methylprop-2-enoic acid 2-oxiranylmethyl ester and 1.3-di(octadecanoyloxy) propan-2-yl octadecanoate reagents for use as an oil adsorbent, obtaining a modified material with a hydrophobicity grade of 87±3%.

In this context, having as motivation the historical of aquatic contaminations involving sea oil spills and their malefic impacts to marine ecosystems, the present article aims to investigate the oily adsorption potential of chemically modified cellulose as a possible alternative of application in environmental remediation of marine ecosystems contaminated by oils.

2. Experimental

The present research mobilized the development of following steps: (1) Characterization of a sample of microcrystalline cellulose via scanning electron microscopy (SEM) and Fourier-transform infrared (FT-IR) analyses; (2) Chemical modification of microcrystalline cellulose; (3) FT-IR analysis of chemically modified cellulose; (4) Hydrophobicity and mass gain tests of chemically modified cellulose; (5) Oil adsorption tests of modified cellulose with soybean, diesel, and residual oils.

2.1 Pre-modification characterization via SEM and FT-IR analyses

A sample of microcrystalline cellulose in nature (Synth) was atomized using gold (Au) and fixed to the sampler using carbon tape. SEM analysis was accomplished in a scanning electron microscope (Joel, model JSM-IT300). Pellets of other sample of microcrystalline cellulose in nature (Synth) were prepared using potassium bromide (KBr), with the aid of pestle, mortar, and hand press (Shimadzu, model 200-64175). The FT-IR spectrum was obtained in the IR solution software of a spectrometer with 2 cm^{-1} resolution and 10 scans for sample (Shimadzu, model IRAffinity-1).

2.2 Chemical modification

Cellulose was functionalized using glycidyl methacrylate (GMA) and stearin in triplicate based on the procedure reported by Pracella[26]. The analytical reagents were: propanone (*Neon*); ethanol (*Synth*); stearin (1.3-di(octadecanoyloxy) propan-2-yl octadecanoate) (*Synth*); GMA (2-methylprop-2-enoic acid 2-oxiranylmethyl ester) (*Aldrich Chemistry*); hexane (*Neon*), triethylamine (*Anidrol*), and distilled water.

The modification montage was made up with the aid of universal support, heating plate, ball condenser, round bottom flask, and thermometer. A solution of triethylamine/GMA (7:3 v:v) was prepared in a 250.0 mL volumetric flask. 60.0 mL of this solution and 12.0 g of dried microcrystalline cellulose, previously weighed on analytical balance (Shimadzu, model AW220), were added to the

Eclética Química Journal, vol. 45, n. 2, 2020, 54-63
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round bottom flask. The chemical reaction was kept under constant stirring at 90 °C (363.15 K) for a period of 4 h.

At the end of stirring and heating period, the material was filtered and washed with ethanol and propanone. Then, the flask was transferred to a beaker and was stored at room temperature for 24 h. Subsequently, it was dried in a kiln (Infinit, model EMT-200) and maintained at 100 °C (373.15 K) for a period of 4 h. A solution of ethanol/stearin (95:5 mL g^{-1}) was prepared in a 250.0 mL volumetric flask. 100.0 mL of this solution was added to the functionalized cellulose. Next, the modification montage was again made up and the reaction was maintained at (50 °C) 323.15 K for 4 h. After the modification steps, the modified material was filtered again. Figure 2 (step I) shows the reaction scheme for the grafting of GMA onto cellulose (Cell-GMA) and (step II) the reaction scheme for the grafting of stearin onto Cell-GMA (Cell-GMA-Stearin).

