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Review Article

Running header: Plant-Based Anxiolytic Psychopharmacology

Plant-Based Medicines for Anxiety Disorders, Part 1

A Review of Preclinical Studies

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Abstract

Research in the area of herbal psychopharmacology has revealed a variety of promising medicines that may provide benefit in the treatment of general anxiety and specific anxiety disorders. However, a comprehensive review of plant-based anxiolytics has been absent to date. This article (part 1) reviews herbal medicines for which only preclinical investigations for anxiolytic activity have been performed. In part 2, we review herbal medicines for which there have been clinical investigations for anxiolytic activity. An open-ended, language-restricted (English) search of MEDLINE (PubMed), CINAHL, Scopus and the Cochrane Library databases was conducted (up to 28 October 2012) using specific search criteria to identify herbal medicines that have been investigated for anxiolytic activity. This search of the literature revealed 1,525 papers, from which 54 herbal medicines were included in the full review (having at least one study using the whole plant extract). Of these plants 21 had human clinical trial evidence (reviewed in part 2), with another 33 having solely preclinical studies (reviewed here in part 1). Preclinical evidence of anxiolytic activity (without human clinical trials) was found for *Albizia julibrissin, Sonchus oleraceus, Uncaria rhynchophylla, Stachys lavandulifolia, Cecropia glazioui, Magnolia* spp.*, Eschscholzia californica, Erythrina* spp.*, Annona* spp.*, Rubus brasiliensis, Apocynum venetum, Nauclea latifolia, Equisetum arvense, Tilia* spp.*, Securidaca longepedunculata, Achillea millefolium, Leea indica, Juncus effusus, Coriandrum sativum, Eurycoma longifolia, Turnera diffusa, Euphorbia hirta, Justicia* spp., *Crocus sativus, Aloysia polystachya, Albies pindrow, Casimiroa edulis, Davilla rugosa, Gastrodia elata, Sphaerathus indicus, Zizyphus jujuba* and *Panax ginseng*. Common mechanisms of action for the majority of botanicals reviewed primarily involve GABA, either via direct receptor binding or ionic channel or cell membrane modulation; GABA transaminase or glutamic acid decarboxylase inhibition; a range of monoaminergic effects; and potential cannabinoid receptor modulation. Future research should focus on conducting human clinical trials on some of these plants with promising anxiolytic activity.

1. Introduction

Anxiety disorders are prevalent and disabling conditions that are often chronic and highly co-morbid (1). While conventional pharmacotherapies and psychological interventions are front-line approaches, plant-based medicines may offer an additional safe and effective option. Phytotherapeutic interventions that may benefit anxiety disorders are classed as 'anxiolytics', and usually have effects on the GABA system (2), either via inducing ionic channel transmission by voltage-gated blockage or through alteration of membrane structures (3), or less commonly via binding with benzodiazepine receptor sites (e.g. GABA-α)(4), GABA transaminase or glutamic acid decarboxylase inhibition (5), or interactions with a range of monoamines.

Over the past few decades clinical trials have been conducted on a range of plant-based medicines for various anxiety and mood disorders, with preclinical research in this area being expansively engaged, in particular by countries such as China, India, Brazil, USA, Spain and Germany. Importantly, preclinical trials often build upon traditional knowledge about the historical use of plant medicines, and inform potential applications for use in humans. Reviews often centre on human clinical trials; however, reviewing both preclinical and clinical data may inform researchers and clinicians to a better degree. By outlining the current evidence from preclinical studies, researchers are provided with direction as to which plant medicines should be studied in phase II and phase III trials, while clinicians may consider the potential for new therapeutic applications (especially if traditional evidence also exists).

While there are general reviews in the area (6-8), there is currently no specialized review comprehensively detailing both preclinical and clinical evidence for an extensive list of psychotropic plant medicines with anxiolytic activity. In this two-part paper we provide an expansive review of herbal preclinical and clinical studies, with the aim of identifying and analysing current evidence concerning herbal medicines studied for anxiolytic activity. In this paper (part 1), we review plant-based medicines for which only preclinical studies have been

performed. In part 2 (9), we provide a comprehensive review of plant-based medicines that have been assessed in human clinical trials.

An open-ended, language-restricted (English) search of MEDLINE (PubMed), CINAHL, Scopus and the Cochrane Library databases was conducted for all available literature up to 28 October 2012, to review preclinical evidence of plant-based medicines with anxiolytic activity. Databases were searched for *in vitro* and *in vivo* data of plant medicines studied specifically for anxiolytic activity. The search terms included 'anxiety' OR 'anxiety disorder' OR 'generalized anxiety disorder' OR 'social phobia' OR 'post traumatic stress disorder' OR 'panic disorder' OR 'agoraphobia' OR 'obsessive compulsive disorder' and were combined with the search terms 'Herb*' OR 'Medicinal Plants' OR 'Botanical Medicine' OR 'Chinese herb*' in addition to 53 individual herbal medicines (both common names and Latin binomial names where appropriate). Papers that met the inclusion criteria included any study that used preclinical methodology assessing an anxiolytic effect. Studies using isolated constituents without additional study of whole plant extracts (except for cannabinol from cannabis and valepotriates from valerian; reviewed in part 2) were omitted from the main results section; in addition, we do not provide an in-depth discussion of traditional Chinese and Kampo formulations. For the preclinical section, studies for inclusion had to have used established animal models, for example, the elevated plus maze (EPM). The term 'significant' was regarded as a *p*-value of <0.05.

