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## Antidepressant-like effect of *Campomanesia xanthocarpa* seeds in mice: Involvement of the monoaminergic system



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### ABSTRACT

**Background and aim:** *Campomanesia xanthocarpa* Berg. (Myrtaceae) present several pharmacological actions, but there are no reports on its antidepressant-like potential. This study investigated the antidepressant-like effect and mechanism of action of *Campomanesia xanthocarpa* seeds extract obtained from supercritical CO<sub>2</sub> (40 °C, 250 bar).

**Experimental procedure:** Mice were orally treated with the extract 1 h before the TST. To investigate the involvement of the monoaminergic system in the antidepressant-like activity of the extract, pharmacological antagonists were administered prior to the acute oral administration of the extract (60 mg/kg). Also, the interaction of the extract with antidepressants was assessed in the tail suspension test (TST). The *in vitro* inhibitory potential of *C. xanthocarpa* seeds extract towards MAO A and MAO B enzymes was tested *in vitro*.

**Results and conclusion:** Animals treated with *Campomanesia xanthocarpa* seeds extract showed a significant reduction in the immobility time in the TST. Mice pretreatment with SCH23390, sulpiride, prazosin, yohimbine, and *p*-chlorophenylalanine prevented the anti-immobility effect of the extract in the TST. The combined administration of sub-effective doses of the extract with imipramine, bupropion and fluoxetine significantly reduced mice immobility time in the TST. The extract showed MAO A inhibitory activity (IC<sub>50</sub> = 151.10 ± 5.75 µg/mL), which was greater than that toward MAO B (IC<sub>50</sub> > 400 µg/mL).

The extract of *Campomanesia xanthocarpa* seeds obtained by supercritical CO<sub>2</sub> shows antidepressant-like activity, which relies on the activation of the monoaminergic neurotransmission (serotonergic, dopaminergic and noradrenergic), suggesting that this species might represent a resource for developing new antidepressants.

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### 1. Introduction

Depression is considered the most disabling disease and, therefore, a public health problem which affects more than 322 million people worldwide (World Health Organization). It is also known that the total number of depressed people increased by 18.4% between 2005 and 2015.<sup>1</sup> This disease presents heterogeneous characteristics and is explained by a combination of genetic,

**Abbreviations**

BUP	bupropione
FLU	fluoxetine
GC/MS	gas chromatography/mass spectrometry
IMI	imipramine
OFT –	open field test
pCPA	<i>p</i> -chlorophenylalanine
SCCO <sub>2</sub> –	supercritical carbon dioxide
SCH 23390	(R)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine
SEM –	Standard error of the mean
TST –	Tail suspension test

neurobiological and environmental factors.<sup>2</sup> Among these factors, the most highlighted and most segregated one to explain the pathophysiology of depression is the neurobiological, which involves the monoaminergic system, that is, the central reduction of neurotransmitters such as serotonin, noradrenaline and dopamine, since they show a direct link with the stimulus and humor.<sup>3</sup>

The drugs used in the pharmacotherapy of depressive mood disorder act on the monoaminergic system, increasing the central concentration of the neurotransmitters involved in the genesis of depression, either by inhibiting their reuptake or degradation.<sup>4</sup> Furthermore, drugs that inhibit monoamine oxidase (MAO) activity (MAOIs), a key enzyme implicated in monoamine (serotonin, noradrenaline and dopamine) metabolism, have been used for decades in the treatment of depression. The antidepressant effect of MAOIs is a consequence from MAO A inhibition in the central nervous system, that could elicit increased brain levels of monoamines.<sup>5,6</sup>

There is a wide range of antidepressant medications available for depression treatment; however, the rate of refractory patients to the treatment is still significant, ranging from 30 to 35%, and the adverse effects also limit the adherence to treatment.<sup>7</sup> Thus, it is necessary to search for new compounds and strategies that could improve conventional therapies.<sup>8</sup>

There is a growing number of natural products being adopted in psychiatric practice.<sup>9</sup> Depression is one of the main indications for the use of alternative and complementary medicines, mainly due to the lower prevalence of side effects.<sup>10</sup> In this perspective, the research of natural products that act on the central nervous system represents a possibility for the development of psychoactive drugs that act through differentiated mechanisms, due to the high prevalence of bioactive molecular models in plant species.<sup>11</sup>

Besides the use as substratum in the manufacture of medicines, plants are used in the traditional medicine.<sup>12</sup> The plant species *Campomanesia xanthocarpa* Berg. (Myrtaceae), popularly known as “guabiroba”, a common species in the forests of the South, Southeast and Central-West regions of Brazil, is also found in Uruguay, Paraguay and Argentina. The infusion prepared from its leaves is commonly used as depurative, antidiarrheal, antirheumatic and to lower blood cholesterol levels.<sup>13</sup> This plant has considerable potential as an alternative source of vitamin C and phenolic compounds<sup>14</sup> and its seeds are enriched in terpenoids, especially β-caryophyllene.<sup>15</sup> Studies have shown that *C. xanthocarpa* leaves present anti-inflammatory,<sup>16</sup> hypotensive<sup>17</sup> antiplatelet<sup>18</sup> and antidiarrheal<sup>19</sup> effects. However, the pharmacological potential of the plant seeds has not been explored yet.

Among the neuroactive species from *Campomanesia* genus are *C. pubescens*<sup>20</sup> and *C. adamantium*,<sup>21</sup> which antidepressant-like activity has been demonstrated in rodents. In this sense, the

study of the antidepressant-like effect of *C. xanthocarpa* seeds represents an unprecedented pharmacological approach in relation to this species.

