Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 1538-1548
Received: 16-01-2018
Accepted: 18-02-2018

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Neuropharmacological potential of the genus *Citrus*: A review

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Abstract
The genus *Citrus* (family: Rutaceae), comprising of evergreen aromatic shrubs and small trees, is cultivated throughout the tropical and temperate regions of the world. *Citrus* fruits, flowers and leaves are store house of secondary metabolites like essential oils, flavonoids, limonoids, carotenoids, coumarins, glucarates, anthocyanins and phenolic acids. *Citrus* species have been attributed with a wide range of biological activities such as antioxidant, anticancer, anti-inflammatory, hypolipidemic, antihypertensive, antiatherosclerotic, antithrombotic, antiulcer, antiallergy and antimicrobial activities. *Citrus* species have been explored for their central nervous system related activities by different groups of researchers. The present review summarizes the ethno-medicinal uses of genus *Citrus* in relation to mental health disorders as reported in traditional medicinal systems and aromatherapy. The scientific literature, till the February 2018, has been searched thoroughly in order to examine available evidence on various neurobiological activities (like anxiolytic, sedative, antidepressant, anticonvulsant, memory enhancing and neuroprotective) of extracts/constituents of different *Citrus* species.

Keywords: *Citrus*, ethno-medicinal, aromatherapy, neurological

Introduction
Mental and neurological disorders have emerged as one of the leading global health concerns during the 21st century affecting one in four people at some point in their lives [1]. Anxiety and mood disorders, often showing co-morbid pattern, are the most prevalent mental disorders associated with immense psychological, social and economic burden and increased risk of physical illness [2, 3, 4]. Neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD), represent the fourth highest source of overall disease burden in the high-income countries. These are chronic and progressive disorders associated with severe functional debility in terms of cognitive dysfunction, dementia and loss of motor coordination [1, 5, 6]. Conventional pharmacotherapy for PD is based on dopamine replacement strategies that provide short term symptomatic relief without improving cognitive dysfunction and halting the progression of the disease [7, 8]. Also, therapeutic agents for AD have failed to modify underlying disease pathology and have numerous adverse effects ranging from psychomotor impairment to severe organ toxicities [9]. Drug therapy for anxiety and mood disorders comprises of beta blockers, benzodiazepines, monoamine oxidase inhibitors, tricyclic antidepressants and selective serotonin reuptake inhibitors. Common limitations of these drugs include occurrence of co-morbid psychiatric disorders, cases of acute poisoning, drug interactions, physical dependence and tolerance leading to intolerable side effects [10, 11, 12]. Development of novel therapeutic interventions that can prevent, delay or modify the course of these neurodegenerative and neuropsychiatric disorders with minimum side effects, is the need of the hour. The present scenario has prompted scientists to explore plants which are commonly employed in traditional and alternative systems of medicine for neurological disorders with a view to find cheap, effective and safer drugs [13, 14, 15, 16]. Plants are one of the greatest healers gifted by nature to mankind. All the indigenous systems of medicine like Ayurveda, traditional Chinese medicine and folk medicinal systems of various tribes and cultures largely make use of plants in one form or another. This ethno-medicinal treasure created by experiences and efforts of generations has constantly provided a healing touch to human civilization and has served as a great source for discovery of numerous currently prescribed drugs [17]. One invaluable gem of this treasure is genus *Citrus* whose presence can be felt in folklore of Chinese, Greek and Roman cultures as ornamental tree, delicious fruit, flavouring and medicinal agent, ingredient of ceremonies and rituals, and above all as symbol of happiness and prosperity.

The genus *Citrus* belongs to family Rutaceae (sub-family: Aurantioideae) comprising 16 species, numerous cross-fertile natural and horticultural varieties of evergreen aromatic shrubs

and small trees native to South-East Asia and China. It is cultivated throughout the tropical and temperate regions, between approximately 40°N and 40°S, for the fruit. The northern boundary encompasses (west to east) California, Florida, Spain, Italy, Turkey, Iran, India, China and Japan. On the southern limit are Argentina, Brazil, South Africa, Australia (east and south coast), and the northern island of New Zealand [18, 19]. Citrus fruits contain a range of key nutrients such as vitamin C, vitamin A, folate, potassium, selenium and dietary fiber. Citrus fruits, flowers and leaves are a store house of secondary metabolites like flavonoids (hesperidin, naringin, narirutin, nobiletin, tangeretin), essential oil (limonene, linalool), limonoids (limonin, nomilin), alkaloids (synephrine), carotenoids, coumarins, glucarates, anthocyanins and phenolic acids [20, 21, 22, 23, 24]. A lot of research has been done on *Citrus* species due to presence of these phytochemicals during last two decades and have been attributed with a wide range of biological activities such as antioxidant, anticancer, antiinflammatory, hypolipidemic, antihypertensive, antiatherosclerotic, antithrombotic, antiulcer, antiallergy and antimicrobial activities [20, 25, 26, 27, 28, 29]. In addition to this, *Citrus* species have been explored for their central nervous system (CNS) related activities by different groups of researchers and quite promising results have been obtained. However, any review study regarding therapeutic potential of genus *Citrus* for management of neurological disorders has not yet been published.