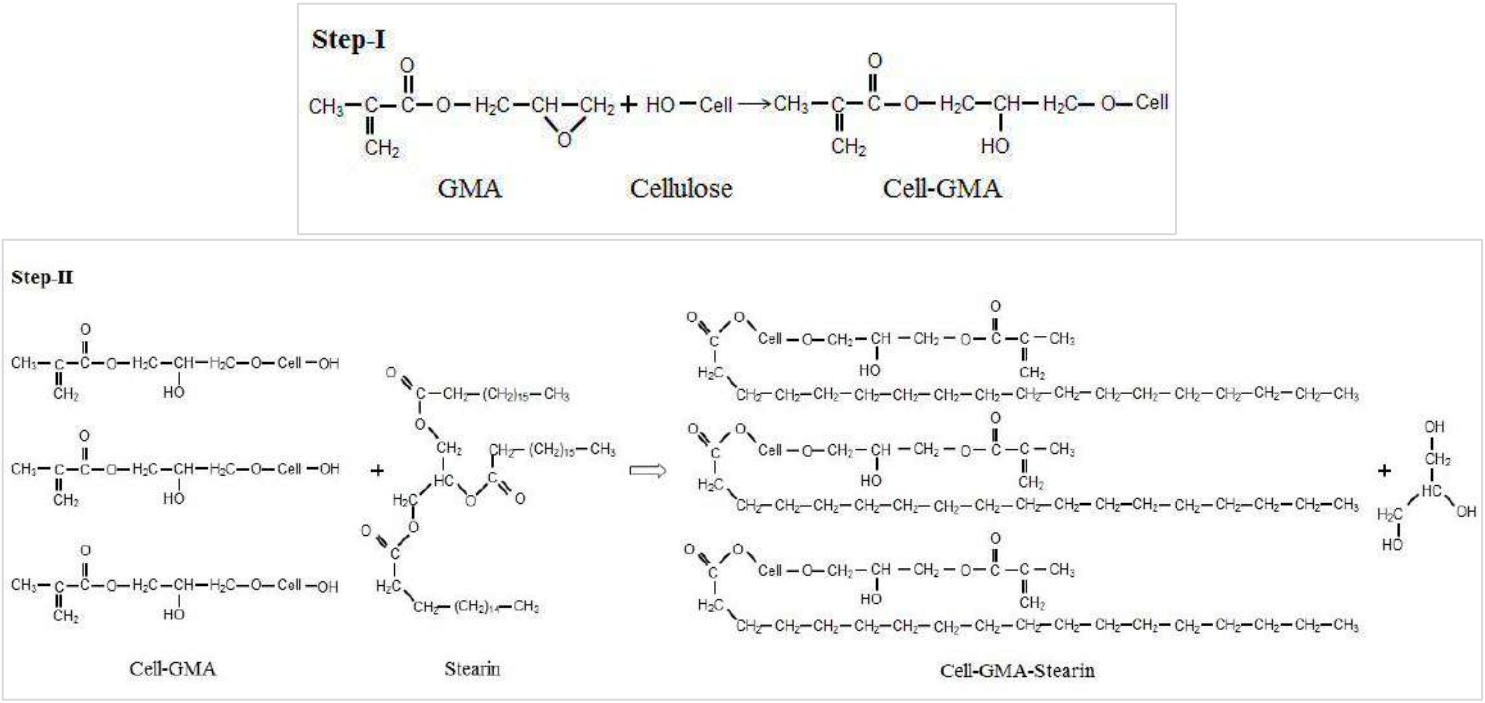

Figure 2. Reaction scheme.

2.3 Post-modification characterization via FT-IR Analysis

Modified cellulose pastilles were prepared using KBr, pestle, mortar, and hand press (Shimadzu, model 200-64175). The FT-IR spectrum was obtained from the IRsolution software in the same spectrometer used for pre-modification characterization (Shimadzu, model IRAffinity-1).