2. Anxiolytic Plants with Preclinical Evidence

2.1 Studies Reviewed

Our search of the literature revealed 1,525 papers germane to the area, of which the majority were omitted (not related to anxiety, used only isolated constituents, review paper), leaving a total of 33 plants with solely preclinical evidence (see Table 1) for review in this paper. The dosing regimen of the herbal medicines consisted mainly of a single dose (unless otherwise specified as a chronic dose). The most common animal model used was the EPM, while other behavioural models were less common such as marble-burying, light/dark, anti-conflict,

grooming, open-field, head-dipping and staircase tests. The universally used EPM model consists of two closed and two open arms, with the number of animal entries into the arms being recorded. Increased entry into the open spaces reflects reduced anxiety, and increased entry into closed spaces reflects increased anxiety (due to an animal's innate aversion to open unprotected spaces) (10).

2.2 Individual Plant Medicines

2.2.1 African Peach (*Nauclea latifolia*)

The roots, fruits, bark and leaves of the African peach are used in Western Africa for a range of ailments for its purported 'tonic' and 'aphrodisiac properties', due in part to isoquinoline alkaloids. A decoction of the root bark of the African peach (16, 40, 80 and 160 mg/kg, intraperitoneally [i.p.]) has been evaluated in a mice model (11). Acute administration of the extract was found to dose dependently increase the total sleep time induced by diazepam, in addition to significantly increasing the number of entries and percentage of time spent in the open arms of the EPM, compared with a control, while also significantly reducing anxiety behaviours of rearing and head dipping.

2.2.2 *Aloysia* (*Aloysia polystachya*)

The flowers of *Aloysia* spp. are abundant with volatile oils, and traditionally used in South America as a digestive and relaxant. Mice orally (p.o.) treated with an ethanolic extract of *Aloysia* (1, 1 and 100 mg/kg) spent more time and had an increased number of entries into the open arms of the EPM (12). Flunitrazepam binding to the benzodiazepine binding site was not affected by the extract. Another model found that intraperitoneal administration of *Aloysia* (25 and 50 mg/kg) caused a significant decrease in total motility, locomotion and rearing and grooming behaviour (13). All doses injected (from 1.56 to 50 mg/kg) increased the exploration of the EPM open arms in a similar way to diazepam. It was reported that phenolic compounds thujone and carvone may be responsible for the sedative and anxiolytic activity.

2.2.3 *Annona* (*Annona* spp.)

The fruits and aerial parts of the *Annona* spp. are used in Mexico and South America for therapeutic effects on the nervous system. A hexane extract of the leaves of *Annona cherimolia* (6.25, 12.5, 25 and 50 mg/kg, i.p.) when administered produced anxiolytic activity in mouse avoidance exploratory behaviour and the burying behaviour tests (14). Picrotoxin, a GABA-gated chloride ion channel blocker, antagonized the anxiolysis of the extract, while a sub-effective dose of the selective $GABA_A$ receptor agonist, muscimol, enhanced the effects of *Annona*. In addition, the extract enhanced the duration of sodium pentobarbital-induced sleeping time. Chromatographic separation isolated palmitone and β-sitosterol as major constituents. Another rat study found that repeated administration of injected *A. diversifolia* hexane (100 mg/kg, i.p. or p.o.), ethanol (100 mg/kg, p.o.) leaf extract and isolated palmitone (10 mg/kg, i.p.) exhibited anticonvulsant properties and delayed establishment of a 'kindling state' as observed with diazepam (15). Parmitone (16) and annomontine (17) have been found in other preclinical work to be active constituents of *Annona*.

2.2.4 Asthma Weed (*Euphorbia hirta*)

Asthma weed whole plant is rich in sterols, alkaloids, tannins, phenolic acids and glycosides, and has traditional use across Central America and Southeast Asia for respiratory disorders. Lyophilized aqueous extract of the plant (12.5 and 25 mg/kg, i.p.) has revealed anxiety-like effects on the staircase test and in the light/dark choice situation test in mice (18). A more recent study on rats using a hydroalcoholic extract of asthma weed for 7 days (200 mg/kg, p.o.) showed marked anti-anxiety activity in chronic immobilization stress (19). The researchers also revealed that the plant may provide anxiolysis via $GABA_A$ receptor-benzodiazepine receptor channel modulation, as demonstrated by co-treatment of rats with GABA receptor competitors flumazenil (0.5 mg/kg, i.p.), bicuculline (1 mg/kg, i.p.) and picrotoxin (1 mg/kg, i.p.) diminishing results.

2.2.5 Bandicoot Berry (*Leea indica)*

This plant is native to Indo-Asia and is used in Bangladeshi medicine for nervous and digestive system disorders. The leaves of the plant have been found to contain hydrocarbons, phthalic

acid esters, β-sitosterol and ursolic acid (20). In a mice model, a whole methanol extract of leaves (200 and 400 mg/kg, p.o.) displayed dose-dependent anxiolytic effects (in hole cross and open-field tests), and suppression of motor activity, while prolonging thiopental-induced sleeping time (21).