The use of supercritical fluids extraction has been consolidated with extracts obtained from natural products, since this technique requires low operating temperatures (in a non-oxidizing medium, allowing the extraction of thermosensitive compounds, besides providing solvent-free analytes).<sup>22</sup> It is also important to consider that extracts obtained with supercritical solvents present an improvement in the biological activity when compared to extracts obtained with organic solvents, mainly due to the obtention of more purified extracts, considering the good solubility of secondary metabolites in supercritical fluids (carbon dioxide - CO<sub>2</sub> - is the most commonly used).<sup>23–26</sup>

To the best of our knowledge, although the antidepressant-like of some species from the *Campomanesia* genus has been demonstrated in rodents,<sup>20,21</sup> there are no scientific preclinical studies demonstrating the pharmacological potential of the *C. xanthocarpa* seeds so far, neither its effects on the central nervous system. This study investigates for the first time the antidepressant-like effect of *Campomanesia xanthocarpa* seeds supercritical CO<sub>2</sub> extract, as well as the time-course and the contribution of monoaminergic neurotransmission (serotonergic, noradrenergic and dopaminergic) to this effect. Finally, the monoamine oxidase (MAO) inhibitory activity of the extract and its interaction with conventional antidepressant drugs was also investigated.

## 2. Materials and methods

### 2.1. Drugs and treatments

The following antidepressants were used: imipramine (from Apsen Pharma®), bupropion (from EMS Pharmaceuticals®) and fluoxetine (from Medley Pharmaceuticals®). In addition, sulpiride (from Sanofi-Aventis®), prazosine (from Pfizer®) and yohimbine (from Apsen Pharma®), were used. SCH23390 and pCPA were purchased from Sigma Aldrich®. The drugs were diluted in saline (0.9% NaCl) or suspended in distilled water with 1% Tween 80 (vehicle).

### 2.2. Plant material

*C. xanthocarpa* fruits were collected in 2016 (during the spring, in November), from Quilombo city, located in the Southern Brazil (26°47'23.6" S, 52°45'42.41" W) and deposited in the Herbarium from Community University of Chapecó Region (Unochapecó Herbarium, SC, Brazil - access number 3153). After collection, the fruits were stored in glass flasks under nitrogen atmosphere, protected from the light, at 8 °C, until the extraction. Seeds were manually separated from the fruits just before the extraction.

### 2.3. Preparation and characterization of the extract

The experimental apparatus employed for supercritical carbon dioxide extractions consists basically of a solvent reservoir, two thermostatic baths, a syringe pump (ISCO 260D), a 130 cm<sup>3</sup> jacketed extraction vessel, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201) with a precision of 0.12 bar, a collector vessel with a glass tube, and a cold trap. Amounts of around 30 g (±0.05 g) of seeds, first dried in an oven at 40 °C to constant weight and then comminuted in a blender (average particle size of 0.14 mm), were charged into the extraction vessel. The solvent was pumped at a constant flow rate of 2 mL/min into the bed, which was supported by two 300 mesh wire disks at both ends and was kept in contact with the herbaceous matrix for

at least 30 min to allow system stabilization. Afterward, the extract was collected by opening the micrometering valve, and the solvent flow rate was accounted for by the pump recordings. After that, the mass of the extract was weighed, and the glass tube was reconnected to the equipment. This procedure was performed until no significant mass was extracted. The working temperature was 40 °C and pressure of 250 bar (for 150 min).<sup>15,23</sup>

A whole experimental run lasted in general 6 h, including all steps involved: sample weighing, temperature stabilization (baths, extractor), extraction, and depressurization. Based on triplicate experiments carried out for all the experimental conditions for both compressed solvents, the overall average standard deviation of the yields noticed was about 0.2 wt%.

The components of *C. xanthocarpa* seeds extract obtained by supercritical CO<sub>2</sub> were determined by Agilent GC/MS (7890B) gas chromatography coupled to a quadrupole mass spectrometer (5977A) (Agilent Technologies, Palo Alto, CA, USA). The GC analysis were performed using Agilent 19091S capillary column, dimension: 30 m × 250 μm × 0.25 μm. The temperature of the injector and detector was set at 250 °C; the oven temperature was programmed from 60 °C (8 min) to 180 °C (4 °C/min), 180–230 °C (20 °C/min), and then 230 °C (for 20 min). Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The chemical components present in the extract were identified by comparison to the equipment library (Agilent P/N G1033A). The relative amounts of each individual component were calculated using their respective peak areas in the chromatogram. The procedures were performed according to Capelleto et al.<sup>15</sup>

The chemical composition of the extract was elucidated in 96%. The major components of *C. xanthocarpa* extract obtained by SCCO<sub>2</sub> were: β-caryophyllene (11.67%), followed by γ-cadinene (9.58%), α-cadinol, (7.17%), viridiflorol (6.70%) and δ-gurjunene (6.48%), as described by Capelleto et al.<sup>15</sup>

#### 2.4. Experimental animals

Male Swiss mice (25–35 g), from the Animal Facility of Community University of Chapecó Region were used. The animals were kept in acrylic cages (28 × 12.5 × 19 cm), with 4–5 animals per cage, in a room under a 12 h light/dark cycle, constant temperature (23 ± 1 °C), humidity (40–60%) and free access to rodent standard diet (Biobase®) and tap water. The experimental protocols using animals were approved by the Committee on Ethics in Animal Care (CEUA-Unochapecó, approval protocol numbers: 013/17; 015/18). All animals were used only once.

#### 2.5. Tail suspension test (TST)

The TST was performed according to Steru et al.<sup>27</sup> with minor modifications.<sup>28</sup> Mice were suspended approximately 1 cm from the tip of the tail 60 cm above the floor by adhesive tape. The duration (in seconds) of immobility was recorded in a 6 min session. Mice immobility was considered when they hung passively and motionless. The animals (n = 7–8/group) were orally treated 1 h before being submitted to the TST; independent groups of mice received vehicle (10 mL/kg), fluoxetine (30 mg/kg, positive control) or the extract from *C. xanthocarpa* seeds (30, 60 and 120 mg/kg). Considering that there are no reports about the antidepressant-like effect of *C. xanthocarpa* seeds extract in the literature, we performed pilot experiments in order to set the doses of the extract used in our study.