2.4 Hydrophobicity and mass gain tests

Cellulose hydrophobicity percentage before and after chemical modification was estimated according the methodology proposed by Brum *et al.*[18]. In a separation funnel, 1.000 g of microcrystalline cellulose, previously weighed on analytical balance (Shimadzu, model AW220), and 20.0 mL of distilled water were added. Then, 20.0 mL of hexane was added. The mixture contained in the funnel was kept under constant stirring for 3 min and thereafter, allowed to stand for 5 min. The aqueous phase was removed, and the dried material was weighed on analytical balance. The test was performed in triplicate. The same test was made with modified cellulose. Percentage hydrophobicity (H%) of both materials, as well the mass gain achieved in polymeric functionalization were evaluated by the methodology described by Brum *et al.*[18]. Equation 1 was used in H% calculation and Eq. 2 was used in mass gain calculation (MG%).

Eclética Química Journal, vol. 45, n. 2, 2020, 54-63
ISSN: 1678-4618
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$$H(\%) = \left(\frac{material\ final\ mass\ (g)}{material\ initial\ mass\ (g)}\right) x\ 100 \quad (1)$$

$$MG(\%) = \left(\frac{modified\ material\ mass\ (g) - in\ microcrystalline\ material\ mass\ (g)}{in\ microcrystalline\ material\ mass\ (g)}\right) x\ 100 \quad (2)$$

2.5 Oil adsorption tests

Oily adsorption tests of microcrystalline cellulose and modified cellulose were accomplished according to methodologies proposed by Zimmermann[24] and Brum *et al.*[18]. Both materials were tested for their ability to absorb three types of oils: soybean oil, diesel oil, and residual oil. An aliquot resulting from the remaining of frying oil used in the college snack bar was utilized like residual oil. The adsorption capacity was tested for volumes of 20.0 mL, 40.0 mL, and 60.0 mL of oil, individually and separately, in a beaker containing 100.0 mL of water and 1.000 g of material. For that, each oil volume was previously weighed on analytical balance. Adsorption was promoted under stirring for 10 min at room temperature (25 °C), in triplicate. Subsequently, the materials were allowed to stand to drain oil excess and were weighed analytically. The adsorption efficiency was calculated by Eq. 3:

$$Adsorption\ efficiency\ (\%) = \left(\frac{final\ mass\ (adsorbed + adsorbent)\ (g) - adsorbent\ mass\ (g)}{oil\ mass\ (g)}\right) x\ 100 \quad (3)$$

3. Results and Discussion

3.1 Characterization analyses

The morphology and structure of cellulose were analyzed by SEM and FT-IR analyses. Figure 3 represents the scanning electron micrographs of microcrystalline cellulose referring to the magnification of 100, 500, 1000, and 3000 times. SEM analysis allowed analyzing the morphological structure of the polymer. It was possible to observe a characteristic surface of regular, fibrous, lignocellulosic material with an elongation of the rod type. An agglomerate appearance of sample was verified, which may have been occasioned by fixation of cellulose to the carbon tape, during the preparation of the sample for analysis in the scanning electron microscope (Fig. 3b and d).

FT-IR analysis allowed studying the structure of the polymer before and after the chemical modification. Figure 4 shows FT-IR spectra of microcrystalline cellulose and modified cellulose.

The defined band existing in range 3600 to 3000 cm^{-1} reports the vibrational modes of hydroxyl (OH) groups (3400-3200 cm^{-1}) and stretches of intramolecular (OH) groups (3570-3450 cm^{-1}) presents in microcrystalline cellulose molecule. Comparing both two spectra, can be seen (i) intensification of the peak in region 3000 to 2800 cm^{-1}, suggesting the presence of new alkane (CH) groups in the molecule (2924 cm^{-1}); (ii) an intense extra carboxyl (CO) peak centered at 1716 cm^{-1}, supporting the formation of bonding between the epoxy moiety of glycidyl methacrylate and cellulose molecule; (iii) an extra peak at 1458 cm^{-1}, corresponding to the stretches of carboxyl (COO) groups (presents in stearin molecule), suggesting the occurrence of reaction between stearin and Cell-GMA; (iv) extra peaks in the range 1190-1070 cm^{-1} (1165 cm^{-1}; 1114 cm^{-1}) supporting the hypothesis of insertion of new CO groups, arising from glycidyl methacrylate and stearin reagents, onto cellulose molecule. Similar results were evidenced by Pracella[26].

