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Chapter

Neuropharmacology of Secondary Metabolites from Plants with Anxiolytic and Antidepressant Properties

Rosa Isela García-Ríos, Armando Mora-Pérez, Ana Raquel Ramos-Molina and Cesar Soria-Fregozo

Abstract

Depression and anxiety currently rank as the second and fifth most common causes worldwide of years lived with disability-a reality that has intensified the search for new treatments. There are many studies of herbal extracts and secondary metabolites from plants used in traditional medicine due to their antidepressant and anxiolytic properties. Clinical and preclinical studies about some of the mechanisms of action of metabolites like alkaloids, terpenes, flavonoids, and sterols, among others, have documented effects similar to those produced by clinically effective drugs. These metabolites have shown anxiolytic and antidepressant effects in various experimental models of anxiety by interacting with γ-aminobutyric acid subtype A receptors ($GABA_A$-receptors) and by stimulating the serotonergic, noradrenergic, and dopaminergic neurotransmitter systems. These pharmacological effects can be attributed to plant metabolites that share structural similarities with monoamines, which allow them to bind to receptors. The objective of this chapter is to summarize the various mechanisms of action that have been identified in secondary metabolites with anxiolytic and antidepressant properties. Terpenes, alkaloids, flavonoids, and sterols can interact at different levels of the neurotransmission systems involved in the neurobiology of anxiety and depression, suggesting their potential for treating these mental illnesses.

Keywords: antidepressant, anxiolytic, active metabolites, plant extracts, herbal medicines

1. Introduction

According to the Global Burden of Disease, depression and anxiety are currently the second and fifth most common causes worldwide of years lived with disability in both sexes in the age range of 15–49 years [1]. In 2015, 4.4% (322 million people) of the world's population suffered from depressive disorders, while 3.6% (264 million) were affected by anxiety [2]. In that year, the World Health Organization (WHO) estimated that by 2020 depression would be the second leading cause of

disability; thus, its prediction has been confirmed. Depression is characterized by persistent sadness and a loss of interest in activities that an individual normally enjoys, accompanied by periods of at least 2 weeks marked by the inability to perform everyday activities [2]. Anxiety, in turn, is defined as an emotion expressed in response to stressful, dangerous, or unfamiliar situations, or some unidentified factor, that is, the feeling of unease, distress, or dread that one feels in the face of a significant event. A certain level of anxiety is necessary to keep us alert and aware, but for those who suffer from anxiety disorders, it can be totally debilitating [3]. Current pharmacological treatments for depressive disorders are mainly based on selective serotonin reuptake inhibitors (SSRIs), serotonin (5-HT) and noradrenaline (NE) reuptake inhibitors (SNRIs), and monoamine oxidase inhibitors (MAOIs), all of which act by increasing short-term levels of neurotransmitters in the brain. One consequence of treatment is the desensitization of receptors, for example, 5-HT_{1A}, with a downregulation of autoreceptors, but no changes in the postsynaptic receptors, which leads to the recovery of neuronal activity in the long term [4]. These changes are associated with the long latency to the onset of antidepressant effects. However, up to 70% of depressed patients have residual symptoms [5], and few options exist for transitioning treatment-resistant sufferers to alternative therapies that operate through distinct mechanisms [6]. It is important to note that conventional antidepressants produce significant side effects, such as nausea and vomiting, insomnia, agitation, fatigue, sedation, sexual dysfunction, headaches, and weight gain, which contribute to poor patient compliance and, in some cases, abandonment of treatment [7]. This occurs under such anxiolytic treatments as benzodiazepine (a $GABA_A$ receptor agonist) and SSRIs [8] and is the main cause of the increasing demand for alternative medicines, such as medicinal plants, to alleviate the symptoms of these psychiatric disorders. However, reports of adverse reactions to products of this kind have increased [9], leading WHO to publish the document, "The WHO's traditional medicine strategy: 2014-2023", which outlines a global approach to fomenting the appropriate integration, regulation, and supervision of natural substances. This paper will be useful in countries seeking to develop a proactive policy toward this important and expanding area of health care and will contribute to the use of herbal medicines of proven quality, safety, and efficacy, providing quality medical care to all people [10]. Recent decades have witnessed efforts to gather scientific evidence that validates the efficacy of plants commonly used for their antidepressant and anxiolytic properties [11], but research has been insufficient because of the wide range of plants available worldwide. We lack solid scientific data on the neurochemical pathways and mechanisms of action of medicinal plants or their active metabolites because few clinical studies have addressed these issues. Also, reports of adverse reactions to medicinal plants [9] may reflect the broad variety of active metabolites they contain, thus highlighting the need for preclinical and clinical studies that evaluate the possible biological activity of compounds isolated from plants or standardized crude extracts, their mechanisms of action, and possible toxicity.

Fajemiroye et al. [12] proposed a hypothetical model for identifying medicinal plant extracts and phytoconstituents with anxiolytic and/or antidepressant properties that is currently used by most researchers: *(i) select medicinal plants with anxiolytic and/or antidepressant potential based on local reports; (II) prepare standard crude extracts; (III) perform phytochemical studies that include sequential partitioning of crude extracts, purification and isolation of phytoconstituents, chemical elucidation or characterization of the isolates, structural modifications or syntheses of new compounds based on chemical structure of their isolates; and (IV) conduct pharmacological analyses of the anti-anxiety and antidepressant properties of the standard crude*

extracts, fractions, isolated compounds, or derivatives using ex vivo, in vitro, and in vivo assays (e.g., preliminary pharmacological screening, classic animal models of anxiety like the light dark box (LDB) or elevated plus-maze (EPM) tests, etc., or the forced swim test (FST) and tail suspension tests (TST), among others). These tools have allowed researchers to analyze the possible metabolites responsible for the anxiolytic or antidepressant properties of plants used by different populations, and identify how their mechanisms of action affect the functioning of the central nervous system (CNS). Their studies contribute to advancing scientific understanding of the neurobiology of depression and anxiety, and to developing new pharmacological treatments that may favorably impact public health.

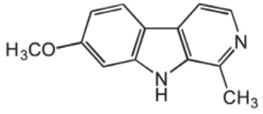
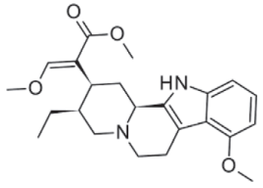
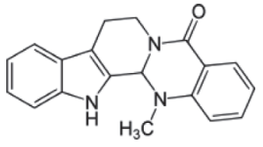
Plants used in traditional medicine contain compounds in their secondary metabolism [13] such as alkaloids, phenols, sterols, carbohydrates, tannins, terpenes, and phytoalexins, all of which have important biological activities [14]. The most widely studied metabolites are terpenes, flavonoids, alkaloids, and sterols, whose mechanisms of action stimulate the serotonergic, noradrenergic, dopaminergic, or GABAergic neurotransmission systems, acting on receptors or the synthetic pathways of neurotransmitters and their transporters. However, they may also stimulate other neurotransmission systems. For example, terpenes can stimulate at the same time serotonergic, dopaminergic, and noradrenergic neurotransmission systems [12] that can produce a similar effect on mood regulation, perhaps leading to an overstimulation that may generate undesirable collateral effects.

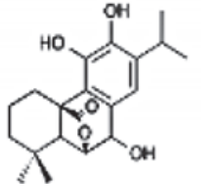
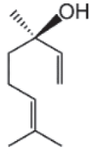
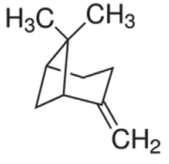
This chapter reviews and discusses the findings from research on several metabolites of medicinal plants that have shown potential anxiolytic and antidepressant activities once screened for their biological mechanisms at various levels: receptor, transporter, synthesis, gene, protein, or metabolic. The studies analyzed were identified by a preliminary search in PubMed, Scopus and Ovid for articles on (i) the dose effects and possible mechanisms of action of metabolite(s) isolated from parts of plants with previously identified anxiolytic or antidepressant effects; and (ii) standard chemical tests performed with specific metabolites.

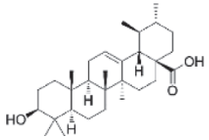
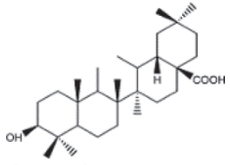
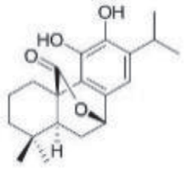
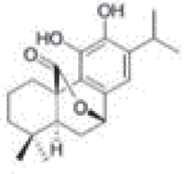
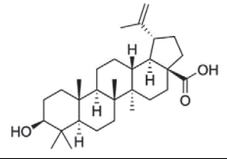
2. Terpenes with antidepressant effects

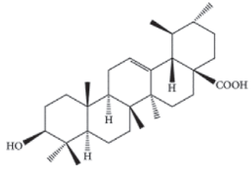
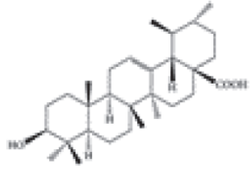
Terpenes are formed by the union of isoprene units (5 C atoms). Their classification depends on the number of units they contain: 10 C terpenes (two units) are called monoterpenes, while 15 C terpenes (three units) are called sesquiterpenes, and those with 20 C are diterpenes. Triterpenes have 30 C, tetraterpenes have 40 C, and polyterpenes are those with over 8 isoprene units. Studies have evaluated the effect of terpenes isolated from plants, including rosmanol from *Rosmarinus officinalis*, ursolic acid, and oleanolic acid; carnosol from *Artemisa indica*; and linalool and β-pinene from *Litsea glaucescens*. All these terpenes have proven antidepressant effects. Abdelhalim et al. [15] isolated rosmanol, an ethyl acetate diterpene, from *R. officinalis*. A single acute dose of 30 or 100 mg/kg i.p. of rosmanol in male Swiss mice produced an antidepressant effect on the FST and TST. The 100-mg/kg dose produced an effect similar to that of a 60-mg/kg dose of imipramine on the FST. Their study also tested the acute toxicity of administering 50, 150, and 200 mg/kg, i.p., of rosmanol. Some signs of toxic effects on grooming behavior were observed, as well as hyperactivity, sedation, respiratory arrest, convulsions, and locomotor activity. However, no cases of lethality or variations in the amount of food and water ingested were reported.