2.2.6 Brazilian Raspberry (*Rubus brasiliensis*)

Native to Brazil, Brazilian raspberry has been used for a range of therapeutic activities including for nervous system disorders. Preclinical studies by Nogueira and colleagues (22, 23) used an oral application of ethanolic extracts (aqueous, butanolic, hexanic, ethanolic and wax fractions) of Brazilian raspberry (50, 100 and 150 mg/kg) 30 min before behavioural evaluation in mice and rats using the EPM. Both infuse and wax ethanolic fraction at the dosage of 150 mg/kg increased the percentage of time spent in and number of entries in the open arms by the animals. However, the aqueous and butanolic fractions obtained from the ethanolic extract failed to induce an anxiolytic effect. The treatment of mice with flumazenil blocked the anxiolytic effects, indicating that anxiolysis may occur by interaction with GABA receptors. The hexanic fraction at 300 mg/kg was able to induce hypnotic, anticonvulsant and muscle relaxant effects, while decreasing sleep latency, increasing sleeping time and inducing muscle relaxant effects. Interaction of the hexanic fraction from Brazilian raspberry with the $GABA_A$ system was blocked by flumazenil (24).

2.2.7 Californian Poppy (*Eschscholzia californica*)

The Californian poppy is a member of the same family as the opium poppy (*Papaveraceae*) and is native to Western North America. However, unlike the opium poppy, it is non-narcotic and only acts as a mild sedative, due to a chemical composition that consists of a different set of alkaloids (25, 26). Animal research has revealed that the Californian poppy produces anxiolytic and sedative effects. An aqueous extract of the plant showed anxiolytic effects in a familiar environment test in mice at oral doses above 100 mg/kg, in the staircase test at doses above 200 mg/kg and the time spent by animals in the lit box of the light/dark choice test (27). These

effects have been found to be caused by an affinity for GABA receptors, as evidenced by suppression of anxiolytic and sedative effects following pre-treatment with flumazenil (28).

2.2.8 Chinese Cat's Claw (*Uncaria rhynchophylla*)

The stems and aerial parts (primarily the 'hooks') of Chinese cat's claw have been used for a variety of ailments in traditional Asian medicine, including as a 'tonic', and for cardiovascular and nervous system conditions. The genus is rich in alkaloids, with the *Rhynchophylla* spp. containing the alkaloid rhynchophylline, which has been shown to have psychoactive effects (29). In an experiment using the EPM and the hole-board apparatus, it was found that single and repeated treatments (200 mg/kg/day, p.o.) for 7 days of an aqueous extract of the hooks and stem of Chinese cat's claw significantly increased the time mice and rats spent in the open arms (and number of entries) of the EPM over the control, while it also significantly increased the number of head dips in the hole-board apparatus. No alterations of locomotor activity or myorelaxant effects were observed. The anxiolytic effects of the extract found in the EPM were abolished by a serotonin 5-HT_{1A} receptor antagonist, WAY-100635, indicating a serotonergic effect.

2.2.9 Coriander (*Coriandrum sativum*)

While coriander (leaf and root), which is rich in volatile oils, is traditionally incorporated into cooking in many cultures for its fragrant properties, it has been used as a digestive spasmolytic in folk medicine. In Persian folk medicine it is also used for anxiety and insomnia. The aqueous extract (10, 25, 50 and 100 mg/kg, i.p.) at 100 mg/kg revealed an anxiolytic effect in mice exposed to the EPM (30). A recent study using different mice models comparing coriander fruit (50, 100 and 200 mg/kg, i.p.) with diazepam showed an extract of the plant extract at 100 and 200 mg/kg doses produced anxiolytic effects almost equal to diazepam (31).

2.2.10 Damiana (*Turnera diffusa*)

The leaves and aerial parts of damiana have been traditionally used in Southern and Central American cultures as a treatment for mood and anxiety disorders, and as an aphrodisiac. One

preclinical study used several damiana extracts (petroleum ether, chloroform, methanol and water) in mice exposed to the EPM (32). Results revealed that only the methanol extract exhibited significant anti-anxiety effects (at 25 mg/kg, p.o.) over the control, an effect that was comparable to diazepam. The butanol fraction of the methanolic extract of damiana was found to exhibit anxiolytic activity in mice (at 10 and 75 mg/kg, p.o.). The compound apigenin has been found thus far to be a key constituent in the anxiolytic activity of damiana (33).

2.2.11 Davilla (*Davilla rugosa*)

The stems from the climbing shrub davilla are commonly used in Brazilian folk medicine for digestive and CNS conditions, being rich in alkaloids, flavonoids and tannins. A hexane or hydroethanolic extract was given p.o. to rats (7.5, 15, 30 or 60 mg/kg) using the EPM. Results revealed that the percentage of time spent and number of entries in the open arms of the EPM were increased only with 15 mg/kg of the hexane extract (34).

2.2.12 East Indian Globe Thistle (*Sphaeranthus indicus*)

Various plant parts from the East Indian globe thistle, which is rich in sesquiterpene lactones, flavonoids and essential oils, have been used in Ayurvedic medicine for the treatment of a range of diseases, including mental illness (35). An isolated mice study using an injected hydroalcoholic extract of the plant (100, 200 and 500 mg/kg, p.o.) found an increase in the number of entries and the time spent in the open arms of the EPM at a dose of 100 mg/kg (36). However, higher doses (200 and 500 mg/kg, p.o.) decreased open-arm entries and time spent in the open arms of the EPM, while decreasing locomotor activity and protecting against induced convulsions.

2.2.13 *Erythrina* (*Erythrina* spp.)