Eclética Química Journal, vol. 45, n. 2, 2020, 54-63
ISSN: 1678-4618
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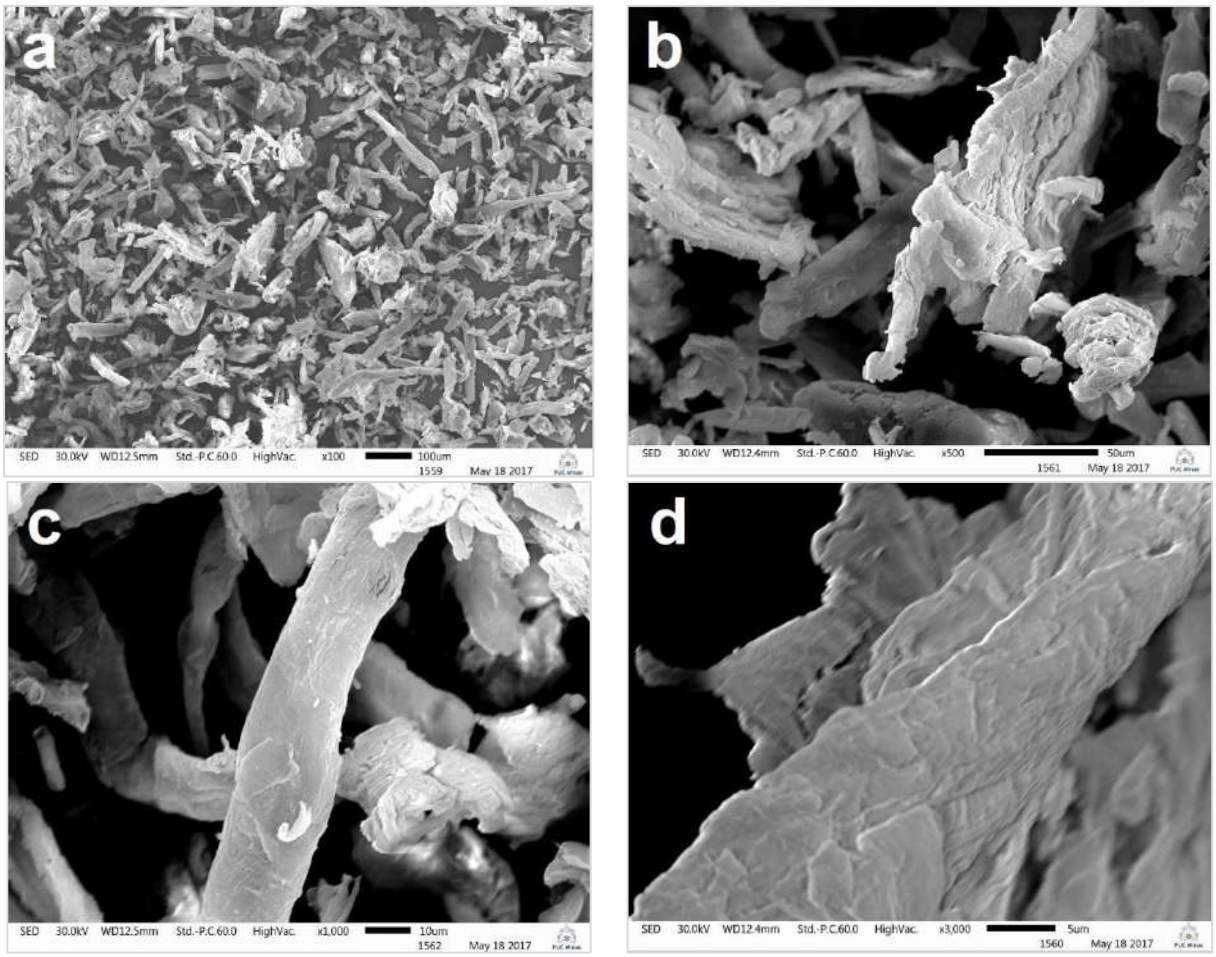

Figure 3. Scanning electron micrographs of microcrystalline cellulose (a) 100-fold magnification, (b) 500-fold magnification, (c) 1000-fold magnification, and (d) 3000-fold magnification.