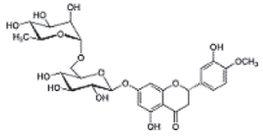
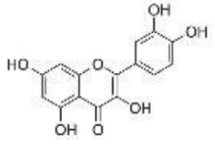
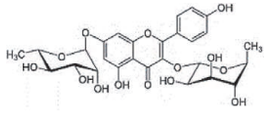
Other terpenes with antidepressant properties include phenolic diterpene, carnosol, and pentacyclic triterpenoids like betulinic, oleanolic, and ursolic acids.

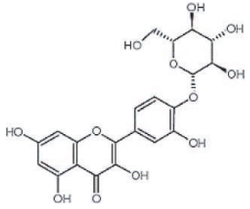
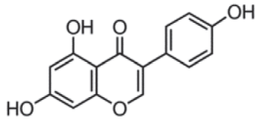
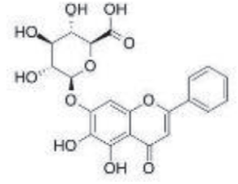
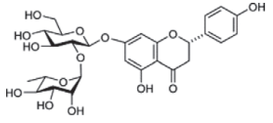
Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses, and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
Alkaloids							
Harmine 	Harmine	i.p. 15 mg/kg/day for 7 days i.p. 5, 10, and 15 mg/kg/day for 7 days	Stressed rats CUMS (60 days old) No stressed rats (60 days old)	• Sucrose preference test • Forced swim test	Antidepressant-like effect in the CUMS model Antidepressant effect with both doses	NE NE	[62]
Mitragynine 	Separated and purified from *Mitragyna speciosa*	i.p 5, 10, and 30 mg/kg a single dose	Male mice	• Tail suspension test • Forced swim test	Antidepressant effect with 10 and 30 mg/kg	Modulating neuroendocrine axis HPA	[67]
Evodiamine 	Evodiamine (*Evodia fructus*, *Evodia rutaecarpa* Benth., Rutaceae)	v.o 10–20 mg/kg for 14 days	Male Sprague–Dawley rats (180–220 g)	• Sucrose preference test • Forced swim test	Antidepressant-like effect in the CUMS model	NE	[69]
Protopine	Protopine hydrochloride was synthesized from Protopine *Dactylicapnos scandens*	Doses of 3.75 mg/kg, 7.5 mg/kg and 30 mg/kg	Male BALB/cj mice (20–24 g)	• Tail suspension test	Antidepressant effects with 30 mg/kg	Inhibitor of serotonin transporter and noradrenaline transporter	[71]

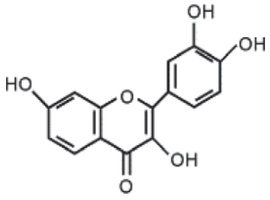
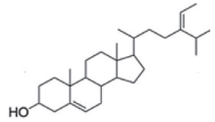
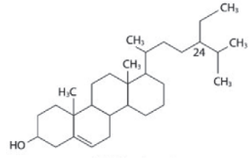
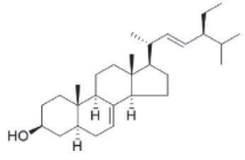
Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses, and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
Terpenes							
Rosmanol 	Ethyl acetate extract of *Rosmarinus officinalis* (Rosemary)	i.p. 30 and 100 mg/kg a single dose	Male Swiss mice (20–30 g)	• Forced swim test • Tail suspension test	Antidepressant effect with both doses 100 mg/kg like with imipramine Antidepressant effect with both doses	NE	[15]
Linalool  (−)-Linalool 	Found shrub such as *Litsea glaucescens* in these studies used chemical standard	i.p. 100 mg/kg a single dose i.p. (10, 50, 100, and 200 mg/kg, a single dose)	Male ICR mice (27–33 g) Male Swiss mice (30–40 g)	• Forced swim test • Tail suspension test	Antidepressant effect with 100 mg/kg Antidepressant effect with 100 and 200 mg/kg	Serotonergic mechanism by 5-HT1A receptors Noradrenergic mechanism by α2-adrenoceptor NE	[22] [21]
(1S)-(−)-β-pinene 	Found shrub such as *Litsea glaucescens*	i.p. 100 mg/kg a single dose	Male ICR mice (27–33 g)	• Forced swim test	Antidepressant effect with 100 mg/kg	Serotonergic mechanism by 5-HT_{1A} receptors Noradrenergic mechanism by activation of the β-adrenoceptor and regulate noradrenergic neurotransmission Dopaminergic mechanisms by activation of D1 receptors	[22]

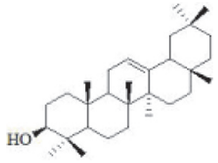
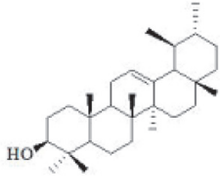
Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses, and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
Ursolic acid  Oleanolic acid  Carnosol 	Methanol extract of *Artemisa indica* in the chloroform fraction (Mugwort)	i.p. 10, 30, and 100 mg/kg in a single dose for metabolite independently	Male Swiss mice (20–30 g)	• Forced swim test • Tail suspension test	Antidepressant effect with all doses of three metabolites without effect in locomotor activity 100 mg/kg of ursolic acid was similar to imipramine (60 mg/kg) Antidepressant effect with all doses of three metabolites	Suggest a GABAergic mechanisms in α1β2γ2L $GABA_A$ receptors	[17]
Carnosol  Betulinic acid 	Crude extract of stems and leaves of *Rosmarinus officinalis* and identified and isolation of the hexane fraction (carnosol) and of the ethyl acetate fraction (betulinic acid)	p.o. 0.01, 0.1, 1, and 10 mg/kg, in a single dose p.o. 0.1, 1, and 10 mg/kg, in a single dose	Male Swiss mice (45–50 g, 60–70 days old)	• Tail suspension test	Carnosol produced an antidepressant effect with 0.01 and 0.1 mg/kg, while betulinic acid only with 10 mg/kg	NE	[16]

Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses, and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
Ursolic acid 	Crude extract of stems and leaves of *Rosmarinus officinalis* and identified in ethyl acetate fraction	p.o. 0.1, 1, and 10 mg/kg, in a single dose p.o. 0.001, 0.01, 0.1, 1, and 10 mg/kg, in a single dose	Male Swiss mice (20–30 g, 60–70 days old)	• Forced swim test • Tail suspension test	Antidepressant effect with 10 mg/kg similar to bupropion (10 mg/kg) Antidepressant effect with 0.01 and 0.1 mg/kg were similar to fluoxetine (10 mg/kg), imipramine (1 mg/kg), and bupropion (10 mg/kg)	NE Suggest a probable dopaminergic mechanism by D1 and D2 receptor	[18]
Ursolic acid 	Chemical standard	0.001 and 0.1 mg/kg	Swiss mice (35–45 g, 55–60 days old) of either sex homogeneously distributed	• Tail suspension test	Antidepressant effects with 0.1 mg/kg alone and in combination with pretreatment with PCPA 100 mg/kg, i.p., 4 days 0.001 mg/kg of ursolic acid and 5 mg/kg of fluoxetine produced a pharmacological synergism	Suggest mechanism serotonergic by synthesis of 5-HT and activation of 5-HT_{1A}	[19]
Terpinene-4-ol γ-terpinene Transsabinenehydrate α-terpinene α-terpinolene Cis-sabinenehydrate β-phellandrene ρ-cymene trans-caryophyllene (E)-p-menth-2-en-1-ol bicyclogermacrene β-myrcene	*Origanum majorana* essential oil (OMEO)	The OMEO was made up of 24 compounds Terpinene-4-ol (32.69%) γ-terpinene (12.88%) Transsabinenehydrate (8.47%) α-terpinene (7.98%) sabinene (6.21%) α-terpineol (5.25%) α-terpinolene (3.36%) Cis-sabinenehydrate (2.92%) β-phellandrene (2.64%) ρ-cymene (2.32%) trans-caryophyllene (2.31%) (E)-p-menth-2-en-1-ol	Male mice (20–30 g)	• Forced swim test	Antidepressant effect with 40 and 80 mg/kg of OMEO 80 mg/kg de OMEO was more effective	Dopaminergic mechanisms by activation of D1 and D2 receptors Serotonergic mechanisms by activation of 5-HT_{1A} and 5-HT_{2A} receptors, increases 5-HT synthesis Noradrenergic mechanism by activation of α1 and α2-adrenoceptors They regulate brain monoamine neurotransmitters	[20]

Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses, and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
		(2.25%) Bicyclogermacrene (1.60%) β-myrcene (1.49%) These anointed for 92.37% of the yield, while the other detected components represented <1.0% in each case. 10, 20, 40, and 80 mg/kg of OMEO in a single dose					
Hesperidin 	Commercial flavonoid (Sigma Chemical)	i.p 0.01, 0.1, 0.3, and 1 mg/kg, for 21 days	Male Swiss mice	• Tail suspension test	Antidepressant effect with all doses evaluated	Increase in BDNF concentrations in the hippocampus	[42]
Quercetin 	Commercial flavonoid (Sigma Chemical)	25 mg/kg, for 14 days	Bulbectomized mice	• Tail suspension test • Forced swim test	Antidepressant effect with 25 mg/kg	Lipid hydroperoxide content (LOOH) levels were reversed by quercetin; antidepressant-like effects seem to occur by modulation of glutamate and nitric oxide	[37]
Kaempferitrin 	Isolation of aerial parts of *Justicia spicigera*	1.0, 5.0, 10, and 20 mg/kg doses	Male Swiss Webster mice	• Tail suspension test • Forced swim test	Antidepressant effect with 5.0, 10, and 20 mg/kg doses	The activation of 5HT1A receptors and the synthesis of 5-HT are mandatory to produce effect of noradrenergic mechanism by α2- adrenoceptor agonism	[95]