The roots and bark from various species of the *Erythrina* genus, e.g. *velutina* and *mulungu*, are used in South American cultures as a sedative. The alkaloids are regarded as the bioactive components (although safety/toxicity issues have been noted), with erythravine and 11α-hydroxy-erythravine being found to have anxiolytic effects (37). A preclinical study evaluating

the effect of acute and chronic (23–26 days) oral administrations of the hydroalcoholic stem bark extract of *E. velutina* to mice found that chronic administration of 100 mg/kg of the extract increased the percentage of open-arm entries in the EPM (38). No effect was seen in the forced swim test, nor was an anxiolytic effect seen via acute administration. This reflective result for the EPM and forced swim tests in rats was also found by Ribeiro et al. (39). In a chronic model using a hydroalcoholic extract (100, 200 and 400 mg/kg, p.o.) of *E. velutina*, they revealed an effect comparable to the reference drug diazepam. Onusic et al. (40, 41) evaluated acute and chronic oral treatment of rats with a water-alcohol extract of *E. mulungu* inflorescences (100, 200 and 400 mg/kg) in different anxiety models. Acute treatment with 200 mg/kg displayed an anxiolytic effect comparable to diazepam in respect to decreased avoidance and escapism behaviours and increased exposure to lit areas (41). This effect was not mediated by alterations in motor function. Chronic oral treatment with the extract at differing doses (50, 100 and 200 mg/kg) found a comparable anxiolytic effect to diazepam in the rat model (40).

2.2.14 Horsetail (*Equisetum arvense*)

The aerial parts of horsetail are rich in silica and flavonoids, and have been traditionally used across Europe for urinary and respiratory complaints. One preclinical animal study compared several extractions of horsetail with diazepam and a control (42). Results revealed that an ethanolic extract of horsetail (50 and 100 mg/kg, i.p.) significantly increased the time spent in, and the percentage of open-arm entries by mice in the EPM that was comparable (though not as potent) to diazepam. Ethanolic extract (100 mg/kg, i.p.) of the plant also increased ketamine-induced total sleeping time, while inhibiting locomotor activity.

2.2.15 Gastrodia (*Gastrodia elata*)

The rhizome and roots from gastrodia, a type of orchid, have been used in traditional Chinese medicine (TCM) for hypertension, dizziness and nervous system conditions. An isolated study administered either an oral aqueous gastrodia extract (50, 100, 200 and 400 mg/kg) or an intraperitoneal injection of the phenolic constituents in mice exposed to the EPM (43). Single treatment of gastrodia significantly increased the percentage of time spent and entries into the

open arms of the EPM at the dose of 400 mg/kg only. Both the phenolic constituents 4-hydroxybenzyl alcohol (50 and 100 mg/kg, i.p.) and 4-hyroxybenzaldehyde (100 mg/kg, i.p.) also had the same significant effect over the control. No changes in locomotor activity or myorelaxant effects were noted. The anxiolytic effects of gastrodia extract were blocked by WAY-100635 and flumazenil, indicating serotonergic and GABAergic activity, respectively.

2.2.16 Korean Ginseng (*Panax ginseng*)

The root of Korean ginseng has been used for millennia across Asia for 'tonic' and adaptogenic properties, being held in particular reverence for use in the elderly. While clinical studies have shown cognitive enhancing (44) and adaptogenic effects (45), preclinical models have also revealed anxiolytic effects. In one study, two varieties of Korean ginseng (red and white) were administered p.o. to rats and mice at two doses (20 and 50 mg/kg) twice daily for 5 days, while diazepam was administered acutely (46). Both doses of ginseng were effective in the open-field test and the EPM, and reduced conflict behaviour, with an effect comparable to diazepam. Ginseng also attenuated a pentylenetetrazole-induced decrease in rat brain monoamine oxidase activity. Red ginseng powder (300, 600 and 1200 mg/kg, p.o.), crude saponin fractions and non-saponin fractions (50, 100 and 200 mg/kg, i.p., for each preparation), and pure ginsenoside Rb1, Rg1 and Ro (2.5, 5 and 10 mg/kg, i.p.) significantly increased the frequency and duration of open-arm entries of mice in the EPM (47). Among the three types of pure ginsenoside, only ginsenoside Rb1 significantly increased both the frequency and duration of open-arm entries. Another study also found a similar result using a butanol extraction of steamed raw and heat-processed forms of Korean ginseng (50 and 100 mg/kg). These extracts significantly increased the number of open-arm entries and the time mice spent in the open arm in the EPM, over that of the saline control, and in a manner similar to diazepam (48).

The anxiolytic effects of ginsenosides Rb1, Rg1, Rg3-R, Rg3-S, Rg5 and Rk isolates (and as a mixed formula) were compared with diazepam in a mice model (49). The oral administration of ginsenoside Rb1, ginsenoside Rg1, and the Rg5 and Rk mixture increased number of entries and time spent in the open arms of the EPM. Ginsenoside Rb1, and the Rg5 and Rk mixture

decreased locomotor activity in a manner similar to diazepam. Another study showed that ginsenosides Rg3 and Rh2 extracted from the steamed root of Korean ginseng significantly increased the time mice spent in the open arms and the number of open-arm entries in the EPM (50). The anxiolytic-like activities of Rg3 and Rh2 were antagonized by flumazenil but not by WAY-100635.