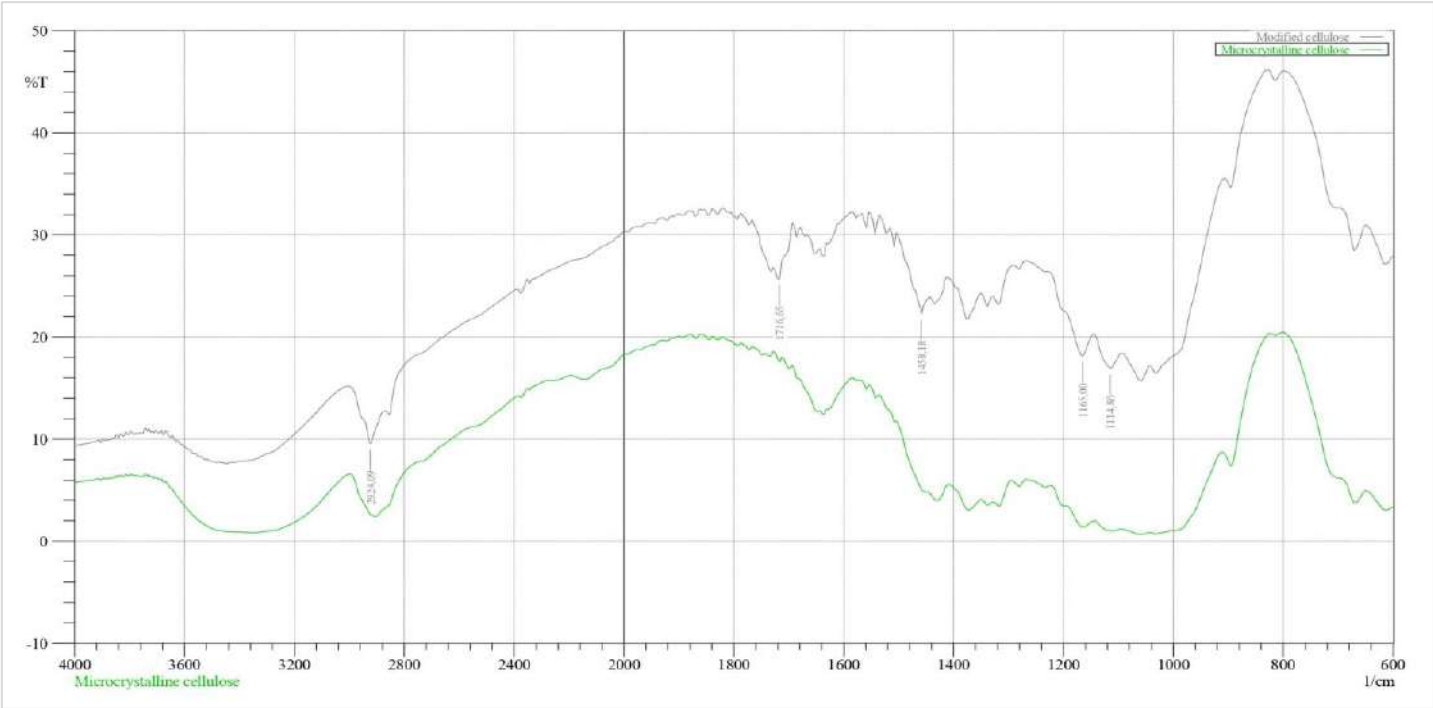

Figure 4. FT-IR spectra of microcrystalline cellulose and modified cellulose.