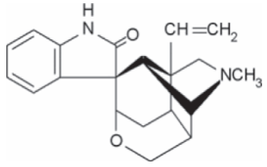
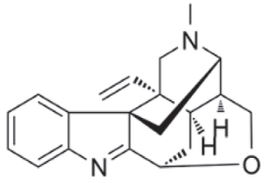
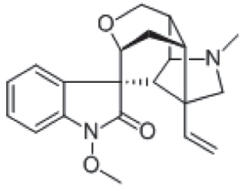
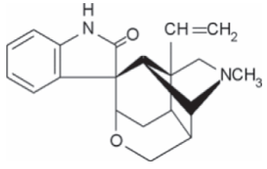
Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses, and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
Quercetin 4'-O-glucoside 	Bulbs of *Allium cepa* var	p.o. 50 and 100 mg/kg extract. Once daily for 7 days	Swiss albino mice of either sex	• Forced swim test	Antidepressant effect with 50 and 100 mg/kg	Effect might be attributed to its anti-oxidant properties, MAO-A inhibition, and consequent increase in brain 5-HT levels	[36]
Genistein 	Commercial Genistein (Ze Lang Biotechnology)	p.o. 5–45 mg/kg, (once per day for 3 weeks)	Male ICR mice	• Forced swim test • Tail suspension test	Antidepressant effect with 15 and 45 mg/kg 45 mg/kg is similar to imipramine 15 mg/kg	Genistein was potentiated by co-treatment with 8-OH-DPAT (5-HT_{1A} receptor agonist)	[33]
Baicalin 	Commercial flavonoid (Nanjing, China)	60 mg/kg	Male ICR mice	• Sucrose preference test	Antidepressant effect	Through a mechanism to promote the differentiation of neurons, and the transformation into mature neurons and their survival via the Akt/FOXG1 pathway	[43]
Naringin 	Commercial Naringin (Sigma-Aldrich)	i.p. 2.5, 5, and 10 mg/kg, once daily for 7 days	Male Swiss mice	• Forced swim test	Antidepressant effects	Maybe by increased neuro-antioxidant and cholinergic activities and it significantly decreased malondialdehyde and nitrite concentrations, suggesting the involvement of oxidative/nitrosative pathways	[44]

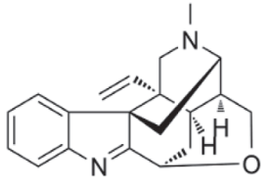
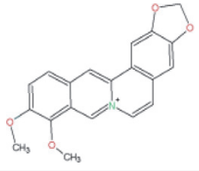
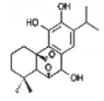
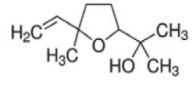
Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses, and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
Fisetin 	Commercial Fisetin (Sigma-Aldrich)	p.o. 5 mg/kg, with 1–2 weeks of treatment	Male adult ICR mice	• Tail suspension test • Forced swim test	Antidepressant effect	Maybe by activation of TrkB signaling in the hippocampus suggesting pro-neurogenesis effects of fisetin in the hippocampus	[40]
Sterols							
Fucosterol 	Ethanol extract of *Sargassum fusiforme* (algas)	i.p. 10, 20, 30, and 40 mg/kg	Male Balb/e mice (20 ± 2 g)	• Forced swim test • Tail suspension test	Antidepressant effect with dose 20 and 30 mg/kg like fluoxetine	Increase in CNS 5HT, NA, and BDNF levels	[92]
β-Sitosterol 	Ethanol extract of *Sargassum horneri*	i.p. 10, 20, and 30 mg/kg, for 7 days	Male Kun Ming mice (20 ± 2 g)	• Forced swim test • Tail suspension test	Antidepressant effect with dose 20 mg/kg β-sitosterol and 200 mg/kg sterols total like fluoxetine	Increase in CNS 5-HT, NA β-sitosterol increases 5-HIAA levels	[93]
α-Spinasterol 	Toronto Research Chemicals Inc., Canada	i.p. 1 and 2 mg/kg	Male albino Swiss mice (23–25 g)	• Forced swim test	Antidepressant effect with dose 1 and 2 mg/kg	TRPV1 antagonistic effects	[90]

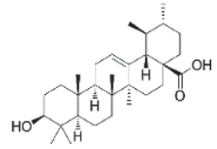
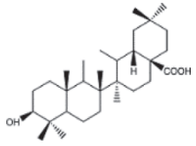
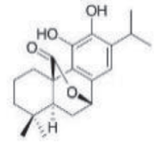
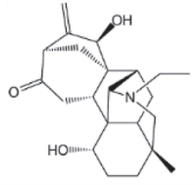
Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses, and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
β-Amirine  α-Amirine 	Hexane–ethyl acetate extracts from *Protium heptaphyllum*	p.o. 1, 2.5, and 5 mg/kg	Male Swiss mice (20–30 g)	• Forced swim test	Antidepressant effect with dose 2.5 and 5 mg/kg	NE	[94]

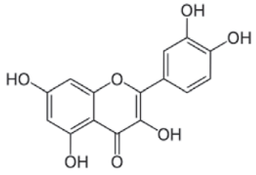
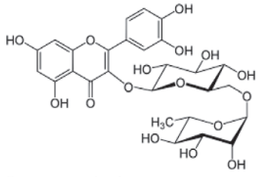
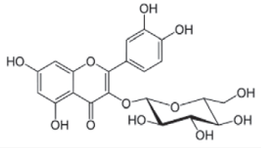
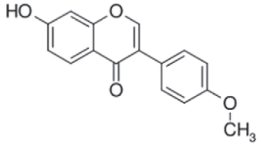
NE = no explorated.

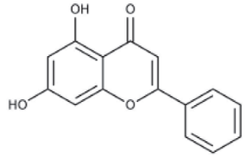
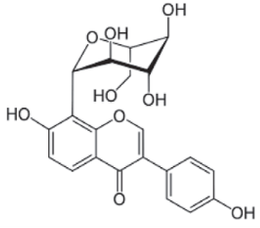
Table 1.
Secondary metabolites with antidepressant properties in preclinical study.

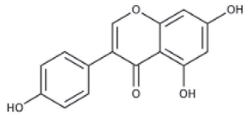
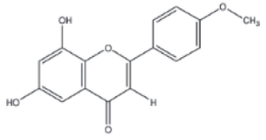
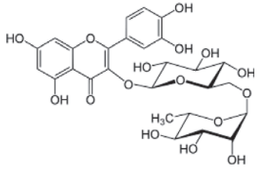
Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
Alkaloids							
Gelsemine  Koumine  Gelsevirine 	Isolated from *Gelsemium elegans* Benth	s.c. gelsemine, koumine and gelsevirine 0.4, 2, and 10 mg/kg s.c. gelsenicine 0.8, 4, or 20 μg/kg	Male mice (24–26 g)	• Elevated plus maze • Light/dark box	Anxiolytic activity with all doses	Mechanism may be involved in the glycine receptor	[72]
Gelsemine 	Hydroalcoholic solution of *Gelsemium elegans* Benth	i.p. 500 μl (10^{-6}, 10^{-10}, or 10^{-14} M) for 7 days	Male Sprague–Dawley rats (250–300 g)	• Elevated plus maze	Anxiolytic activity with 10^{-6} and 10^{-10} M	NE	[73]

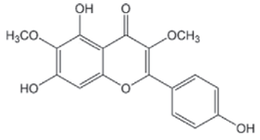
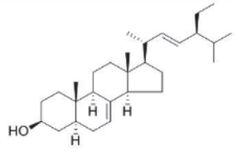
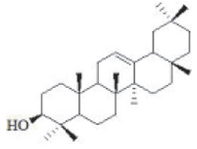
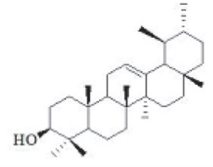
Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
Koumine 	Separated and purified from *Gelsemium elegans*	s.c. 0.167, 0.5, or 1.5 mg/kg Only one administration	Male Wistar rats (6–8 weeks and 180–220 g)	• Vogel conflict test	Anxiolytic effect with all doses	NE	[74]
Berberine (isoquinoline alkaloid) 	Berberine hydrochloride	v.o. 100 mg/kg/day for 21 days	Male Wistar rats (200–250 g)	• Elevated plus maze	Anxiety-like behaviors in addiction	Modulation of neuropeptide oxytocin and its receptor	[59]
Terpenes							
Rosmanol 	Ethyl acetate extract of *Rosmarinus officinalis* (Rosemary)	i.p. 1, 10, 30, and 100 mg/kg Only one administration	Male Swiss mice (20–30 g)	• Elevated plus maze • Light/dark box	Anxiolytic effect with 10, 30, and 100 mg/kg 10 and 30 mg/kg like diazepam (1 mg/kg)	Suggest a GABAergic mechanisms in $GABA_A$ receptors PTZ (20 mg/kg), but not Flumazenil (2.5 mg/kg) blocked the anxiolytic effect of 10 mg/kg of rosmanol in the elevated plus maze	[15]
Linalool oxide 	Frequently found aromatic plants such as *Lavandula angustifolia* Mill., *Melissa officinalis* L., Rosmarinus officinalis L., and *Cymbopogon citratus* DC	Inhalation of linalool oxide emulsion 0.65%, 1.25%, 2.5%, and 5.0% w/w. 7 min of exposure to the inhalation chamber	Male Swiss mice (20–30 g)	• Elevated plus maze • Light/dark box • Rota-rod test	Anxiolytic effect with all doses without effect in coordination motriz	NE	[28]

Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
Ursolic acid  Oleanolic acid  Carnosol 	Methanol extract of whole *Artemisa indica* in the chloroform fraction (Mugwort)	i.p. 1, 10, 30, and 100 mg/kg Only one administration	Male Swiss mice (20–30 g)	• Elevated plus maze • Light/dark box	Anxiolytic effect with 10, 30, and 100 mg/kg of the three metabolites without effect in locomotor activity 30 and 100 mg/kg of the three metabolites are like diazepam (1 mg/kg)	Flumazenil (2.5 mg/kg) blocked the anxiolytic effect of 10 mg/kg of the three metabolites in elevated plus maze test Suggest a GABAergic mechanisms in α1β2γ2L $GABA_A$ receptors	[17]
Songorine 	The chloroform extract obtained from the aerial part of wolfsbane (*A. barbatum* Pers.)	p.o. 2.5 and 0.25 mg/kg, for 5 days	Male CBA/CaLac mice (20–22 g)	• Vogel conflict test	Anxiolytic activity with 0.25 mg/kg produced exceeding that of phenazepam Without sedative effect	NE	[29]
p-Cymene + thymol	Ethyl acetate extract of *Lippia graveolens* leaves	i.p. 3 mg/kg	Male CD-1 mice (25–30 g)	• Hole-board test • Elevated plus maze	Anxiolytic effect	NE	[24]

Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
Flavonoids							
Quercetin  Rutin  Isoquercitrin 	Flowers and bracts of *Tilia americana* L.	All i.p. mixes of quercetin (20 mg/kg), isoquercitrin (2 mg/kg), and rutin (15.70 mg/kg)	Male CD-1 mice	• Hole-board test • Elevated plus maze	Anxiolytic-like effects producing a significant diminution in head dips during the hole-board test, an increase in time spent at the open-side arms in the plus-maze	The involvement of GABAergic and serotonergic receptors. Flumazenil and WAY100635, inhibited the anxiolytic-like effects of the flavonoid mixture in the plus-maze test, while WAY100635 showed a significant decrease in the number of explorations in the hole-board test	[46]
Formononetin 	Formononetin from *Trifolium pratense L.*	25 mg/kg for 8 consecutive days	C57BL/6 male mice	• Elevated plus maze	Formononetin relieved CFA-induced anxiety-like behaviors in mice	A mechanism based on the inhibition of hyperexcitability and inflammation in the basolateral amygdala is suggested through the inhibition of NMDA receptor and CREB-binding protein (CBP)	[47]

Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
Theaflavins	TF40, a crude theaflavin extract	p.o 1 or 5 mg/kg theaflavins, once a day for 5 days for EPM and once a day for 6 days for LDB	DDY male mice	• Elevated plus maze • Light/dark box	5 mg/kg theaflavins show anxiolytic-like effects in both models. In EPM, the time spent in the open arms was significantly increased, while the time spent and the number of entries in the light box increased	Theaflavin increased the levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and the ratios of DOPAC/DA and (DOPAC + homovanillic acids)/DA indicating DA turnover, in the frontal cortex	[48]
Chrysin 	Commercial Chrysin (Sigma-Aldrich)	(0.5, 1, 2, and 4 mg/kg)	Adult female Wistar rats in a model of surgical menopause	• Light/dark box • Elevated plus maze	2 and 4 mg/kg produced anxiolytic-like effects. Increased the total time spent in the light compartment in rats with the long-term absence of ovarian hormones. With respect to the elevated plus maze, chrysin (2 mg/kg) increased the time spent on the open arms	$GABA_A$ receptor activation partial, pretreatment with picrotoxin (1 mg/kg), did not block the anxiolytic-like effects of chrysin	[49]
Puerarin 	Commercial Puerarin (Sigma-Aldrich)	p.o. 30, 60, and 120 mg/kg	Male Sprague–Dawley rats	• Elevated plus maze • Vogel conflict test	Anxiolytic-like effects were produced by puerarin (60 and 120 mg/kg, i.g)	It's suggested that puerarin (60 and 120 mg/kg, i.g.) produced an increase of allopregnanolone and serotonin (5-HT) in the prefrontal cortex	[50]

Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
Genistein 	Commercial Genistein (Sigma-Aldrich)	i.p. 2–8 mg/kg, for 7 days	Sprague–Dawley male rats	• Elevated plus maze	Anxiolytic-like effects were produced by genistein (2–8 mg/kg)	Significantly increased total time spent in open arms in a dose-dependent manner	[53]
6-Methoxyflavanone 	Commercial 6-methoxyflavanone (Sigma-Aldrich)	i.p. 10, 30, 50, and 100 mg/kg	BALB/c mice of both sex	• Elevated plus maze • Staircase test	6-methoxyflavanone (10, 30, and 50 mg/kg) spent appreciably longer in the open and arms and on the central platform like diazepam. In staircase test, both diazepam and flavonoid 6-MeOF (10 and 30 mg/kg) reduced the incidence of rearing without decreasing the number of steps ascended	α1-subunit containing $GABA_A$ receptor mediated sedative action of the 6-methoxyflavanone	[56]
Rutin 	Commercial Rutin (Sigma-Aldrich)	(i.p.) 30, 100, 300, 562, and 1000 mg/kg microinjected into the basolateral amygdala (16 nmol/4 μl, intracerebral)	Male Wistar rats	• Elevated plus maze	Anxiolytic-like effects in rutin (300–1000 mg/kg) significantly and dose manner dependently increased (3–6-fold) the number of entries to the open arms and the time spent in this significantly increased in a dose-dependent manner	Anxiolytic-like effects are partly modulated by $GABA_A$ receptors in the basolateral amygdala. Flumazenil partly antagonized the effects of systemic rutin	[57]

Metabolite	Extract from plant/part of plant (common name)	Posology (administration way, doses and duration of treatment)	Experimental subject	Experimental model	Identified effect	Mechanisms of action	References
Viscosine 	*Dodonaea viscosa* (Linn)	i.p. 10, 30, and 100 mg/kg	Male Swiss mice	• Elevated plus maze • Light/dark box	Viscosin increased the % entries and % time spent in the open arms	Anxiolytic effect of viscosine are likely mediated via its positive allosteric modulatory action of GABA at different $GABA_A$ receptor subtypes	[58]
Sterols							
α-Spinasterol 	Toronto Research Chemicals Inc., Canada	i.p. 1 and 2 mg/kg	Male albino Swiss mice (23–25 g)	• Elevated plus maze • Light/dark box	No effects were found (0.5, 1, and 2 mg/kg)	NE	[90]
β-Amirine 	Hexane–ethyl acetate from *Protium heptaphyllum*	p.o. 10, 25, and 50 mg/kg	Male Swiss mice (20–30 g)	• Elevated plus maze	Anxiolytic effect with dose 10–25 mg/kg like diazepam	Mechanisms GABAérgic by $GABA_A$ receptors in the subunit α1	[94]
α-Amirine 							

NE = no explorated.

Table 2.
Secondary metabolites with anxiolytic properties in preclinical study.

Carnosol generated an antidepressant-like effect at doses of 0.01 and 0.1 mg/kg [16] on TST, and 10, 30, and 100 mg/kg, i.p., on TST and FST, as did the oleanolic and ursolic acids. Meanwhile, 100 mg/kg of ursolic acid showed an effect similar to that of imipramine at 60 mg/kg [17]. Betulinic acid at 10 mg/kg was evaluated in male mice on TST [16]. None of those metabolites had effects on locomotor activity. Ursolic acid at doses of 0.01 and 0.1 mg/kg produced an effect similar to that of fluoxetine (10 mg/kg), imipramine (1 mg/kg), and bupropion (10 mg/kg) on TST [18]. Only the 10-mg/kg dose had an antidepressant effect on FST, which was similar to that of bupropion at 10 mg/kg. Studies exploring the mechanism of action of ursolic acid found the involvement of D1 and D2 receptors and a pharmacological synergism with bupropion at 1 mg/kg, p.o. (dual dopamine/noradrenaline reuptake inhibitor, DDNRI) [18], 5 mg/kg of fluoxetine, or 2 mg/kg of reboxetine (SRNI) [19]. They also observed a related increase in noradrenaline and dopamine synthesis on TST [19]. These findings are noteworthy because they suggest the possibility that various mechanisms of action may be stimulated by the same metabolite.

A study of the essential oil of *Origanum majorana* (OMEO) identified 14 component terpenes that represented 92.32% of yield. The five components that were more abundant in OMEO were terpinene-4-ol (32.69%), γ-terpinene (12.88%), trans-sabinene hydrate (8.47%), α-terpinene (7.98%), and sabinene (6.211%). Administered in a single acute dose of 40 or 80 mg/kg, OMEO increased swimming and climbing times in male mice on FST. These findings were interpreted as showing an antidepressant-like effect. In that study [20], pretreatment with antagonist drugs demonstrated that the terpenes act through several mechanisms: first, by activating the dopaminergic receptors D_1 and D_2, the noradrenergic α1- and α2-adrenoceptor, and the 5-HT_{1A} and 5-HT_{2A} receptors, and, second, by activating or increasing 5-HT synthesis and monoamine vesicular storage involved in reducing the immobility time produced by 80 mg/kg of OMEO [20]. A pharmacological synergism at the combined subthreshold OMEO doses of 10 mg/kg plus fluoxetine or imipramine (5 mg/kg, i.p.) was also seen. It reduced immobility time but increased swimming and climbing times, similar effects to those produced by OMEO at 80 mg/kg [20]. (A more detail view about the OMEO components can be reviewed at **Tables 1** and **2** as summary). (R)-(−)-linalool produced antidepressant effects in male mice on TST at a single dose of 100 or 200 mg/kg, i.p., and on FST when administered 3 times at 100 mg/kg, i.p. [21]. That effect was produced by activation of the 5-HT_{1A} receptor and α2-adrenoceptors [22], while (1S)-(−)-β-pinene produced an antidepressant effect after three applications at a dose of 100 mg/kg, i.p., by activating β-adrenoceptors, 5-HT_{1A} and D1-receptors, and noradrenergic neurotransmission in the cerebral cortex [22].

However, due to the terpene's capacity to stimulate several neurotransmission systems—especially high doses of monoterpenes—possible collateral or undesirable effects as serotonergic syndrome need to be explored. The monoterpenic oxide, 1,4-cineole, for example, produced a prodespair effect at doses of 200 mg/kg, i.p., on FST and 400 mg/kg, i.p., and FST and TST, but did not induce any significant deficit in motor coordination on the rota-rod test (RRT). It did, however, have an anxiolytic effect at a dose of 400 mg/kg in male mice evaluated in EPM that was not associated with any sedative effect [23]. These findings require additional study in light of potential depressor effects on the CNS, and to elucidate the mechanisms of action involved.

3. Terpenes with anxiolytic effects: preclinical research

Anxiolytic properties have also been attributed to terpenes. A combination of two monoterpenoids, p-cymene + thymol, both at doses of 3mg/kg i.p., produced

anxiolytic effects on the hole-board test (HBT) and EPM [24]. Studies have also demonstrated that (−)-myrtenol, a monoterpenoid alcohol, produced an anxiolytic effect on EPM at doses of 25, 50, and 75 mg/kg, i.p., though only the 25- and 50-mg/kg doses did so on LDB. On both tests, the anxiolytic effect of 25 mg/kg of (−)-myrtenol was mediated by $GABA_A$ receptors [25]. In another study, rosmanol produced an anxiolytic effect at doses of 10, 30, and 100 mg/kg in EPM and LDB, and the 10- and 30-mg/kg doses showed an effect similar to those of diazepam at 1 mg/kg [15]. This mechanism of action acts on $GABA_A$ receptors at a distinct site from the high-affinity benzodiazepine-binding region [15]. Triterpenes as ursolic acid, oleanolic acid, and carnosol produced anxiolytic effects at doses of 10, 30, and 100 mg/kg in EPM and LDB. The effect of the 30- and 100-mg/kg doses of these three metabolites was similar to that of diazepam at 1 mg/kg. No significant effect was seen on locomotor activity. We know that this effect was produced through $GABA_A$ receptors of the α1β2γ2L conformation because 2.5 mg/kg of flumazenil blocked the anxiolytic effects of 10 mg/kg of all three in EPM [17].