2.2.17 Lime Blossom (*Tilia* spp.)

The aerial parts of lime blossom (in particular the flowers) have been used in traditional South and Central American medicine for anxiety and insomnia. The anxiolytic and sedative effects have to date been studied in three preclinical models. An experimental mice model showed that oral administration of the aqueous extracts (10, 30, 100 and 300 mg/kg) potentiated the hypnotic effect of sodium pentobarbital (51, 52). Moreover, a significant attenuation in the anxiety response in the EPM and a diminution in both the head-dipping response and ambulatory activity were observed, resembling the response to diazepam. This reflects an increase in the animal's confidence and a lessening of perceived threat. Another study examining the acute intraperitoneal application of a 100 mg/kg dose of hexane, ethyl acetate and methanol crude Mexican lime blossom extracts, also found the plant produced a significant and dose-dependent sodium pentobarbital-induced hypnotic potentiation, while an anxiolytic effect in the EPM and head-dipping mice models was also observed (53). Tiliroside was the major ingredient from the active fraction in the methanol extract, also consisting of quercetin, quercitrin, kaempherol and their glycosides. Another study compared the flavonoid content and anxiolytic effect of material from three different regions of Mexico. Lime blossom extracts (10–300 mg/kg, i.p.) were administered to mice in experimental models (open-field, hole-board and EPM tests, as well as sodium pentobarbital-induced hypnosis) (54). Although differences were found in flavonoid content between species, the anxiolytic and hypnotic activities were similar, with their effects being attributed in part to the quercetin and kaempferol glycosides.

2.2.18 Luobuma (*Apocynum venetum*)

Luobuma is a plant medicine used in TCM for its calming and mood-elevating effects. A leaf extract of luobuma applied to cultured mouse neuroblastoma N2A cells has been found to inhibit the voltage-gated inward Na(+) current (reversible and concentration-dependent), and to also exert a mild inhibitory effect on voltage-gated K(+) channels (potentially reducing neuronal excitability) (55). Single treatment of an ethanolic extract of luobuma leaves (30 and 125 mg/kg p.o) significantly increased the time mice spent in the open arms of the EPM (56). This effect was comparable to diazepam and the 5-HT_{1A} agonist buspirone, and was antagonized by flumazenil.

2.2.19 Magnolia (*Magnolia* spp.)

The bark of magnolia is used in Oriental traditional medicine for anxiety and mood disorders, being a major ingredient of Kampo formulas Saiboku-to and Hange-koboku. In vitro assays have revealed that whole magnolia extract has an affinity with adenosine and GABA pathways, in addition to exerting minor 5-HT_6 receptor antagonism (57). Crude extract of magnolia (30, 100 and 300 mg/kg, p.o.) has shown in rats significant and dose-dependent diminution in the anxiety response in the EPM, head-dipping and exploratory rearing tests (58). Magnolia also prolonged sodium pentobarbital-induced hypnosis and reduced seizures.

The active anxiolytic constituents of magnolia are considered to date to be honokiol (59), 4-O-methylhonokiol (60), magnolol (61) and obovatol (62). An animal study injected mice for 7 days with honokiol (1 mg/kg, p.o.) versus the same regimen of diazepam, and found an increase in both time spent in the open arms and the number of open-arm entries in the EPM (63). Hippocampal glutamic acid decarboxylase of honokiol-treated mice was significantly increased compared with diazepam-treated groups (63). Single treatment of mice with 4-O-methylhonokiol (0.1, 0.2 and 0.5 mg/kg, p.o.) or oral treatment for 7 days revealed an increase in the percentage of time spent in the open arms and the number of open-arm entries in the EPM, with the anxiolytic effects being abolished by flumazenil (60). The α1-subunit of $GABA_A$ receptors was over-expressed in the cortex of mice brains after treatment. In addition, GABAergic transmission in cultured cortical cells was increased due to enhanced Cl(-) channel

opening. Oral administration of obovatol to mice (0.2, 0.5 and 1 mg/kg) significantly increased the number of open-arm entries and the time spent in the open arm of the EPM, and increased head dipping, compared with the control (62). These effects were comparable to those of diazepam, and were reversed by flumazenil. Obovatol also increased the $GABA_A\,\alpha^1$ expression in the amygdala of mouse brain, and increased Cl(-) influx.

2.2.20 Mimosa (*Albizia julibrissin*)

The bark and cortex of mimosa have been used in TCM and traditional Persian medicine to improve mood and treat insomnia. Mimosa is known as 'shabkhosb' (night sleeper) due to leaves closing during the night. Modern in vitro and in vivo pharmacological studies have revealed a range of effects including antidepressant and anxiolytic activity. Pharmacological rat models have found that constituents from mimosa extract bind to $5\text{-}HT_{1A}$ receptors, with the p.o. administered aqueous extract (200 mg/kg) demonstrating a marked increase of activity in the frontal cortex, hippocampus, lateral septum and dorsal raphe region (64). Another rat model demonstrated that both single and repeated oral treatment of an aqueous extract (100 or 200 mg/kg) significantly increased time spent in open-arm entries of the EPM (65). In addition, the anxiolytic activity of the extract was abolished by pindolol, indicating this effect was meditated in part via the serotonergic system. The flavonoid and triterpenoid saponin constituents are considered to be partly involved in this activity.

2.2.21 Saffron (*Crocus sativus*)

Saffron is native to Western Asia, with its stigma having been used in traditional medicine to treat a range of health conditions, including mood disorders, muscular spasms, pain and menstrual disorders. The active constituents involved in the therapeutic activity of saffron stigma include an estimated 40–50 major constituents. High-quality saffron contains approximately 30 % crocins, 5-15 % picrocrocin and over 5 % volatile compounds including safranal (66).

Preclinical animal models using ethanolic extracts of saffron and its constituents safranal and crocin, have shown antidepressant, anxiolytic and hypnotic effects (67). The antidepressant activity of crocin purportedly acts via re-uptake inhibition of dopamine and noradrenaline (norepinephrine), and the antidepressant activity of safranal is via serotonin reuptake inhibition (66). In vitro, crocin was found to have an affinity for NMDA receptor binding (68). Safranal (145.5 mg/kg, i.p.) has been shown to attenuate the convulsant effects of pentylenetetrazol, being considered to achieve this via $GABA_A$ complex interaction as the effect was abolished with flumazenil (69). Chronic administration of saffron and its active constituent crocin to rats has protected against stress-induced impairment of learning and memory, as well as oxidative stress damage to the hippocampus (70, 71) and from imipramine (72).