#### 2.6. Time-course of the antidepressant-like effect

The time-course of the antidepressant-like activity of the extract

from *C. xanthocarpa* seeds (60 mg/kg, p.o.), was assessed in the TST. The immobility time (s) of independent groups of mice (n = 9–10/group) was registered 1, 2 and 3 h after the extract (or vehicle) administration.

#### 2.7. Antidepressant-like mechanism of action

In order to assess the involvement of the noradrenaline-mediated mechanism in the anti-immobility effect of the extract, mice (n = 6–8/group) were pretreated with prazosin (1 mg/kg, i.p., an α<sub>1</sub> adrenoceptor antagonist), yohimbine (1 mg/kg, i.p., an α<sub>2</sub> adrenoceptor antagonist) or vehicle (0.9% NaCl, 10 mL/kg, i.p.) 30 min before the administration of the extract (30 mg/kg p.o.) or vehicle (0.9% NaCl + 1% Tween 80, 10 mL/kg, p.o.). One h later, the animals were observed in the TST.

To establish the involvement of the dopaminergic neurotransmission in the extract's mode of action, mice (n = 6–8/group) were pretreated with sulpiride (50 mg/kg, i.p., a D<sub>2</sub> antagonist), SCH 23390 (15 μg/kg, s.c., a D<sub>1</sub> antagonist) or vehicle (0.9% NaCl, 10 mL/kg, i.p.). Thirty min later, the animals received the extract (30 mg/kg p.o.) or vehicle (0.9% NaCl + 1% Tween 80, 10 mL/kg, p.o.) and were submitted to the TST after 1 h.

The contribution of the serotonergic neurotransmission in the mechanism of the extract's action was investigated by pretreating mice (n = 6–8/group) with pCPA (100 mg/kg/day – for 4 consecutive days - i.p., an inhibitor of serotonin synthesis) or saline. Twenty-four h after the last pCPA or saline injection, animals were treated with the extract (30 mg/kg, p.o.), or vehicle (0.9% NaCl + 1% Tween 80, 10 mL/kg, p.o.) and were observed in the TST 1 h later.

All the drug doses were chosen based on previous studies.<sup>8,29,30</sup>

#### 2.8. Interaction of the extract with antidepressants

The anti-immobility effect of sub-effective doses of antidepressants along with a sub-effective dose of the extract (30 mg/kg, p.o.) was also investigated in the TST. The animals (n = 9–10/group) received sub-effective doses<sup>28</sup> of fluoxetine (a serotonin reuptake inhibitor, 15 mg/kg, p.o.), imipramine (a noradrenaline and serotonin reuptake inhibitor, 10 mg/kg, p.o.) or bupropion (a dopamine and noradrenaline reuptake inhibitor, 3 mg/kg, p.o.) and immediately after, a sub-effective dose of the extract (30 mg/kg, p.o.) or vehicle was administered. After 1 h, the animals were submitted to the TST.

#### 2.9. Open field test (OFT)

The OFT was carried out with the objective of evaluating the possible effects of the extract of *C. xanthocarpa* seeds, as well as its association with sub-effective doses of antidepressants on mice locomotor and exploratory activities. For that, independent groups of mice were submitted to OFT immediately after the TST.

The animals were placed individually in an acrylic box (40 × 30 × 30 cm), with the floor divided into 24 equal squares. The number of squares crossed with the four legs (crossing), the frequency of rearings and groomings and the amount of fecal bolus expelled by the animal were recorded for 10 min. The apparatus was cleaned with 10% ethanol after each animal exposure.<sup>28</sup>

#### 2.10. Measurement of MAO inhibitory activity

MAO assays were performed as described elsewhere.<sup>31–33</sup> The *in vitro* inhibitory potential of *C. xanthocarpa* seeds extract on MAO A and MAO B activities was tested in mice brain mitochondrial preparations using kynuramine as a substrate, as described elsewhere.<sup>33–35</sup> Briefly, the mitochondrial fractions were preincubated

at 37 °C for 5 min with the selective and irreversible inhibitor selegiline (250 nM) or clorgyline (250 nM) to pharmacologically isolate MAO A or MAO B activity, respectively. The protein content was determined according to Bradford<sup>36</sup> and set to 0.5 mg in a final reaction volume of 500  $\mu$ L. The extract was solubilized in DMSO (5%, v/v) and added to the medium from 0 to 400  $\mu$ g/mL. The non-selective substrate kynuramine was added (50  $\mu$ L) to the reaction mixture (containing mitochondrial fractions, the extract and inhibitors) at a concentration equal to its Km (90  $\mu$ M for MAO A; 60  $\mu$ M for MAO B). The solutions were incubated at 37 °C for 30 min. Finally, the formation of 4-hydroxyquinoline (the product of Kynuramine deamination by MAO) was monitored spectrophotometrically at 314 nm using an UV/Vis spectrophotometer (Hitachi, U-2001).

The experiments (n = 3) were carried out in duplicate. The percentage of inhibition was determined and the IC<sub>50</sub> value was calculated using GraphPad Prism 5.0.

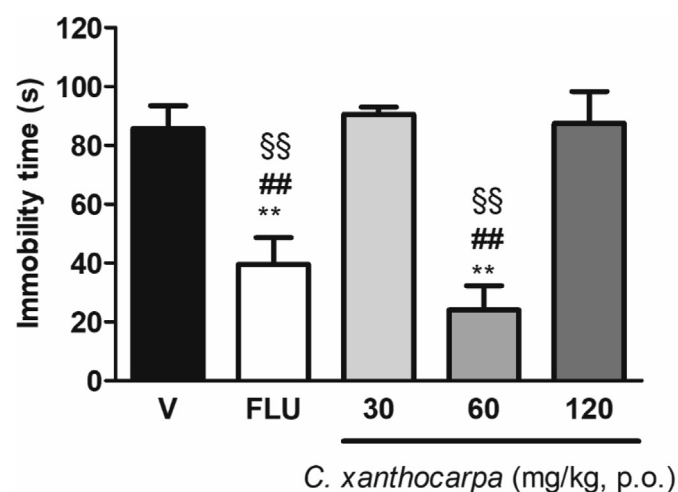
### 2.11. Statistical analysis

Data from the investigation of *C. xanthocarpa* action mechanism were analyzed by two-way ANOVA and the other results were evaluated by one-way ANOVA followed by Student-Newman-Keuls test, using GraphPad Prism 5.0 software. Data are expressed as mean  $\pm$  standard error of the mean. The level of significance was accepted at  $p < 0.05$ .