Although most terpenes have a GABAergic mechanism, their action may also occur through the serotonergic system, as evidenced in the study by Costa et al. [26]. In an experiment with male rats, those authors identified that acute administration of 5 mg/kg, p.o., or 14-day repeat doses (1 mg/kg/day), of the essential oil of ripe fruits of *C. aurantium* (Rutaceae) (whose chemical composition includes 98.66% limonene, 0.53% β-myrcene, 0.41% β-pinene, and 0.41% unidentified compounds) produced an anxiolytic-like effect in LDB that was mediated by the serotonergic system through 5-HT1A receptors (WAY 100635 0.5 mg/kg, i.p.), not the GABAergic system (flumazenil 2 mg/kg, i.p.). That study also analyzed the antidepressant effect on FST after oral and inhaled treatment, but found that it did not modify immobility. Their results suggest that distinct mechanisms of action exist for the anxiolytic and antidepressant effects [26]. In this regard, some of the terpenes in certain essential oils produce anxiolytic effects and are often used in aromatherapy to reduce anxiety in animals and humans [27]. Inhaling emulsions of linalool oxide (a monocyclic alcohol) at 0.65, 1.25, 2.5, and 5.0% w/w during 7 min of exposure in the inhalation chamber, for example, produced anxiolytic-like effects in EPM and LDB, but no significant motor deficit on RRT [28].

Finally, songorine, a C20 diterpenoid alkaloid, produced an anxiolytic-like effect at 0.25 mg/kg v.o. for 5 days with greater efficacy than phenazepam on Vogel's conflict test (VCT) [29]. For the terpenes described so far, we know that both the serotonergic and GABAergic systems are involved in their mechanisms of action, and these are the same systems that are activated by other groups of metabolites, such as flavonoids and sterols (see **Tables 1** and **2** for summaries).

4. Flavonoids with antidepressant effects: preclinical research

Flavonoids are the most widely studied active metabolites (for a broad review of research, see German-Ponciano et al. [30]). Genistein is an isoflavone that can cross the blood-brain barrier in mice [31] and rats [32]. Acute oral administration of 5, 15, and 45 mg/kg genistein in male mice did not reduce immobility time on FST or TST, but chronic, dose-dependent administration for 21 days produced antidepressant-like effects on both tests, without affecting locomotor activity [33]. This effect was associated with increased NA and 5-HT concentrations in the hippocampus and frontal cortex, and of 5-HT in the hypothalamus, though it decreased the 5-HTIAA/5-HT ratio in the hippocampus and frontal cortex. These results suggest an inhibition effect of genistein on MAO-A in the hippocampus, frontal cortex, and hypothalamus and on MAO-B in the hippocampus, three brain structures involved in the

neurobiology of depression and anxiety. Their results [33] also demonstrate that central depletion of 5-HT reversed the antidepressant effect of genistein, suggesting a critical role of the serotonergic system, specifically through 5-HT_{1A} receptors. It is important to note that these results on the serotonergic metabolic ratio (5-HIAA/5-HT) may be dependent on gonadal hormones. Ovariectomized rats (OVX, surgical removal of both ovaries) showed reduced immobility times on FST after administration of genistein (10 mg/kg, p.o. [34], or by i.m.) [35] for 14 days, but the downward tendency of the serotonergic metabolic ratio caused by FST was only evident in the hippocampus [34]. Sapronov and Kasakova [35] found antidepressant-like effects on FST at the same dose of genistein (10 mg/kg) but in non-ovariectomized rats. That effect was more marked in the metestrus and diestrus phases of the estral cycle, which are characterized by low plasmatic concentrations of ovarian hormones, than during the proestrus and estrus stages with their characteristically high hormone concentrations. This suggests that these hormones play a significant role in the antidepressant effect of genistein.

A similar effect on the serotonergic system and MAO-A activity was found with quercetin 4'-O-glucoside or quercetin administered at doses of 10 and 20 mg/kg, p.o., for 7 days in Swiss albino mice of both sexes. These substances produced antidepressant-like effects in mice on FST as well as those subjected to unpredictable, chronic mild stress (CUMS, a mouse model designed to induce depression) and subsequently evaluated on FST. In that study, a 20-mg/kg dose of quercetin 4'-O-glucoside showed a similar effect to that of fluoxetine at 20 mg/kg, p.o., on FST, with or without prior exposure to CUMS [36]. A consequence of CUMS on the sucrose preference test (SPT, a test used to study anhedonia rodents, the main symptom of depression in humans) is a decrease in the consumption of a sweetened solution. In these sense, both doses of quercetin 4'-O-glucoside reverted this effect, and interpreted as being antidepressant. Other consequences of CUMS are metabolic, for example, excessive production of reactive oxygen species that was evidenced by higher brain thiobarbituric acid reactive species (TBARS). Compromising endogenous anti-oxidants, like reduced glutathione (GSH), enhances MAO-A activity in the brain and, consequently, depletes monoamine levels there, especially serotonin 5-HT. The effects observed in that study were blocked by 21 days of treatment with 10 and 20 mg/kg of quercetin 4'-O-glucoside [36], suggesting a possible mechanism of action with an antioxidant effect that impedes ROS production. Another study [37] found that 10 mg/kg of quercetin administered for 14 days reduced immobility time on TST, but not FST, while doses of 25 and 50 mg/kg produced this effect in female mice on both tests. The mechanisms of action were explored on TST, where i.c.v. administration of N-methyl-D-aspartate (NMDA at 0.1 pmol/site) and L-arginine (at 750 mg/kg, i.p., a nitric oxide inhibitor) blocked the antidepressant effect of quercetin. Hence, the antidepressant-like effect of quercetin may involve inhibiting NMDA receptors to decrease intracellular calcium that, in turn, inhibits the protein calmodulin, which then inhibits neuronal nitric oxide synthase to decrease nitric oxide levels (NO). This hypothesis is supported by the finding that administering methylene blue (a NO synthase inhibitor) at 20 mg/kg, i.p., and soluble guanylate cyclase or 7-nitroindazole (another NO synthase inhibitor) at 50 mg/kg, i.p., improved quercetin's antidepressant-like effect on TST. This indicates that the antidepressant effect may be dependent on limiting NO synthesis, either by inhibiting the enzyme or by reducing NO production, perhaps via decreased cyclic guanosine monophosphate (cGMP), since sildenafil (a phosphodiesterase 5 selective inhibitor that increases cGMP levels) also canceled this effect [37].

A model of depression induced by olfactory bulbectomy (OB, surgical removal of the olfactory bulbs) reduced the latency to immobility and increased immobility

time on FST and TST. This was accompanied by an increase in the levels of the markers of oxidative stress, for example, 116% in the case of lipid hydroperoxide content (LOOH) in the hippocampus. This effect was reverted by 52.25% by administering 25 mg/kg of genistein in the content of LOOH, as observed on the immobility on FST and TST. In sham rats only (*i.e.*, animals subjected to the same surgical procedure but without resection of the olfactory bulbs), genistein reduced glutathione (GSH) levels, in that study by 65.94%. The authors [37] explained that "the reduction of GSH levels caused by OB and, surprisingly, quercetin, can be explained by the fact that glutathione peroxidase, in addition to reducing H_2O_2, decreases lipid and nonlipid hydroperoxides at the expense of GSH, causing it to become oxidized and giving rise to glutathione disulfide. Therefore, it is suggested that LOOH activated glutathione peroxidase which, in turn, oxidized GSH to normalize LOOH levels". In this area, increased levels of the markers of oxidative stress in major depression have been associated with poor response to antidepressant treatment [38]. Therefore, a therapy that reduces the levels of markers of oxidative stress and produces antidepressant effects could be a promising form of treatment.

Additional mechanisms of the antidepressant action of flavonoids have been explored. Administering chrysin for 14 days at a dose of 20 mg/kg, for example, increased grooming time in male OB C57B/6J mice evaluated on the splash test (ST), where 200 ml of a 10% sucrose solution is squirted on the mouse's snout to initiate grooming behavior. Here, greater grooming time is considered an antidepressant effect. Doses of 5 and 20 mg/kg impeded an increase in immobility time by these OB mice on FST, but increased 5-HT and brain-derived neurotrophic factor concentrations in the hippocampus [39]. In another study, fisetin administered at 5 mg/kg v.o. increased activation of the tropomyosin kinase B receptor (TrkB) by signaling and increasing its phosphorylation in the hippocampus. This suggests that fisetin produced pro-neurogenesis [40] related to its antidepressant effect on FST and TST after 1 or 2 weeks of treatment with a relatively short therapeutic latency compared to clinically-effective antidepressants. Fisetin also reversed depression-like behaviors induced by spatial restraint stress in mice evaluated on FST and TST [40]. Other studies have found that the chemical standard dihydromyricetin activated the ERK1/2-CREB pathway and increased glycogen synthase kinase-3 beta (GSK-3β) phosphorylation at ser-9 with upregulation of BDNF expression in the hippocampus, while inhibiting neuroinflammation. These findings may be related to the antidepressant effect seen on TST and FST after once-daily administration of 10 and 20 mg/kg, v.o. for 3 days, but not after a single acute dose [41]. Interestingly, dihydromyricetin reverted the depressogenic effect caused by CUMS in mice subjected to SPT and FST, or TST, only after administration of once daily for 7 days, but not 3 days [41]. Another flavonoid analyzed is hesperidin, which increased BDNF levels in the hippocampus after administration of once daily for 21 days (0.3 and 1 mg/kg, i.p.). These doses produced an antidepressant effect on TST similar to fluoxetine (32 mg/kg i.p.) and imipramine (15 mg/kg, i.p.). Another research has also verified that when applied acutely (1 mg/kg after 30 min) or chronically (0.1, 0.3, and 1 mg/kg for 21 days) hesperidin significantly decreased nitrate/nitrite (NOX) levels in the hippocampus of mice, suggesting a possible inhibition of the L-arginine-NO-cGMP pathway [42].