While human clinical studies have shown saffron to be an effective antidepressant (7), there are only preclinical studies exploring its anxiolytic effects. Saffron aqueous extract and its constituents, crocin and safranal administered i.p. in mice showed that the whole extract dose dependently reduced the locomotor activity (67). At lower doses, saffron significantly increased the time in the open arms of the EPM. At a dose of 0.56 g/kg, saffron increased the total sleep (increased hypnosis of sodium pentobarbital, 30 mg/kg). Crocin however showed no anxiolytic, hypnotic or myorelaxation effects, while safranal in higher doses (0.15 and 0.35 ml/kg), revealed anxiolytic effects. Safranal also increased the total sleep time dose dependently, and demonstrated no effects on motor coordination. In another animal model using injected crocin (50 mg/kg, i.p.) versus diazepam (1.5 mg/kg, i.p.), both treatments significantly prolonged the time rats spent in the lit chamber of the light/dark test (73). This reveals an anxiolytic effect, as the animals spent increased time in a more exposed area where under natural conditions they would potentially be more vulnerable to predators. Lower doses of crocins (15–30 mg/kg, i.p.) however did not have anxiolytic effects.

2.2.22 Soft Rush (*Juncus effusus*)

Preclinical studies have shown that the pith of soft rush, a native of Western North and Central America, is rich in phenanthrenes (74), and possesses anxiolytic effects that are in part

modulated by GABAergic pathways. Injected dehydroeffusol (2.5 and 5 mg/kg, i.p.), a phenanthrene, has revealed anxiolytic effects in the EPM, and significantly increased the head dips of mice in the hole-board test in a dose-dependent manner (75). No inhibitory effect on motor function was found. Effusol and dehydroeffusol were shown in an in vitro study to enhance GABA-induced chloride currents (independent of the benzodiazepine binding site) (76).

2.2.23 Sour Date (*Zizyphus jujuba*)

The seeds from sour date are used in TCM as an anxiolytic and hypnotic, and are prescribed in the formula Suanzaoren for anxiety and tension, or for sleep disorders. The jujubosides are regarded as active constituents in sour date, displaying inhibition of glutamate-mediated pathways in the hippocampus in an in vivo model (77). An isolated mice study using p.o. administered sour date seed (between 0.5 and 2.0 g/kg) found the extract to increase the percentage of time spent in and the percentage of arm entries into the open arms of the EPM, while decreasing the percentage of time spent in and the percentage of arm entries into the closed arms (78). In this study, at the dosage of 1.0 g/kg, sour date seed prolonged hexobarbital-induced sleeping time in mice, and decreased the locomotor activity in rats.

2.2.24 Sow Thistle (*Sonchus oleraceus*)

The leaves and aerial parts of sow thistle, which is native to Asia and Europe, are rich in nutrients and polyphenols and are considered to have similar therapeutic qualities to the herb dandelion, another member of the Asteracea family. A mouse study was conducted to establish the anxiolytic effect of hydroethanolic and dichloromethane extracts (30 and 300 mg/kg, p.o.) from the aerial parts of sow thistle in the EPM and open-field tests (79). Results showed that both extracts significantly increased the percentage of open-arm entries and time spent in the open-arm portions of the maze, providing a similar anxiolytic effect to clonazepam.

2.2.25 Tilo (*Justicia* spp.)

The leaves of tilo have been used traditionally in the Americas as an antispasmodic and anti-inflammatory. A standardized aqueous tilo extract administered intragastrically in an acute mice model (50, 100 and 200 mg/kg) found similar effects to diazepam in the EPM, without altering the general motor activity (80). Flumazenil reversed anxiolytic effects of both diazepam and tilo. Elenoside derived from *Justicia hyssopifolia* and injected (25 and 50 mg/kg, i.p.) in rats reduced locomotor activity (Varimex test), muscular relaxant activity and permanence time on muscular relaxant activity (traction test), while open-field test, ambulation and rearing were reduced compared with the control group (81). It should be noted however that elenoside is cytotoxic.

2.2.26 Tongkat Ali (*Eurycoma longifolia*)

Tongkat ali, a shrub-tree native to South-East Asia, has come to prominence in recent years, with root extracts being used as an erectile and ergogenic aid in men, with animal models finding it to exert 'aphrodisiac' properties. The anxiolytic effect of p.o. administered tongkat ali (0.3 g/kg, three times daily for 5 days) was found to significantly increase the number of entries and time mice spent in the open arms of the EMP (82). Furthermore, a range of injected fractions of the plant significantly decreased the fighting episodes in mice when compared with control animals. In addition, results were found to be consistent with an anxiolytic effect produced by diazepam.

2.2.27 Violet Tree (*Securidaca longepedunculata*)

The roots of violet tree are used in parts of Africa for a range of conditions, including for the belief it improves sexual function. One animal study used an aqueous root extract (100, 200 and 400 mg/kg, p.o.) in mice using the strychnine- and picrotoxin-induced seizure models (83). Results revealed that the extract (100–400 mg/kg, p.o.) produced a significant dose-dependent increase in onset of convulsion, while increasing the prolongation of the cumulative time spent in the open arms of the EPM compared with the control. The extract also significantly reduced the time of onset of sleep induced by hexobarbitone, with the result being comparable to that

produced by diazepam. At doses of 100–400 mg/kg, the violet tree extract produced a dose-dependent decrease in exploratory activity of the mice (83).