## 3. Results

The result of the extract's antidepressant-like activity in the TST is presented in Fig. 1. The extract of *C. xanthocarpa* administered at 60 mg/kg (p.o.) significantly reduced ( $p < 0.01$ ) the immobility time of the animals (72.5%). As expected, the positive control, fluoxetine (30 mg/kg, p.o.), caused a significant ( $p < 0.01$ ) reduction in the animals' immobility time compared to the group treated with vehicle (saline + 1% Tween 80, p.o.).

In contrast, the immobility time of the extract-treated animals at 30 or 120 mg/kg was not statistically different from those treated



**Fig. 1.** Effects of *Campomanesia xanthocarpa* seeds extract obtained by supercritical CO<sub>2</sub> in mice tail suspension test. V: vehicle-treated group (0.9% NaCl + 1% Tween 80, 10 ml/kg p.o., n = 8). FLU: fluoxetine (30 mg/kg p.o., n = 7) or *C. xanthocarpa* extract (30, 60 or 120 mg/kg, p.o., n = 7), 1 h before TST. Data were analyzed by one-way ANOVA followed by the Student-Newman-Keuls test and are expressed as mean  $\pm$  SEM. \*\* different from vehicle-treated group  $p < 0.01$ ; ## different from the group treated with *C. xanthocarpa* extract at 30 mg/kg,  $p < 0.01$ ; §§ different from the group treated with *C. xanthocarpa* extract at 120 mg/kg,  $p < 0.01$ .

with vehicle. Therefore, the minimal antidepressant dose of the extract was set at 60 mg/kg. This dose was chosen to be used in the other behavioral experiments.

In the open field test (Fig. 2), the extract of *C. xanthocarpa* seeds at 60 mg/kg (p.o.) did not change the number of crossings (Fig. 2A), groomings (Fig. 2B), rearings (Fig. 2C) and fecal bolus expelled by the mice (Fig. 2D). However, the administration of fluoxetine to the animals elicited a decrease in the number of crossings when compared to the vehicle-treated ( $p < 0.05$ ) and *C. xanthocarpa* extract-treated ( $p < 0.01$ ) groups.

The results of the time-course investigation of the *C. xanthocarpa* seeds extract antidepressant-like effect in the TST are shown in Fig. 3A. The immobility time of the extract-treated group (60 mg/kg, p.o.) was significantly different from the vehicle-treated group 1 h after the extract administration ( $p < 0.01$ ), whereas, after 2 and 3 h, this effect was abolished. Additionally, the animals treated with *C. xanthocarpa* extract presented the same number of crossings (Fig. 3B), rearings (Fig. 3C), groomings (Fig. 3D) and amount of fecal bolus expelled (Fig. 3E) in the open field test as the vehicle-treated groups at 1, 2 and 3 h after treatment.

Considering these data, the time point of 1 h and the dose of 60 mg/kg were chosen for all further experiments. Also, the extract sub-effective dose of 30 mg/kg was selected to be combined with antidepressants sub-effective doses in the TST.

The results depicted in Fig. 4 show the mechanism of action involved in the antidepressant-like effect elicited by *C. xanthocarpa* seeds extract.

The previous administration of monoaminergic receptors antagonists sulpiride (D2 receptor antagonist, Fig. 4A,  $p < 0.05$ ), prazosin ( $\alpha$ 1 receptor antagonist, Fig. 4B,  $p < 0.001$ ), yohimbine ( $\alpha$ 2 receptor antagonist, Fig. 4C,  $p < 0.001$ ), pCPA (inhibitor of serotonin synthesis, Fig. 4D,  $p < 0.05$ ) and SCH23390 (D1 receptor antagonist, Fig. 4E,  $p < 0.05$ ) to mice, significantly prevented the anti-immobility effect of *C. xanthocarpa* seeds extract in the TST.

Fig. 5A shows the results of the combined administration of sub-effective doses of the *C. xanthocarpa* seeds extract with conventional antidepressants' sub-effective doses in mice TST.

The oral administration of sub-effective doses of fluoxetine (15 mg/kg), bupropion (3 mg/kg) or imipramine (10 mg/kg) with a sub-effective dose of *C. xanthocarpa* seeds extract (30 mg/kg, p.o.) significantly ( $p < 0.01$ ) reduced the immobility time of mice when compared to the vehicle-treated group.