Another flavonoid that has shown effects on the CNS is baicalin, which may promote neuronal differentiation through neuronal maduration and ensure their survival via the associated Akt/FOXG1 pathway, which stimulates dendrite elongation. This is related to findings that indicated that, after 6 weeks of treatment, a 60-mg/kg dose of baicalin had an effect similar to that of fluoxetine (15 mg/kg, v.o.), because it reverted the decrease of sucrose intake on SPT and the increase in immobility on TST produced by CUMS [43]. Another flavonoid that associates

antidepressant and antioxidant effects is naringin, which reduced immobility on FST at doses of 2.5, 5, and 10 mg/kg given for 7 days. The antidepressant effect of these doses correlated with enhanced cholinergic transmission due to a decrease in the activity of the enzyme acetylcholinesterase and of the antioxidant defense systems caused by higher GSH levels, as well as an increase in the activity of superoxide dismutase (SOD) and catalase (CAT) in mice brains.

Studies have demonstrated that naringin inhibits lipid peroxidation and nitrosative processes by reducing levels of ROS and nitrogen species [44]. Finally, the extract of *Cirsium japonicum* at doses of 200 and 400 mg/kg has shown the ability to reduce immobility time on FST in a similar manner to that of 5 mg/kg of the antidepressant imipramine. A major component of this plant is the flavonoid luteolin, which at doses of 5 and 10 mg/kg produced a similar effect to that of the complete extract, likely through a positive modulating effect on the $GABA_A$ receptor complex. This was proven in an *in vitro* study where extracts of both *Cirsium japonicum* and luteolin increased Cl^- influx in an effect impeded by pretreatment with bicuculline, a competitive $GABA_A$ receptor antagonist [45].

The varied mechanisms seen in flavonoids make them an important object of study, especially in the search for side effect-free treatments that can compromise their effectiveness or produce toxicity by interacting with other medications or food. This is another area of research that remains to be explored.

5. Flavonoids with anxiolytic effects

A particularly important fact concerning the potency of the biological activity of plants is that it depends on several factors, for instance, the part of the plant used, the region where it is gathered, the season, and harvesting time, among others [46]. For example, in male mice evaluated by HBT and EPM, a single dose of 100 mg/kg i.p. of the methanolic extract of inflorescences of *Tilia americana* var. mexicana collected in Morelia, Mexico, produced a more effective anxiolytic effect than those gathered in Honey, Puebla, though the leaves collected in Honey were more effective than those from Morelia or Santa María Ahuacatitlan, Mexico. These three Mexican states are located at different elevations with distinct humidity and soil types. That study quantified quercetin, rutin, and isoquercitrin in the inflorescences and leaves, determining that the concentrations of these substances differed with the part of the plant used and the collection area [46]. It also tested several standard commercial flavonoids: kaempferol (10 mg/kg), quercetin (20 mg/kg), astragalin (10 mg/kg), isoquercitrin (2 mg/kg), quercetin (10 mg/kg), and rutin (15.7 mg/kg), and a mixture of flavonoids (MIX) composed of quercetin 20 mg/kg, rutin 15.7 mg/kg, and isoquercitrin 2 mg/kg and quercetin (20 mg/kg). Results showed that a mixture of quercitin (20 mg/kg), rutin (15.70 mg/kg), and isoquercitrin (2 mg/kg) produced an anxiolytic effect in male mice tested in HBT and EPM [46] by reducing the number of head-dippings but increasing the time spent in the open arms, respectively. Finally, upon testing the anxiolytic effect of the methanolic extract of *Tilia americana* var. mexicana, those authors found that this produced an effect in EPM through the participation of GABA/BDZ (flumazenil 5 mg/kg) and $5HT_{1A}$ serotonergic receptors (WAY 100635 0.5 mg/kg), though they were not involved in the anxiolytic effect on HBT [46].

Another flavonoid known to have anxiolytic effects is formononetin, an active metabolite of traditional Chinese medicine red clover (*Trifolium pratense L.).* Wang et al. [47] observed that administering 25 mg/kg of this metabolite to male mice once daily for 8 days blocked the anxiogenic effect on the open field test (OFT) produced by administering Freund's complete adjuvant (CFA), reduced the time

spent and distance traveled in the central area, and decreased the time spent in the open arms of EPM [47]. Formononetin did not modify behavior compared to the control group on either test, indicating that it demonstrated an anxiolytic effect. However, it seems that these results should be understood as a neuroprotective effect more than an anxiolytic one, at least under those study conditions. The notion of a neuroprotector effect is supported by the fact that the study found that formononetin attenuated inflammation and neuronal hyperexcitability by inhibiting NMDA receptors and the CREB signaling pathway in the basolateral amygdala (BLA) [47].

Other anxiolytic mechanisms of action seen in flavonoids are dopaminergic in nature. Theaflavins, for example, increased dopamine (DA) turnover to induce activation of the dopaminergic system in the frontal cortex in male mice in EPM and LDB [48], while chrysin at 2 and 4 mg/kg produced an anxiolytic effect in rats at 12 weeks postovariectomy on LDB by increasing the time spent in the light compartment. Those findings resembled the effect of diazepam. At doses of 1, 2, and 4 mg/kg, this flavonoid increased the frequency of entries into, and the time spent in, the open arms of EPM partially through action on $GABA_A$ receptors (pretreatment with 1 mg/kg picrotoxin) [49]. On the other hand, neurosteroids and the serotonergic system have also been implicated in the anxiolytic effect of flavonoids, as in the case of puerarin, which increased 5-HT and allopregnanolone levels in the prefrontal cortex and hippocampus in male rats. These results have been associated with the finding that puerarin increased the time spent in the open arms and the percentage of entries into the open arms of EPM, whereas on the VCT test, it produced an increase in the number of shocks received. In both cases, the effect was similar to that of sertraline, which was used as a positive control drug to generate an anxiolytic effect on both tests [50].

In an animal model of surgically-induced menopause, genistein at 0.09 and 0.12 mg/kg, s.c., for seven consecutive days, or the same treatment regimen but with 17β-estradiol, increased the time spent in, and the percentage and frequency of entries into, the open arms of EPM. These effects were caused by activation of the β-estrogenic receptor (ERβ) since pretreatment with tamoxifen (5 mg/kg, an ERβ antagonist) blocked the anxiolytic effect. Also, genistein and 17β-estradiol increased the frequency of rearing and grooming behaviors on the locomotor activity test (LAT), associated with an anxiety-reducing effect manifested in EPM [51]. Genistein tested at doses of 0.25, 0.5, and 1 mg/kg increased the time spent in, and the frequency of exploration of, the light compartment of LDB, while doses of 0.5 and 1 mg/kg increased rearing frequency, and 1 mg/kg increased grooming time. Those studies used rats at 12 weeks postovariectomy [52]. The authors suggest that "genistein is considered a phytoestrogen that acts in a dose-dependent manner with a broader margin of safety at anxiolytic doses. However, more studies are required to take advantage of its potential therapeutic anxiolytic effects" [51].

In a distinct approach, a post-traumatic stress disorder (PTSD) model used a chamber with a grid floor connected to a system that delivered foot shocks, exposing rats to 5 shocks per day. There, an increase in the contextual freezing time on days 7, 14, and 21 indicated the induction of anxiety-like behaviors. The time spent in freezing behavior was calculated with the shock-administering system turned off. In that study, genistein at 4 and 8 mg/kg i.p. administered to male rats from day 7 reduced freezing time at 7, 14, and 21 days. Interestingly, only the 8-mg/kg dose returned freezing times to control levels on day 21 [53]. The stressed rats were also tested in EPM, where they spent less time in the open arms, indicating an anxiety-like effect that was reverted by administering 4 and 8 mg/kg of genistein. This reduced anxiety-like behavior in the stressed rats occurred in association with enhanced tryptophan hydroxylase (TPH) and 5-HT levels, but also promoted the

5-HT receptor-related CaMKII/CREB signaling pathway in the amygdala [53], likely reflecting the fact that the amygdala receives serotonergic projections from the raphe, two brain structures to which emotional valence and 5-HT synthesis, respectively, are attributed [54].

A study evaluated the pharmacokinetic profile of 6-methoxyflavanone and calculated the K_P value (*i.e.*, the tissue-to-serum partition coefficient). Molecules with K_P values >0.30 are thought to be readily distributed in the brain [55]. That study determined that 30 min postadministration of a 30-mg/kg i.p. dose, the 6-methoxyflavanone had crossed the blood-brain barrier (BBB) to reach the amygdala with K_p = 0.47, and the cerebral cortex with K_p = 0.437. These two cerebral structures are known to be involved in the neurobiology of anxiety [56], so these properties were associated with the anxiolytic effect of 6-methoxyflavanone in male and female mice in EPM at doses of 10, 30, and 50 mg/kg, and on the staircase test (ScT). In this model, the lower frequency of rearings, but no reduction in the steps climbed in a 3-min period, was interpreted as indications of an anxiolytic effect [45]. Those authors verified that 6-methoxyflavanone produced its effect by activating $GABA_A$ receptors with the α2-subunit, perhaps in the amygdala and brain cortex, since pretreatment with PTZ blocked this anxiolytic effect in EPM and on ScT [56].

Another flavonoid with anxiolytic effects is rutin at doses of 300 and 562 mg/kg, i.p., or 16 nmol/site, in the basolateral amygdala of male rats tested in EPM. This involves partial GABAergic neurotransmission that was not associated with BDZ binding in the $GABA_A$ receptors [57]. Finally, viscosine administered to male mice assessed in EPM and LDB was seen to exert its action through the α1β2γ2L and α2β2γ2L modulates of the $GABA_A$ receptors at a site distinct from the one classically associated with benzodiazepine [58].

6. Alkaloids with antidepressant activity

Alkaloids purified from crude acid-base extracts have diverse chemical structures. They may contain one or more nitrogen atom(s) (in the heterocyclic ring) in the form of salt [59]. Pseudoalkaloids that possess nitrogen exist. They are not synthesized from amino acids, but by nitrogen transfer in the form of ammonia to a compound of terpenic origin, steroids, polyketides, monosaccharides, or fatty acids [59].