Eclética Química Journal, vol. 45, n. 2, 2020, 54-63
ISSN: 1678-4618
DOI: 10.26850/1678-4618eqj.v45.2.2020.p54-63

3.2 Hydrophobicity and mass gain tests

Table 1 shows the hydrophobicity results achieved. After the hydrophobicity test, 100% of the microcrystalline cellulose mass used was in the aqueous phase, and thus, presented 0% of hydrophobicity. Zimmermann[24] and Brum *et al.*[18] also obtained the same hydrophobicity grade for microcrystalline cellulose. Meanwhile, it was possible to verify the increase of hydrophobicity for the modified material. The chemical modification route proposed using GMA/stearin suggested the insertion of alkyl groups onto cellulose molecule, enabling considerably increase the hydrophobic character of the lignocellulosic material. The increase of hydrophobicity making possible its application in the production of oil adsorbents or manufacture of composites. An average hydrophobicity grade of 78.3±0.9% was obtained, confirming the possible substitution of polar groups, particularly hydrophilic, by apolar groups (hydrophobic). Zimmermann[24] also functionalized cellulose using GMA/stearin obtaining similar hydrophobicity grade 87±3%.

It is expected that after reaction, the modified material will present greater molecular mass, due to the grafting of glycidyl methacrylate (MW = 142.15 g mol^{-1}) and stearin (MW = 891.48 g mol^{-1}) onto cellulose molecule. In general, the higher the mass gain, the higher the alkyl groups' grafting rate[18]. According to obtained results, it was observed a mass gain of MG = 2.89%, suggesting other indicative of the occurrence of chemical modification and, therefore, the possible substitution of hydrophilic groups by hydrophobic groups. In Tab. 2 are given the results of the mass gain test.

Table 1. Hydrophobicity test results.

Material	Test	Initial mass (g)	Final mass (g)	Hydrophobicity (%)	Average Hydrophobicity (%)
Microcrystalline cellulose	I	1.0	0.001	0.0	0.0 ± 0.0
	II		0.000	0.0	
	III		0.001	0.0	
Modified cellulose	I		0.794	79.4	78.3 ± 0.9
	II		0.781	78.1	
	III		0.775	77.5	

Table 2. Percentage of mass gain achieved.

Microcrystalline Cellulose Mass (g)	Modified Cellulose Mass (g)	Mass Gain (%)
36.000	37.041	2.89

3.3 Oil adsorption tests

Oil-adsorbing materials can be used in environmental remediation of marine ecosystems contaminated by oil spills. Oily adsorption capacities of microcrystalline cellulose and modified cellulose were tested in the presence of water. Soybean oil, diesel oil, and residual oil were used because of their easy obtaining and high hydrophobicity. The obtained results in oily adsorption tests were presented in Tabs. 3 and 4.

The microcrystalline cellulose, when placed in the water/oil system, immediately passed to water, which makes its use for oil absorption in systems containing water impossible. This result was already expected based on the results obtained by the hydrophobicity test. The ability to absorb soybean, diesel and residual oils was minimal, which corroborates its majority hydrophilic character. Resembling results were evidenced by Brum *et al.*[18].

The modification of cellulose resulted in a substantial increase in the presence of alkyl groups in its molecule, and consequently, an increase in its hydrophobicity/oleophilicity. The results demonstrated that the mass of oil adsorbed per gram of modified cellulose decreases in the order of oils residual > soybean > diesel, indicating the similar behavior for either oil volumes tested. This suggests a larger affinity of modified cellulose to adsorb residual oil to the detriment diesel oil. The adsorption efficiency of modified cellulose decreases in the order of 20 mL > 40 mL > 60 mL of oil volume, suggesting the smaller the amount of oil in test, bigger the adsorption effectiveness.