2.2.28 Western Himalayan Fir (*Abies pindrow*)

The leaves of Western Himalayan fir are used in traditional Ayurvedic medicine for a range of ailments. Initial preclinical pilot studies showed that single-dose administration of extract had little to no acute behavioural effects; however, when an ethanolic extract (50 and 100 mg/kg, p.o.) was injected in mice once daily for 3 consecutive days, the extract induced a significant increase in entries and time spent in the open arms of the EPM, in addition to a slight decrease in anxiety behaviours (head dips and stretched attend postures) (84). However, the effects induced by the ethanolic extract were less potent than the positive control lorazepam.

2.2.29 White Sapote (*Casimiroa edulis*)

The leaves and fruit of white sapote have been used in traditional Central American medicine, and contain histaminergic compounds that may be of value in CNS disorders. In an animal model, a hydroalcoholic extract of the leaves (doses 1.56, 3.12 and 12.5 mg/kg, i.p.) administered to rats caused a reduction in locomotion and increased the exploration of the EPM open arms in a similar way to diazepam (85). However, a reduced effect was seen in mice, with only one oral dose (160 mg/kg) demonstrating a small but significant percentage increase of entries into the open arms. Further, the extract prolonged pentobarbital-induced hypnosis. Another study demonstrated that white sapote extract (25 and 35 mg/kg, i.p.) was similar to diazepam in increasing open-arms exploration by rats in the EPM over the control (86), with higher doses (45 and 55 mg/kg) showing a reduction in exploration.

2.2.30 Wood Betony (*Stachys lavandulifolia*)

The flowers and aerial parts of wood betony, which are rich in volatile oils and flavonoids, have folkloric use in Western Asia for headaches, and as a relaxing sedative for a range of nervous

system conditions. One EPM model showed that wood betony extract, or its isolated essential oil, administered to mice i.p. (100 mg/kg) increased the percentage of time spent in and the percentage of entries into the open arms (87). The extract also prolonged ketamine-induced sleeping time, while also decreasing the locomotor activity in mice. No significant effect was found for the extract at doses lower than 100 mg/kg on any outcome (87).

2.2.31 Yarrow (*Achillea millefolium*)

The aerial parts of yarrow have been used in Eurasian and American folk medicine for gastrointestinal, respiratory and skin conditions. In an isolated preclinical study, a hydroalcoholic extract from the aerial parts of the plant (88) was administered in mice that underwent tests in a range of anxiety models. Yarrow (at oral doses of 300 and 600 mg/kg) was found to exert anxiolysis in the EPM and a marble-burying test after both acute and chronic (25 days) use that was partially blocked by flumazenil (though not altered by picrotoxin pre-treatment). Flunitrazepam binding was unaffected by treatment, indicating that these effects were not mediated by GABA receptor interaction. Current mechanistic evidence has revealed antioxidant and anti-inflammatory effects, which are posited to occur via the plants flavonoids, sesquiterpene lactones and dicaffeoylquinic acids (89).

2.2.32 Yarumo (*Cecropia glazioui*)

The leaves of yarumo are used in South American countries for cardiovascular and respiratory conditions. In a preclinical study, mice were treated with an aqueous extract of yarumo (0.25–1 g/kg, p.o.) acutely or repeatedly (24, 7 and 1.5 h before the test). It was found that after repeated administration of the extract, the frequency of entries into the open arms of the EPM increased (90). A similar profile of action was observed after administration of the butanolic fraction, but not with the aqueous fraction. The main active constituents are regarded as being the flavonoids and terpenes, with a biochemical analysis of the hippocampal neurotransmitters showing a significant increase in monoamine levels, with an inhibition of the reuptake of serotonin, dopamine and noradrenaline by synaptosomes of different brain regions (91).

3 Conclusions

In summary, our review of preclinical studies revealed a total of 32 plant medicines researched using at least one established anxiolytic animal model. While the preclinical studies detailed above reveal many plants with encouraging in vitro or in vivo evidence, none of these have been studied in humans to treat general anxiety or any specific anxiety disorder. This remains an area of potential future interest. The literature revealed that the most commonly reported mechanism of action was via modulation of the GABAergic pathway. However, aside from exploration of this pathway and occasional study of potential serotonergic effects, many other neurological pathways were not studied for the majority of plants, and in many cases there is an absence of any mechanistic data.

While many plants reviewed revealed anxiolytic effects preclinically, it should be highlighted that these have not yet been studied in any human trial as a potential treatment for an anxiety disorder. The preclinical evidence is encouraging; however, it is important to consider how these results transfer to humans. The in vitro mechanistic effects cannot always be extrapolated to clinical efficacy in humans, as plant constituents undergo significant metabolism via enzymatic and hepatic processes, being biotransformed into new metabolites. In addition, while many animal models such as the EPM are considered a sound model for anxiety, these results may not translate to human experience, especially in cases of clinical anxiety. However, there are many plant medicines with evolving preclinical evidence of anxiolytic activity that can be studied in humans. Some of these medicines with a growing body of evidence include *Eschscholzia californica, Tilia* spp.*, Albizia julibrissin, Erythrina* spp.*, Magnolia* spp. and *Zizyphus jujuba*. It is advisable to base human clinical studies of herbal medicine interventions on a combination of in vitro*,* in vivo and traditional knowledge, to develop replicable standardized monotherapies and formulations. However, a counter consideration is that as preclinical data for many of these plant medicines is sparse, judgement for which constituents to standardize for is currently restricted.