The present review has been carried out with an aim to enlist all the ethno-medicinal uses of genus *Citrus* in relation to mental health disorders as reported in various traditional medicinal systems and aromatherapy. Secondly, it has been tried to locate the *Citrus* species which have been scientifically evaluated for various neurobiological activities like anxiolytic, sedative, antidepressant, anticonvulsant, memory enhancing and neuroprotective activities in order to validate the traditional claims by examining scientific literature. Also, an attempt has been made to critically review the level of success achieved by reported experimental studies and identify the research gaps that need to be filled so that neuropharmacological potential of these *Citrus* species can be properly utilised and put in the service of mankind.

Review of Literature

Ethno medicinal uses of Citrus for CNS disorders

Earliest evidence of *Citrus* orchards dates back to 2200 BC in China. The beauty and fragrance of *Citrus* attracted the attention of traders, travellers and invaders that led to migration of these plants from South East Asia to the Middle East, eventually to European countries and later on America. Over time, *Citrus* became an integral part of cuisine, religious ceremonies, perfumery and folk medicine of all these regions [18, 30]. Citrus fruit and its juice, peel oil, leaves and flowers are reported to be used as folk remedies for central nervous system disorders in various indigenous medicinal systems as mentioned below:

Fruit

Fruit peel and juice of *C. aurantifolia* is used as tranquiliser and anxiolytic in Chinese, Brazilian and Mexican folk medicine [31, 32]. Peel and juice of *C. aurantium* is used as a folk remedy for anxiety, emotional shock, insomnia and seizures in tropical regions of Africa and Latin America [33, 34, 35]. Peel oil of *C. aurantium* var. *bergamia* administered in drops on a sugar lump is a popular remedy for insomnia in European countries [36]. Peel of *C. limon* and *C. maxima* is used as sedative in nervous affections throughout Mediterranean region [37, 38]. Lemon juice is considered a neurotonic [39]. Pulp of *C. medica* is used as pulmonary sedative [37]. Fruit peel of *C. macroptera* is used to treat anxiety and depression in Assam [40]. Peel of *C. reticulata* is a popular ingredient of many formulations used to treat psychological disorders in Chinese-Japanese traditional medicine [36]. Peel extract and pulp of *C. sinensis* is popularly used as sedative and antianxiety agent in Arabian Peninsula and its juice along with honey is a remedy for nervousness and anxiety in Ayurveda [41, 42].

Leaves

Pounded leaves of *C. aurantifolia* are used for headache in Malay and leaf infusion is used as tranquilizer in Yucatan [32]. Leaf infusion of *C. aurantium* is a remedy for anxiety, epilepsy and nervous disturbance in Iranian folk medicine and is used as sedative by Afro-brazilians [43, 44]. Leaves of *C. limetta* are used to improve mood and sadness by Latin American tribes [31]. Leaves of *C. maxima* are used as sedative for nervous affections as well as antiepileptic in southeast Asia and Philippines [45, 46]. Boiled leaves of *C. paradisi* are also used as sedative in Carribean [47]. Leaf extracts of *C. sinensis* are used for neurological disorders by tribes of Easter Island in the South Pacific while leaf infusion is used as sedative by various Brazilian tribes like Cabaclos and Quilombolas [48, 49].

Flowers

Also, flowers of *C. aurantifolia* are used for anxiety and nervousness in Mexican Traditional Medicine [31]. An infusion of dried flowers of *C. aurantium* is used as nerve sedative in Africa and Latin America while aqueous extract of flowers is used in nervous affections in Oriental Medicine [31, 32, 47]. Aromatic waters of *C. maxima* and *C. reticulata* are used to relieve stress and insomnia [38, 50]. A decoction made from flowers of *C. sinensis* is used as sedative in China, Italy, France and Mexican Traditional Medicine [32, 51, 52].