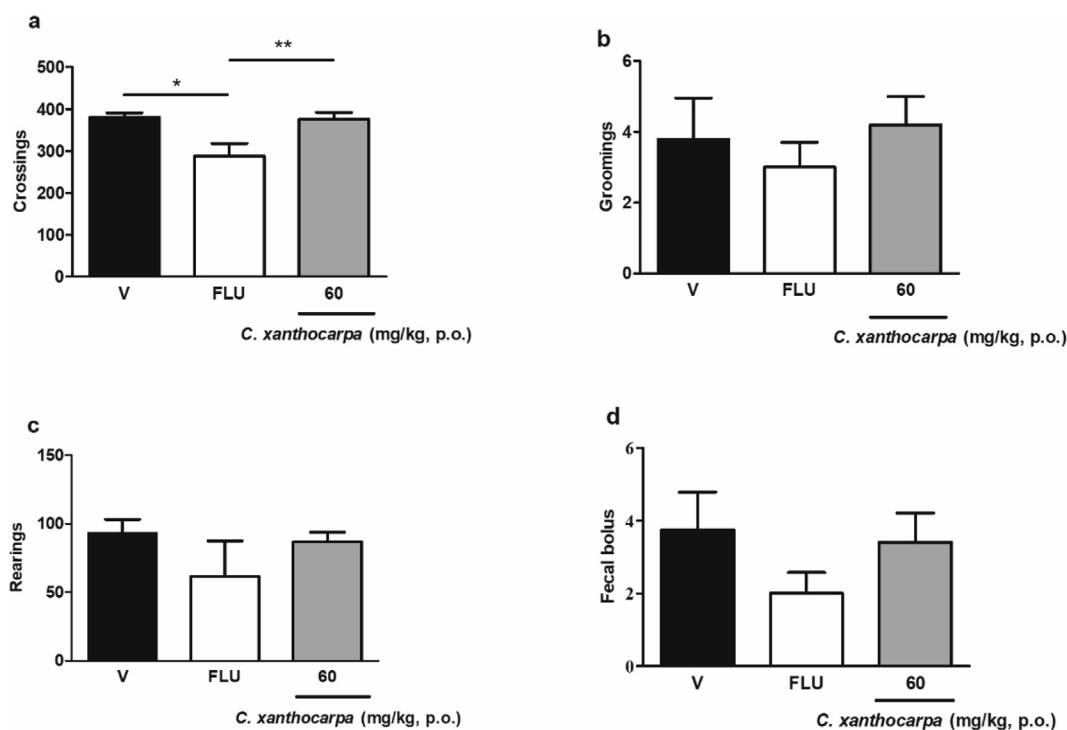
Furthermore, the association of the antidepressants with the extract did not affect the locomotor and exploratory activities of mice in the OFT (Fig. 5B).

The results of MAO A and MAO B inhibition are presented in Table 1. The extract of *C. xanthocarpa* seeds showed MAO A inhibitory activity at the tested concentrations (IC<sub>50</sub> = 151.10  $\pm$  5.75  $\mu$ g/mL), which was greater than that toward MAO B (IC<sub>50</sub> > 400  $\mu$ g/mL).

## 4. Discussion

In the present study, we demonstrated for the first time the antidepressant-like effect of *C. xanthocarpa* seeds extract obtained by supercritical CO<sub>2</sub>. The results were assessed by using mice TST, which is a predictive test of antidepressant-like activity.<sup>37</sup> In addition, the involvement of the monoaminergic system in the mechanism of antidepressant-like action of the extract has been demonstrated, as well as its capacity to inhibit MAO A enzyme activity and to potentiate the anti-immobility effect of sub-effective doses of conventional antidepressants.

Herein, we observed an anti-immobility effect of the extract in the TST; where a bell-shaped dose-response relationship was found. It is known that some antidepressants present a bell-shaped



**Fig. 2.** Effect of *C. xanthocarpa* seeds extract obtained by supercritical CO<sub>2</sub> on mice (n = 6/group) locomotor activity (open field test). A: number of crossings. B: number of groomings. C: number of rearings. D: number of fecal bolus at the end of test. V: vehicle-treated group (0.9% NaCl + 1% Tween 80, p.o.). FLU: fluoxetine (30 mg/kg, p.o.), *C. xanthocarpa* (60 mg/kg, p.o.). Data were analyzed by one-way ANOVA followed by the Student-Newman-Keuls test and are expressed as mean ± SEM. \*p < 0.05; \*\*p < 0.01.

dose-response curve, where increasing dose leads to increasing efficacy up to a point, and so further increases lead to decreasing efficacy. This finding could be related to the effects of negative feedback signaling by auto-receptors, that beyond a certain point exceed the effects of post-synaptic neurotransmission and therefore inhibits the synthesis and secretion of neurotransmitters in the synaptic cleft, such as serotonin.<sup>38</sup>

In the OFT there were no significant changes in the locomotor activity of extract-treated animals. Thus, the effect on the *C. xanthocarpa* seeds in the TST was not related to a nonspecific behavioral alteration and it is possible to affirm that the decrease of the animals' immobility time caused by the extract administration in the TST is not result of a stimulating effect.

One of the main hypotheses of depression's pathophysiology was proposed by Schildkraut.<sup>39</sup> This hypothesis postulates that depression is related to impaired monoaminergic neurotransmission and reduced concentration of cerebral monoamines, especially serotonin, dopamine and noradrenaline.<sup>39</sup> The cerebral connections between the basal ganglia, the limbic system and the frontal lobes are specially affected by monoamines deficiency and the clinical manifestations of these changes are related to mood and sleep alterations, sexual dysfunction among other disorders involved in depressive symptomatology.<sup>40</sup>

Most of antidepressants currently available were developed based on the monoaminergic hypothesis of depression and act by blocking the reuptake of those neurotransmitters (mainly serotonin or noradrenalin-serotonin) or by inhibiting their degradation (monoamine oxidase inhibitors), thus increasing their levels in the synaptic clefts.<sup>4</sup>