The alkaloid berberine (50 mg/kg, i.p.) decreased immobility but increased climbing behavior on FST; results are considered to reflect an antidepressant-like effect in rats after abstinence from repeated morphine administration [59]. Chronic treatment with the extract of *Annona cherimola* produced antidepressant-like effects in tests of mice on FST. *A. cherimola* contains mainly the alkaloids 1,2-dimethoxy-5,6,6a,7-tetrahydro-4H-dibenzoquinoline-3,8,9,10-tetraol, anonaine, liriodenine, and Nornuciferine, which are likely responsible for the increase in 5-HT and DA [60].

In another work, the alkaloid derivatives of the β-carbolines (harmane 5, 10, and 15 mg/kg, norharmane 2.5, 10, and 15 mg/kg, and harmine 5, 10, and 15 mg/kg, all i.p.) showed antidepressant effects in mice that were dose-dependent, suggesting that the effect occurs through an inverse agonist mechanism of the benzodiazepine receptors due to flumazenil antagonism (5 mg/kg, i.p.) [61]. In addition, anhedonia was reversed in rats subjected to the CUMS model after harmine treatment at 15 mg/kg/day for 7 days. They showed increased adrenal gland weight, ACTH levels, and BNDF protein levels produced by the CUMS [62]. Treatment for 14 days with harmine (5, 10, and 15 mg/kg) and imipramine (10, 20, and 30 mg/kg) in rats

subjected to FST produced antidepressant-like effects, while harmine (10 and 15 mg/kg), but not imipramine, increased BDNF protein levels in the hippocampus of rats [62]. These results indicate that the main mechanism involved in harmine's antidepressant effect is an increase in hippocampal BDNF, though this may be dependent on treatment time and the precise region of the hippocampus.

An infusion of harman (1-methyl-beta-carboline) into the hippocampus of rats, or through systemic administration, increased the concentration of 5-HT [63]. In addition, metabolite levels of 5-HT degradation decreased dose-dependently, probably due to inhibition of MAO-A [63]. Another study showed that harman bonds to type 5-HT_{2A} serotonergic receptors but shows no affinity to dopaminergic or BZ receptors [64]. Injections of 2.5 and 10 mg/kg of harmane in rats under fear conditioning have been shown to increase plasma ACTH, corticosterone, 5-HT, and NA levels in limbic system structures. These results suggest that harman can modulate behavioral alterations, brain neurochemistry, and neuroendocrine functions through a mechanism that inhibits MAO-A [65].

Subchronic, oral administration for 21 days of the lyophilized extract of *Rhazya stricta* and alkaloid fractions (akuammidine, rhaziminine, and tetrahydrosecamine) to male rats inhibited the activity of the MAO-B enzyme, a mechanism through which the antidepressant-like effect may occur [66]. Another alkaloid in the *Mitragyna speciosa* plant has shown antidepressant effects. Administered to mice at doses of 10 and 30 mg/kg, i.p., mitragynine decreased immobility time on then TST and FST [67] and the release of corticosterone. Mitragynine's effect appears to be mediated through the neuroendocrine HPA (hypothalamus-adrenal-pituitary) axis [67].

Punarnavine administered at doses of 20 and 40 mg/kg, v.o., for 14 days decreased immobility on FST, MAO-A activity, and corticosterone levels in both stressed and unstressed mice [68], while treatment with evodiamine at 10 and 20 mg/kg in rats exposed to CUMS reversed the decrease in their preference for sugared water and immobility time on FST, but increased 5-HT and NA levels and the protein expression of BNDF in the hippocampus. However, it reduced corticosterone levels, suggesting that it likely modulates monoamines and BDNF-TrkB signaling in the hippocampus [69]. Chronic administration of piperine in rats at 5, 10, and 20 mg/kg has shown antidepressant-like effects on FST, probably due to a serotonergic mechanism [70]. At a dose of 30 mg/kg, protopine produced an antidepressant effect on TST in mice, perhaps by inhibiting the 5-HT and NA transporters, since *in vitro* studies have reported that it produces an inhibitory effect on these elements [71].

Because alkaloids have powerful antidepressant effects, many are used in clinical practice with effective therapeutic results. Preclinical studies have clearly demonstrated the antidepressant effects of alkaloids, but evidence of their mechanisms of action is still deficient or unclear. Alkaloids isolated from plants are an option for treating depression, but more studies are needed at the preclinical level to evaluate their potency, efficacy, and safety before they can be incorporated into clinical practice.

7. Alkaloids with anxiolytic effect

The alkaloids gelsemine, koumine, and gelsevirine exerted anxiolytic effects in single doses of 2 and 10 mg/kg in mice in EPM and LDB [72]. Gelsemine in low doses (10^{-6} M and 10^{-14} M) administered to male rats for 7 days also showed anxiolytic effects in EPM [73]. Koumine has shown this effect on VCT in mice at doses of 0.167, 0.5, or 1.5 mg/kg [74]. Other reports indicate that the decoction of

the African peach root (*Nauclea latifolia*) injected intraperitoneally in mice produces dose-dependent anxiolytic-like effects (16, 40, 80, and 160 mg/kg) in EPM. Its effect has been attributed to isoquinoline-type alkaloids [75], but no reports have yet substantiated this claim. One study reported that the isoquinoline alkaloid berberine hydrochloride has both antipsychotic and anxiolytic properties. In this regard, studies have shown that a dose of 100 mg/kg/day produces anxiolytic effects and can modulate the gratifying effects induced by methamphetamine in rats [76].

The aqueous extract of *Eschscholzia californica* Cham (200 mg/kg, p.o.) has shown anxiolytic-like effects on the LDB test in mice that have been attributed to action on $GABA_A$ receptors [77]. Administration of the aqueous extract of the stem of *Uncaria rhynchophylla* (200 mg/kg), which contains the alkaloid rhynchophylline, in a single dose, or for 7 days, produced an anxiolytic effect in EPM by acting on the $5\text{-}HT_{1A}$ receptor [78]. Another example has been implicated to alkaloids with anxiolytic effects were the hydroethanolic extract of *Davilla rugosa* produced anxiolytic-like effects in EPM when administered to rats at 15 mg/kg [79]. Two other plants that contain alkaloids with anxiolytic effects (erythravine and 11a-hydroxy-eritravine) are *Erythrina velutina and Erythrina mulungu.* A study in mice showed that chronic administration (23–26 days) of the hydroalcoholic extract of the stem of *E. velutina* at 100 mg/kg produced an anxiolytic effect in EPM [80], while acute treatment with 200 mg/kg of *E. mulungu* showed an anxiolytic response in LDB comparable to that of diazepam [81]. Nevertheless, in this study did not identify the content or type of alkaloids in these extracts.

Turning to the species Magnolia (*Magnolia spp.*), we find that at least four anxiolytic components have been identified: honokiol, 4-O-methylhonokiol, magnolol, and obovatol. Administering honokiol (1 mg/kg) for 7 days had an anxiolytic-like effect on mice tested in EPM with results similar to those of diazepam [82]. That treatment increased the activity of the enzyme glutamic acid decarboxylase (GAD-subtype 65) in the hippocampus, but not the cortex of the mice brains. This, in turn, increased the release of GABA and reduced anxiety behavior. GAD65 is located on the terminal nerve and regulates the release of GABA to the synaptic cleft [83]. On this topic, there are reports that GAD65-deficient mice show higher anxiety levels [83]. Administering 4-O-methylhonokiol (0.1, 0.2, and 0.5 mg/kg) to mice in a single dose or during 7 days produced anxiolytic effects in EPM through the benzodiazepine site by binding to the $GABA_A$ receptor [84]. This is similar to observations of obovatol at doses of 0.2, 0.5, and 1 mg/kg [85]. In addition, an increase in the expression of the $GABA_A$ receptor α1 subunit [84] and of the α1 subunit in the amygdala and Cl (−) currents was observed [85]. The diterpene alkaloid songorine has shown anxiolytic effects when male mice were tested on VCT at a dose of 0.25 mg/kg [29], revealing an effect similar to that of phenazepam.

Numerous reports attribute anxiolytic activity to a broad range of plants. However, isolating and identifying the alkaloids responsible for this activity have not advanced substantially. Preclinical reports point to a common mechanism of action that modulates the GABAergic and serotonergic systems. The data described here justifies the need to conduct preclinical and clinical studies using alkaloids as alternative treatments for some anxiety disorders.

8. Sterols with anxiolytic and antidepressant effects

Plants synthesize a class of sterols called phytosterols, whose chemical structure is similar to that of cholesterol. Phytosterols are found in nuts, vegetable oils, cereals, fruits, vegetables, and various plants [86]. Some 40 different types have

been identified, including β-sitosterol, campesterol, fucosterol, and stigmasterol. Due to their lipidic nature and glycosylated forms, they are able to cross the blood-brain barrier and impact the CNS [87]. Trevisan et al. [88] suggest that α-spinasterol has the ability to cross the blood-brain barrier and exert an antagonistic effect on the transient potential receptor V1 (TRPV1). When these receptors are expressed in various areas of the brain—prefrontal cortex, amygdala, hypothalamus, and hippocampus—their activation augments the release of glutamate and, consequently, that of GABA, DA, or other catecholamines [89]. This fact involved TRPV1 receptors in the mechanism underlying the etiology of depression and anxiety. This was corroborated by Socała and Wlaź [90] by administering (1 and 2 mg/kg, i.p.) α-spinasterol to male mice and testing them on FST. Their results suggest an antidepressant effect. Also, intracerebroventricular (i.c.v.) coadministration of 50 μg of the TRPV1 receptor antagonist capsazepine/mouse with an ineffective dose of 0.5 mg/kg, i.p., of α-spinasterol, also reduced immobility time on FST, indicating the involvement of TRPV1 in the neurobiology of depression. However, α-spinasterol itself (0.5, 1, and 2 mg/kg) was unable to produce anxiolytic-like effects in EPM or LDB. In this sense, TRPV1-knockout mice manifested less anxiety behavior on the same tests [91]. Socała and Wlaź [90] proposed that α-spinasterol may be able to activate CB1 receptors with greater affinity because those neurons coexpress these receptors in various brain structures whose activation could activate TRPV1 receptors simultaneously to block their possible anxiolytic effects. In another work, administering fucosterol (10, 20, 30, or 40 mg/kg, v.o.) to male mice produced antidepressant effects on FST and TST, with the 20 and 30 mg/kg doses achieving an effect of comparable efficacy to 20 mg/kg of fluoxetine, a standard dose in humans [92]. Those doses also exerted an acute effect that increased BDNF levels in the hippocampus, a limbic structure involved in mood regulation. Fucosterol also blocked the decrease in 5-HT, 5-HTIIA, and NA levels in mice brains generated by the stress of FST. The effect of fucosterol on that test was similar to that of the positive control drug, but it was unable to prevent the reduction of DA, another factor caused by FST. These findings suggest that the antidepressant mechanism is mediated by increasing monoamines and reducing the rate of 5-HT metabolism. Fucosterol did not modify either motor or exploratory activity and showed no neurotoxic effects [92]. Similar results were found when administering β-sitosterol at 10, 20, and 30 mg/kg for 7 days. In that case, 30 mg/kg exerted effects similar to those of 20 mg/kg of fluoxetine on FST and TST. Finally, the effects on monoamine levels in mice brains confirm that sterols modify the serotonergic and noradrenergic systems but do not impact the dopaminergic system [93].