Eclética Química Journal, vol. 45, n. 2, 2020, 54-63
ISSN: 1678-4618
DOI: 10.26850/1678-4618eqj.v45.2.2020.p54-63

Oil adsorption results of modified cellulose showed agreement with the reported by Duong[27] about the relationship between viscosity and the adsorption efficiency. According to the authors, the higher the oil viscosity, the higher the adsorption rate and oil retention in the adsorbent. The adsorption efficiency of residual oil (most viscous) was higher among the analyzed oils. Already for the soybean (medium viscosity) and diesel (low viscosity) oils, the adsorption efficiency was lower, as expected. Resembling results were evidenced by Zimmermann[24].

Table 3. Adsorption tests data.

Oil	Test	Oil Mass (g)			*Oil Adsorbed Mass* *Microcrystalline Cellulose Gram*			Oil Mass (g)			*Oil Adsorbed Mass* *Modified Cellulose Gram*		
		20.0 mL	40.0 mL	60.0 mL	20.0 mL	40.0 mL	60.0 mL	20.0 mL	40.0 mL	60.0 mL	20.0 mL	40.0 mL	60.0 mL
Soybean	I	17.462	31.004	49.628	0.079	0.015	0.024	17.348	29.078	51.604	10.874	16.697	29.348
	II	16.989	30.076	49.809	0.081	0.015	0.022	17.330	30.934	48.171	10.474	19.973	28.141
	III	17.451	29.200	50.051	0.080	0.016	0.023	17.598	33.000	49.138	10.976	20.240	33.727
Diesel	I	5.313	10.681	15.852	0.028	0.123	0.048	5.250	10.906	14.778	3.791	4.610	5.003
	II	5.287	10.879	16.453	0.026	0.124	0.048	5.245	10.723	16.443	3.807	4.945	7.106
	III	5.304	10.722	15.986	0.027	0.122	0.047	5.214	10.721	16.546	3.662	5.131	6.099
Residual	I	15.715	30.968	49.679	0.107	0.166	0.238	15.895	31.742	48.492	14.465	19.754	31.779
	II	15.609	31.751	50.861	0.105	0.167	0.240	15.435	31.772	50.354	13.976	19.586	26.505
	III	15.468	31.156	50.288	0.106	0.169	0.239	15.772	29.321	51.153	14.282	19.910	32.318

Table 4. Adsorption efficiency results.

Oil	Microcrystalline Cellulose			Modified Cellulose		
	20.0 mL oil (%)	40.0 mL oil (%)	60.0 mL oil (%)	20.0 mL oil (%)	40.0 mL oil (%)	60.0 mL oil (%)
Soybean	0.46 ± 0.19	0.05 ± 0.64	0.05 ± 0.15	62 ± 1	61 ± 4	61 ± 7
Diesel	0.51 ± 0.01	1.14 ± 0.07	0.30 ± 0.22	84 ± 1	52 ± 3	42 ± 5
Residual	0.01 ± 0.09	0.01 ± 0.29	0.005 ± 0.418	90.7 ± 0.3	64 ± 3	61 ± 7

4. Conclusions

SEM and FT-IR analyses results obtained for microcrystalline cellulose allowed to recognize the morphological structure and the functional groups of the studied polymer. The chemical modification route of the polymer structure via GMA/stearin reactions suggested the possible insertion of hydrophobic groups onto the cellulose molecule, corroborated by the reach an average hydrophobicity grade of 78.3±0.9% and a mass gain of MG = 2.89%. FT-IR analysis results of modified cellulose enabled to identify appeared modifications in the chemical structure of the polymer, reinforcing the hypothesis of successful grafting of GMA and stearin onto the structure. The oily adsorption tests proved a satisfactory capacity of the modified cellulose to adsorb small amounts of viscous oils, like residual oil. It is expected which the potential of the oil-adsorbing material can be availed in the development of improvements and solutions of environmental remediation of marine ecosystems contaminated by oil spills.

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Eclética Química Journal, vol. 45, n. 2, 2020, 54-63
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