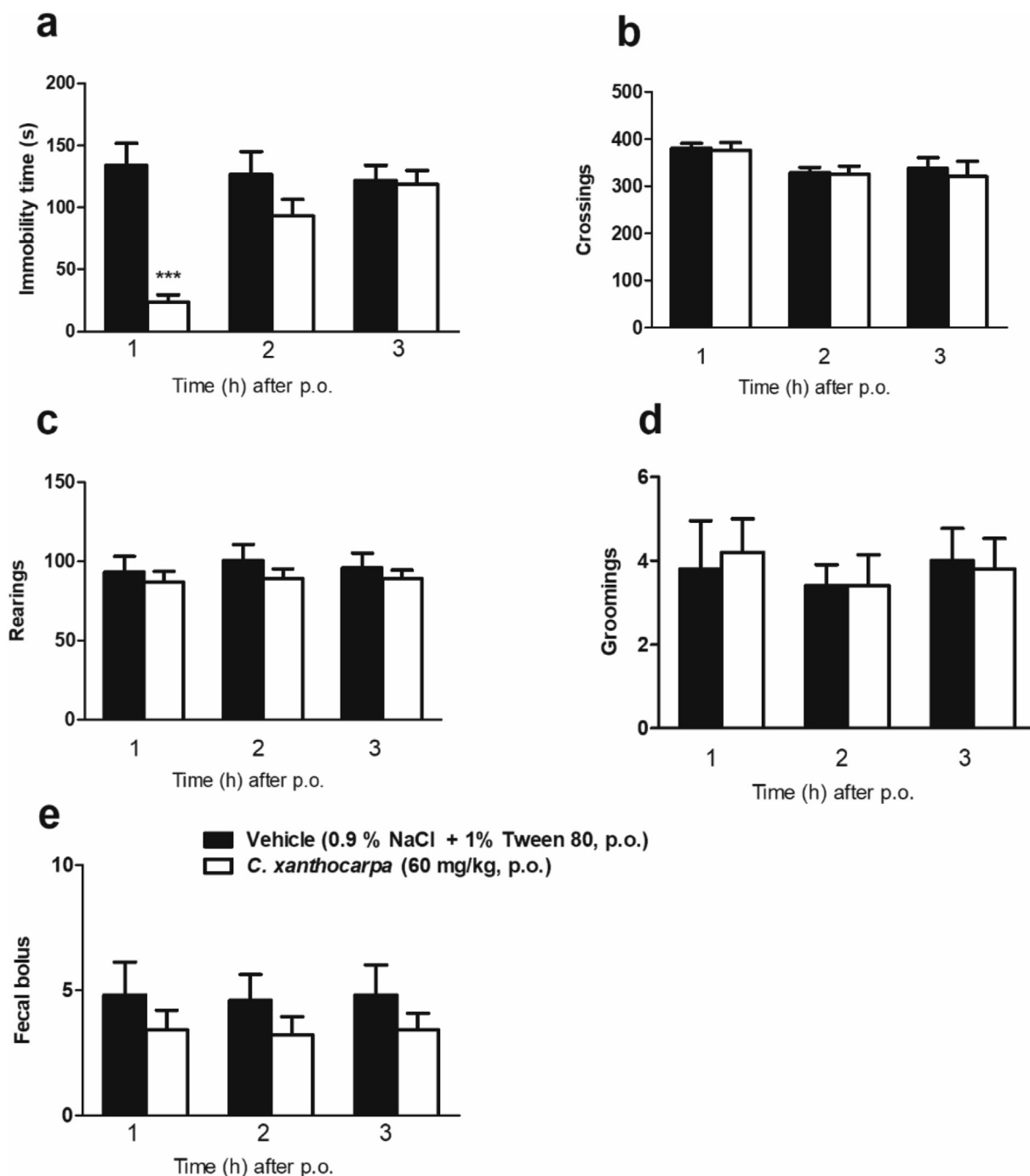
Our results demonstrate that the antidepressant-like effect of *C. xanthocarpa* extract is mediated by an interaction with dopaminergic, noradrenergic and serotonergic neurotransmission. Our data are consistent with other studies demonstrating the antidepressant-like effect of other plant species mediated by

dopaminergic, noradrenergic and serotonergic pathways.<sup>41–43</sup> Indeed, this mechanism of action is innovative and differs from any other conventional antidepressant currently available on the market, since most of them present a dual action mechanism (acting on the noradrenergic and serotonergic or dopaminergic and noradrenergic pathways) or act selectively on serotonergic neurotransmission.<sup>4</sup>

Many antidepressants are available in the market but, despite the seriousness of the scenario involving depression, 30–35% of depressed patients do not show satisfactory response to the treatment, and most drugs have significant adverse effects that limit the adhesion to the pharmacological treatment.<sup>44</sup> Considering that triple monoaminergic transporter inhibitors have been pointed as drugs with improved efficacy in the management of depression<sup>45–47</sup> we may infer that *C. xanthocarpa* extract could represent an alternative for the management of depressed patients who do not respond to any pharmacological intervention. However, this hypothesis deserves further studies.

The main chemical compound present in the supercritical CO<sub>2</sub> extract of *C. xanthocarpa* seeds is β-caryophyllene (11.67%),<sup>15</sup> which antidepressant-like effects were already demonstrated.<sup>48–50</sup> Bahi et al., showed the involvement of cannabinoid CB2 receptors in the mode of β-caryophyllene pharmacological action.<sup>50</sup> In fact, the cannabinoid-dependent activation of monoaminergic signaling has already been demonstrated.<sup>51,52</sup> Therefore, the effects of β-caryophyllene on the cannabinoid receptors system might elicit an indirect interaction with the monoaminergic system. Indeed, the catecholaminergic neurotransmitter system mediates the antidepressant-like effect of β-caryophyllene.<sup>49</sup>

Herein, γ-cadinene (9.58%), α-cadinol (7.17%) and viridiflorol (6.70%), also found in *C. xanthocarpa* seeds extract are presented for the first time as chemical compounds that could contribute to the antidepressant-like activity of the supercritical CO<sub>2</sub> extract of *C. xanthocarpa* seeds. Other authors demonstrated the antioxidant,