Another case involved α- and β-amyrin (αβAMY) isolated from the resin of the stem of *Protium heptaphyllum* plants obtained and identified from hexane-ethyl acetate fractions analyzed by TLC. That process produced 450 mg of αβAMY made up of 67% α- and 33% β-amyrin, which were further purified and tested on FST. Administering 2.5 and 5 mg/kg of αβAMY via i.p. or p.o. decreased immobility time, but the most effective treatment was the 2.5-mg/kg dose via the p.o. route. However, the effects produced by this route were similar to those of imipramine at 30 and 10 mg/kg. Imipramine is a tricyclic antidepressant (TCA) that blocks reuptake of both serotonin and norepinephrine. In addition, a pharmacological synergism between 1 or 2.5 mg/kg of αβAMY and 10 mg/kg of imipramine was observed, but not between 2.5 mg/kg of αβAMY and 4 mg/kg of paroxetine (SSRIs). These effects were blocked by pretreatment with 2 mg/kg of reserpine, an inhibitor of the vesicular catecholamine transporter that facilitates vesicular storage. This result suggests a possible mechanism of action through activation of the noradrenergic system [94]. The base structure that cholesterol and sterols share allows the latter to exert actions at the level of the CNS, as in the case of cholesterol. Cholesterol is a vital

substance for neurons because it is required for vesicle transport and neurotransmitter release and as a precursor to neurosteroids. It is also implicated in synaptic plasticity in relation to the formation of new synapses. For these reasons, studying sterols and their mechanisms of action on the CNS is extremely important because of the anxiolytic and/or antidepressant effects they exert.

9. Final comments and conclusion

This broad review constitutes a significant contribution to our understanding of the mechanisms of action that allow plants to produce antidepressant and anxiolytic effects (see **Figure 1** for a summary). However, most of the studies reviewed were conducted with mice, due to the low yields achieved when isolating the metabolites of plant extracts [95], which limit the amount of testing that can be done. Another

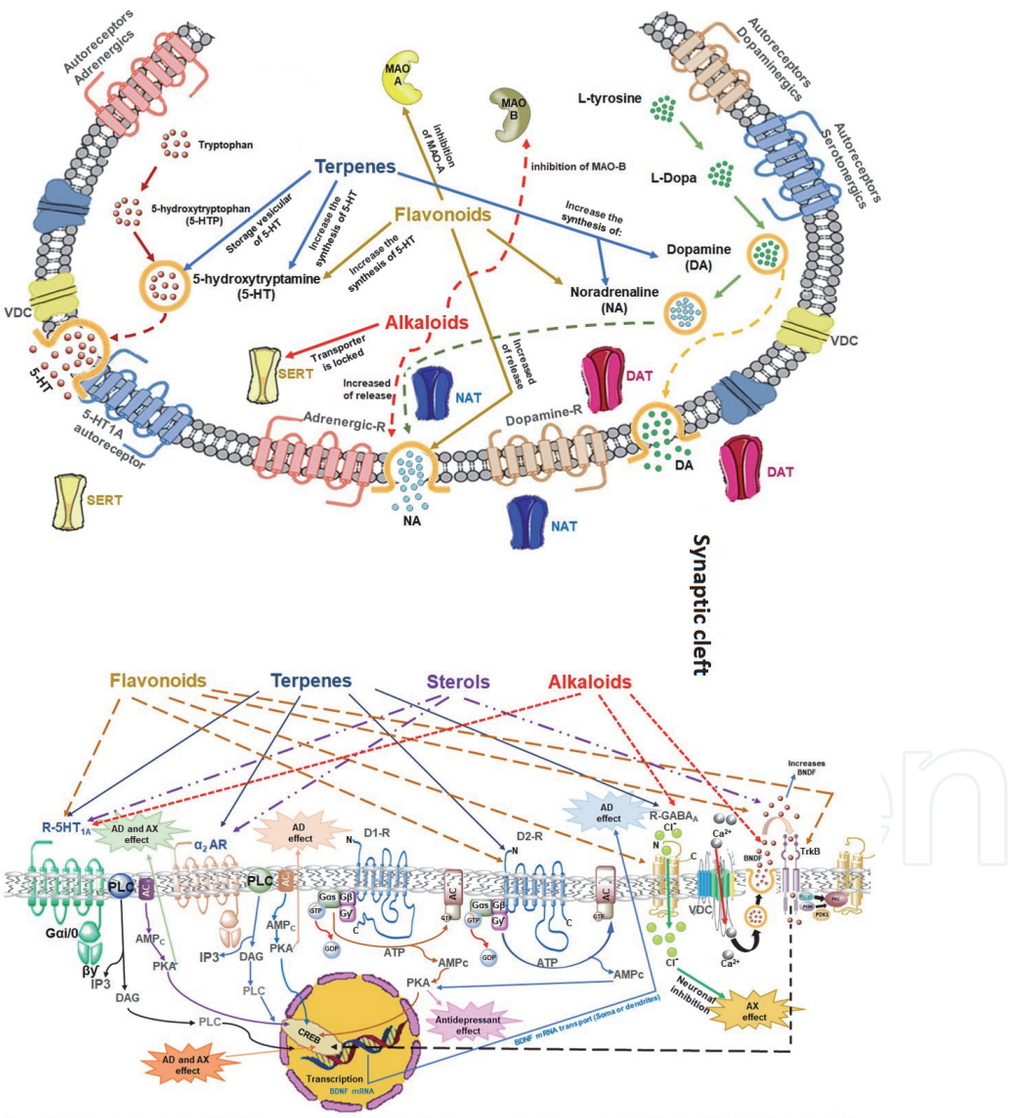


Figure 1.
Principal mechanisms of action of flavonoids, terpenes, sterols, and alkaloids with antidepressant and anxiolytic properties. DAG: diacylglycerol; IP3: inositol triphosphate; AMPC: adenosine monophosphate 3; PKA: protein kinase A; PLC: phospholipase C; AC: adenylyl cyclase; ATP: adenosine triphosphate; GDP: guanosin trifosfato; VDC: canal dependiente de voltaje; BNDF: factor neurotrófico derivado del cerebro; TrkB: tropomyosin receptor kinase B; Ca^{2+}: calcium ion; Cl^{-}: chloride ion; R-5HT$_{1A}$: 5HT$_{1A}$ receptor; α_2AR: alpha 2-adrenergic receptor; D1-R: dopamine receptor D1; D2-R: dopamine receptor D2; DAT: dopamine transporter; NAT: noradrenaline transporter; SERT: serotonin transporter; MAO-A: monoamine oxidase A; MAO-B: monoamine oxidase B; AD: antidepressant; AX: anxiolytic.

concern is that some infusions or extracts used as household remedies lose their antidepressant or anxiolytic effects when fractioned [96]. These findings indicate that in some cases it may be necessary to keep the metabolites together at the concentrations present in the original infusion or extract that has a proven therapeutic effect. As explained herein, some metabolites share pharmacological targets, which explains why they lose their effect when separated and emphasizes the importance of using standardized extracts with demonstrated therapeutic effects in animal and human studies [97, 98]. Unfortunately, very few clinical studies have evaluated the potential antidepressant or anxiolytic effects of isolated metabolites, so a great deal of work remains to be done.

Several observations suggest that active metabolites share the mechanism of action of antidepressant and anxiolytic drugs like SSRIs, SNRIs, MAOIs, DDNRI, and BZDs, but we should emphasize that some metabolites—at least in preclinical studies—produced better effects than conventional drugs, even at lower doses, while others presented a pharmacological synergism between both types at suboptimal doses that improved the effects exerted separately at higher doses. A second shared characteristic is that they contain active stereoisomers and probably, some metabolites, once metabolized, could become more active. This encourages us to consider a significant number of substances with anxiolytic and/or antidepressant pharmacological profiles and invites us to take on the challenge of evaluating their pharmacokinetics, pharmacodynamics, and safety.

In conclusion, terpenes, flavonoids, alkaloids, and sterols share mechanisms of action that include activation of the critical enzyme for catecholamine synthesis (e.g., tyrosine hydroxylase) or the inhibition of their limiting enzymes, MAO-A and MAO-B, and transporters, thus stimulating the vesicular storage monoamine and the release of neurotransmitters toward the synaptic cleft. Finally, they can prevent the production of ROS and inhibit NO synthesis and, further downstream, interact with the 5-HT_{1A}, 5-HT_{2A}, D1, D2, $GABA_A$ receptors, and α1, α2, β-adrenoceptors that contribute to stimulating PKA. One consequence is that CREB increases BDNF levels, which foster the appearance of dendritic contacts that improve cerebral neurotransmission and modulate the emotions.

10. Perspective

This chapter discusses the efficacy of some plant metabolites in treating anxiety and depression disorders, as demonstrated in preclinical studies. In the future, this option for treating such disorders will allow us to reduce treatment costs and moderate the side effects produced by drugs currently in use. However, our review also points out that few clinical studies have focused on the pharmacokinetic and pharmacodynamic processes involving metabolites that would permit the safe use of these extracts. Despite this, research has shown that traditional medicine, especially forms that use medicinal plants that have been passed down through several generations, constitutes an important alternative for health care.

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