As detailed in this review, preclinical data concerning plant-based anxiolytics is encouraging, and justifies further exploration in rigorous human clinical trials and the potential application for

use in treating a range of anxiety disorders. Part 2 of this review will cover 21 plant-based medicines with human clinical trials.

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Table 1 Anxiolytic plant medicines: preclinical studies

Botanical name	Common name	Active constituents[a]	Neurochemical pathways[b]	Animal model[c]	References[d]
Achillea millefolium	Yarrow	Flavonoids, sesquiterpene lactones, sicaffeoylquinic acids	Unknown	Marble-burying test, EPM	(88, 89)
Aloysia polystachya	Aloysia	Thujone, carvone	GABA	EPM, rearing and grooming tests	(12, 13)
Albies pindrow	Western Himalayan fir	Terpenoids, flavonoids, glycosides	Unknown	EPM, head-dipping tests	(84)
Albizia julibrissin	Mimosa	Flavonoids, triterpenoid saponins	Serotonin 5-HT_{1A}	EPM	(64, 65)
Annona spp.	Annona	Palmitone, annomontine, β-sitosterol	GABA, 5-HT, DA	Open-field test, marble-burying test	(14-17)
Apocynum venetum	Luobuma	Flavonoids	GABA, 5-HT	EPM	(56, 92)
Casimiroa edulis	White sapote	Histaminergic compounds	Unknown	EPM	(85, 86)
Cecropia glazioui	Yarumo	Flavanoids, terpenes	5-HT, NE, DA	EPM	(90, 91)
Coriandrum sativum	Coriander	Volatile oils, flavonoids	Unknown	EPM	(30, 31)
Crocus sativus	Saffron	Safranal, crocins, picrocrocin	5-HT, NE, DA, GLU, GABA	EPM, light/dark test	(66, 67, 69, 70, 73)
Davilla rugosa	Davilla	Flavonoids	Unknown	EPM	(34)
Equisetum arvense	Horsetail	Silica, flavonoids	Unknown	EPM	(42)
Erythrina spp.	Velutina, mulungu	Erythravine alkaloids	Unknown	EPM, elevated T-maze	(37-41)

Eschscholzia californica	Californian poppy	Benzophenanthridine alkaloids	GABA	Familiar environment, staircase and light/dark tests	(25, 28, 93)
Eurycoma longifolia	Tongkat ali	Alkaloids	Unknown	EPM, anti-conflict tests	(82)
Euphorbia hirta	Asthma weed	Alkaloids, phenolics	GABA	Staircase test, light/dark choice test	(18, 19)
Gastrodia elata	Gastrodia	Phenolics (4-hydroxy benzaldehyde)	GABA, 5-HT	EPM	(43)
Juncus effusus	Soft rush	Polyphenols, phenanthrenes, dehydroeffusol	GABA	EPM, hole-board and head-dipping tests	(79, 94)
Justicia spp.	Tilo	Elenoside	GABA	EPM, open-field, rearing and ambulation tests	(80, 81)
Leea indica	Bandicoot berry	Triterpenoid glycosides, hydrocarbons, ursolic acid	Unknown	Hole cross and open-field tests	(20, 21)
Magnolia spp.	Magnolia (Saiboku-to, Hange-koboku)	Honokiol, 4-O-methylhonokiol, magnolol, obovatol	GABA	EPM, head-dipping and exploratory tests	(58, 60-63, 95)
Nauclea latifolia	African peach	Isoquinoline alkaloids	Unknown	EPM, head-dipping test	(11)
Panax ginseng	Korean ginseng	Triterpenoid saponins (ginsenosides)	Monoamines, HPA-axis, BDNF	Open-field test, EPM, reduced conflict behaviour test	(96)
Rubus brasiliensis	Brazilian raspberry	Tannins, flavonoids	GABA	EPM	(22-24)
Securidaca longepedunculata	Violet tree	Xanthones	Unknown	EPM	(83)

Sonchus oleraceus	Sow thistle	Polyphenols	Unknown	EPM	(79)
Sphaerathus indicus	East Indian globe thistle	Sequiterpene lactones, flavonoids, essential oils	Unknown	EPM	(35, 36)
Stachys lavandulifolia	Wood betony	Flavonoids, terpenoids, essential oils	Unknown	EPM	(97, 98)
Tilia spp.	Lime blossom	Tiliroside: quercetin and kaempferol glycosides	Unknown	EPM, open-field and hole-board tests	(52-54)
Turnera diffusa	Damiana	Flavonoids (apigenin), essential oils	GABA	EPM	(32, 33)
Uncaria rhynchophylla	Chinese cat's claw	Rhynchophylline alkaloid	5-HT	EPM	(29)
Valeriana spp.	Valerian	Valepotriates: valerenic acid, didrovaltrate, valtrate and acevaltrate	GABA	EPM	(99-103)
Zizyphus jujuba	Sour date	Jujubosides, spinosin	GABA, DA, 5-HT, GLU	EPM	(77, 78, 104)

a Constituents to date found to have relevant biological activity
b Involved in the mechanism of action
c Anxiety-specific tests
d Main studies
5-HT 5-hydroxytryptophan, *ACh* acetyl cholinesterase, *BDNF* brain-derived neurotropic factor, *DA* dopamine, *EPM* elevated plus maze, *GLU* glutamate, *HPA* hypothalmic pituitary adrenal, *MAO* monoamine oxidase, *NE* noradrenaline (norepinephrine)