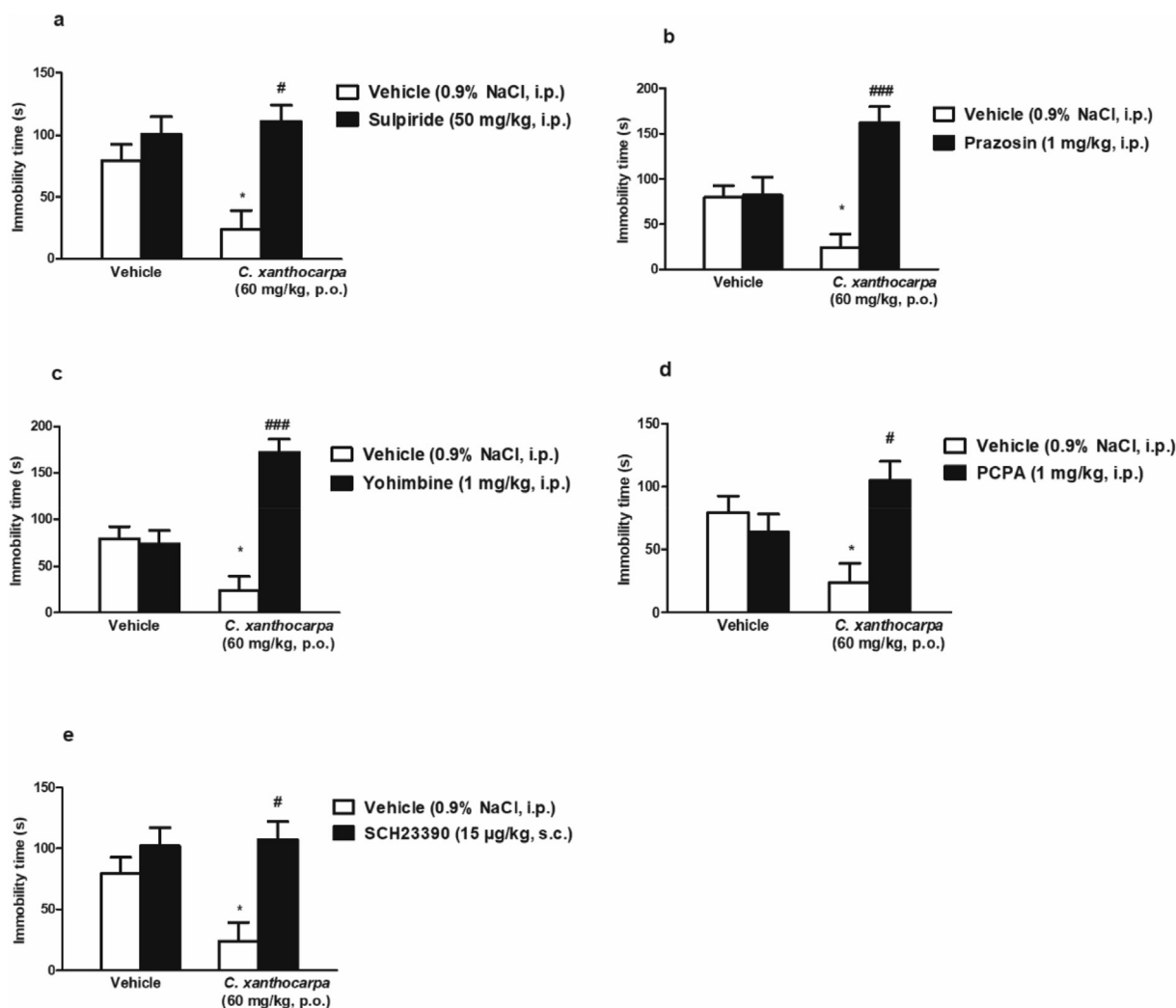
**Fig. 3.** Time course of the *C. xanthocarpa* seeds extract obtained by supercritical CO<sub>2</sub> in mice (60 mg/kg, p.o.) tail suspension test (panel A) and open field test: crossings (panel B), rearings (panel C), groomings (panel D) and fecal bolus (panel E). The extract (60 mg/kg, p.o.) or vehicle (0.9% NaCl + 1% Tween 80, p.o.) were administered to independent groups of mice (n = 9–10 mice/group) 1, 2 or 3 h before the tail suspension test. Each column represents the mean ± SEM. One-way ANOVA followed by Student-Newman-Keuls: \*\*\*p < 0.001 when compared to the vehicle-treated group.

anti-inflammatory and antimicrobial activity of viridiflorol,<sup>53</sup> but no antidepressant-like effects have been described in the literature for this compound. Moreover, the pharmacological effects of isolated  $\gamma$ -cadinene and  $\alpha$ -cadinol have not been studied yet.

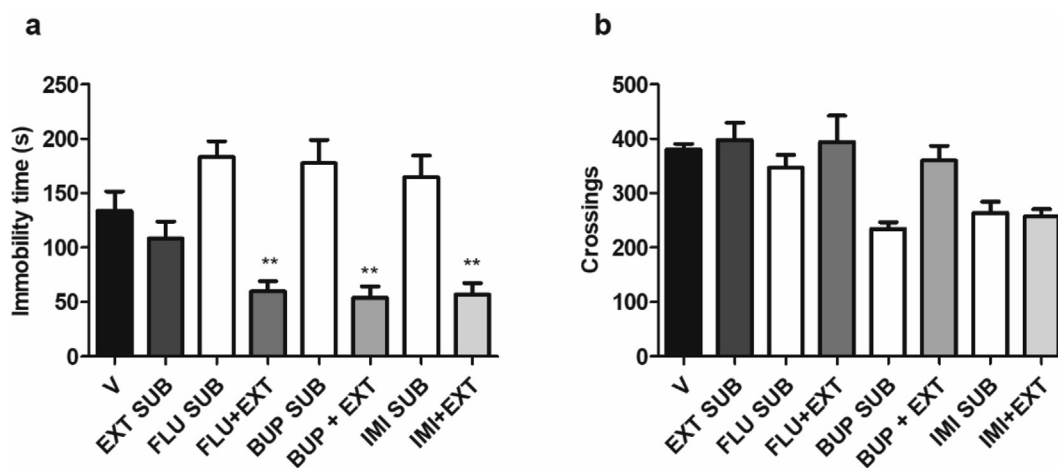
Linalool, one of the constituents of *C. xanthocarpa* seeds extract (5.09%)<sup>15</sup> shows antidepressant-like activity in mice forced swimming test. This effect is mediated by the interaction with serotonergic (5-HT<sub>1A</sub> receptor) and noradrenergic ( $\alpha$ <sub>2</sub> receptor) pathways.<sup>54</sup> These findings suggest that linalool could, at least in

part, contribute to the antidepressant-like effect of *C. xanthocarpa* seeds extract.

The investigation of the time-course of *C. xanthocarpa* antidepressant-like effect revealed that the peak effect of *C. xanthocarpa* effective dose (60 mg/kg) is achieved within 1 h after administration. However, the immobility time of the animals treated with the extract was not different from the immobility time of vehicle-treated animals 2 h after administration. These results indicate that the extract presents a reduced time of



**Fig. 4.** Effect of mice pretreatment with sulpiride (50 mg/kg i.p., panel A), prazosin (1 mg/kg, i.p., panel B), yohimbine (1 mg/kg i.p., panel C), pCPA (100 mg/kg/day, i.p., panel D) and SCH 23390 (15 µg/kg, s.c., panel E) on the anti-immobility effect of *C. xanthocarpa* seeds extract obtained by supercritical CO<sub>2</sub> (60 mg/kg, p.o.) in the TST. Each column represents the mean ± S.E.M (n = 6–8 mice/group). Two-way ANOVA followed by Student-Newman-Keuls: \*p < 0.05, different from vehicle (0.9% NaCl, 10 mL/kg, i.p.) + vehicle (0.9% NaCl + 1% Tween 80, 10 mL/kg, p.o.) - treated group; #p < 0.05; ###p < 0.001, different from the group treated with vehicle (0.9% NaCl, 10 mL/kg, i.p.) + extract (60 mg/kg, p.o.).



**Fig. 5.** Immobility time in the TST (panel A) and number of crossings (panel B) of Swiss mice (n = 10 animals/group) after the administration of vehicle (V: 0.9% NaCl + 1% Tween 80, p.o.) or *C. xanthocarpa* seeds extract obtained by supercritical CO<sub>2</sub> sub-effective dose (EXT SUB: 30 mg/kg, p.o.) with sub-effective doses of antidepressants. (FLU SUB: fluoxetine 15 mg/kg, p.o.; FLU + EXT: fluoxetine 15 mg/kg + *C. xanthocarpa* extract 30 mg/kg, p.o.; BUP SUB: bupropion 3 mg/kg, p.o.; BUP + EXT: bupropion 3 mg/kg + *C. xanthocarpa* extract 30 mg/kg, p.o.; IMI SUB: imipramine 10 mg/kg, p.o.; IMI + EXT: imipramine 10 mg/kg + *C. xanthocarpa* extract 30 mg/kg). Data were analyzed by one-way ANOVA followed by the Student-Newman-Keuls test and are expressed as mean ± SEM. \*\*p < 0.01 when compared to vehicle-treated group.

**Table 1**

MAO inhibitory activity on mice brain mitochondria of *C. xanthocarpa* seeds extract obtained by supercritical CO<sub>2</sub>.

Concentration (µg/mL)	MAO inhibition (% ± S.E.M., n = 3)	
	MAO A	MAO B
50	17.83 ± 0.70	0
100	32.33 ± 3.05	0
200	63.83 ± 3.39	3.83 ± 0.47
400	82.17 ± 3.52	6.17 ± 1.48

pharmacological action, which could be related to a short half-life of its active compounds. Indeed, the preclinical pharmacokinetic evaluation of β-caryophyllene (the major compound of *C. xanthocarpa* seeds supercritical CO<sub>2</sub> extract) demonstrated a short elimination half-life of this terpene.<sup>55</sup> Therefore, extended-release formulations could represent an alternative to accomplish the desired pharmacological effect of the extract, with longer half-life and longer therapeutic effect duration.<sup>56</sup>

In the present study, we found that *C. xanthocarpa* seeds extract was able to potentiate the effect of conventional antidepressants (imipramine, fluoxetine and bupropion), without behavioral stimulation. These results reinforce the ones obtained in the investigation of the extract's mechanism of action with pharmacological antagonisms in the TST. According to Sarris et al.<sup>57</sup> the association of phytotherapies with conventional drugs may result in a beneficial interaction, since the pharmaceutical action of synthetic antidepressants could be potentiated through pharmacodynamic synergism.<sup>57</sup> Indeed, the association of different therapeutic mechanisms is an approach to treat refractory patients and allows the prescription of decreased doses of antidepressants and, consequently, the adverse side effects.<sup>58</sup> Therefore, we may propose that the combined administration of *C. xanthocarpa* seeds extract with conventional antidepressants could be a promising strategy in the treatment of refractory depression.

Monoamine oxidase (MAO) catalyzes the degradation of monoamines (serotonin, noradrenaline and dopamine), and plays a crucial role in psychiatric and neurological diseases and is situated at the mitochondrial membrane of neurons and glia. There are two isoforms of MAO, designated as MAO A and MAO B.<sup>59,60</sup> MAO B is involved in neurodegenerative diseases and MAO A in psychiatric disorders, such as depression.<sup>61</sup> Inhibitors of MAO B are useful as neuroprotectants, whereas inhibitors of MAO A are effective as antidepressants.<sup>61</sup>

Our results demonstrate that the extract of *C. xanthocarpa* seeds inhibits MAO A (IC<sub>50</sub> = 151.10 ± 5.75 µg/mL) in a greater magnitude than MAO B (IC<sub>50</sub> > 400 µg/mL). It is known that an extract has to inhibit MAO at a concentration between 5 × 10<sup>-4</sup> and 5 × 10<sup>-3</sup> mg/mL *in vitro*, in order to elicit *in vivo* effects.<sup>32</sup> Therefore, *C. xanthocarpa* seeds extract accomplishes the criterion for possible inhibition of MAO A *in vivo*. These findings strengthen the antidepressant-like effect of *C. xanthocarpa* seeds and point to this plant species as a potential new source for the development of antidepressants useful in the management of depression.

## 5. Conclusion

Our results demonstrate the first evidence of the involvement of monoaminergic system (dopaminergic, serotonergic and noradrenergic) in the mechanism of antidepressant-like activity of *Campomanesia xanthocarpa* seeds extract obtained by supercritical CO<sub>2</sub>. This is an innovative triple action mechanism of action that differs from the available synthetic antidepressants.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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