Citrus oils

Aromatherapy is one of a very sophisticated traditional practices which utilizes plant based essential oils to balance mind, body and spirit by means of inhalation and dermal application. Nowadays, it is acknowledged that aromas as well as single fragrance compounds possess pharmacological and/or psychological properties and in many cases the combination of both plays an important role [53]. Essential oils from *Citrus* species occupy a prestigious status in aromatherapy for their central nervous system related activity since ages. *Citrus* oils are used for relieving depression and anxiety, improving cognition, reducing pain and stress, relaxing, sedating or stimulating, and restoring both physical and emotional well-being (Table 1).

Table 1: Citrus oils used for mind related disorders in aromatherapy

Plant Source	Name of oil	Properties in aromatherapy	Recommended Use
Peel of *C. aurantium* var. *amara*	Bitter orange oil	Sharp, detoxifying, refreshing, relaxing [54, 55]	Anxiety, insomnia [45, 58]
Peel of *C. aurantium* var. *bergamia*	Bergamot oil	Calming, refreshing, relaxing, joyous [54, 56]	Stress, Anger, Anxiety, Depression [48]
Peel of *C. limon*	Lemon oil	Cheerful, uplifting, increases concentration and awareness, helps to get rid of mental fatigue [54, 55]	Stress, Anxiety, Depression [53, 56]
Peel of *C. nobilis*	Tangerine oil	Calming, soothing, relaxing [57]	Anxiety, insomnia [57]
Peel of *C. limon*	Lemon oil	Cheerful, uplifting, increases concentration and awareness, helps to get rid of mental fatigue [54, 55]	Stress, Anxiety, Depression [53, 56]
Peel of *C. paradisi*	Grapefruit oil	Bright, crisp, refreshing, cheering, energizing, helps release resentment and bottled emotions, uplifting [54]	Bipolar disorder, anxiety, sadness, nervous exhaustion, insomnia [58, 47]
Peel of *C. reticulata*	Mandarin oil	Joyous, uplifting, soothing and calming [56]	Anxiety, insomnia of nervous origin, seasonal affective disorder [56, 57]
Flowers of *C. sinensis*	Neroli oil	Soothing, rejuvenative, calming, imparts peace and purity [55]	Depression, insomnia [56]
Peel of *C. sinensis*	Sweet orange oil	Refreshing, energizing, comforting, relaxing, uplifting joyous, sunny, generous [54]	Anxiety, nervousness, depression [58]

Scientific evidence of effect of Citrus on central nervous system

In past two decades, several studies have been conducted by researchers to scientifically explore the neuropharmacological potential of *Citrus* species as claimed by folk medicine of different cultures. Fruit peel extracts, juice, essential oil, flowers as well as leaves of several *Citrus* species have been evaluated for anxiolytic, sedative, antistress, antidepressant, anticonvulsant and neuroprotective activities using different animal models. Most of the studies have provided promising results in relation to CNS effects of *Citrus* and some of them have attempted to locate the chemical constituents and possible mechanism of action responsible for the corresponding activity. A detailed account is given below:

Anxiolytic, sedative and antistress Activity

***C. aurantium* (Sour Orange):** Essential oil (EO) of peel of *C. aurantium* showed sedative activity at dose 1.0 g/kg as it prolonged pentobarbital sleeping time (PST) in mice after acute oral administration. Essential oil was also found to have significant anxiolytic activity comparable to diazepam (DZP) using elevated plus maze (EPM) in mice at dose 1.0 g/kg after acute oral administration without interfering with motor activity and muscle relaxation. In the same study, hexane and dichloromethane fractions of hydroethanolic (70% w/v) extract of leaves at dose 1.0 g/kg, showed sedative activity in PST test in mice after acute oral administration but failed to show anxiolytic activity [42]. Aqueous flower extract of *C. aurantium* was found to significantly prolong PST comparable to DZP after intraperitoneal (i.p) administration in rats [59]. In another study, anxiolytic activity of EO was evaluated using the light–dark (LD) test and the marble-burying (MB) test, respectively related to generalized anxiety disorder and to obsessive compulsive disorder. Single dose of 0.5 and 1.0 g/kg as well as repeated administration of same dose for 15 days by oral route was able to suppress marble-burying behaviour comparable to DZP without any motor deficit showing anxiolytic activity. The principal constituents of EO were found to be limonene (97.83%) and myrcene (1.43%) by GC-MS analysis [60]. Inhalation of peel EO (limonene-96.24%) vapours at concentration of 2.5% increased both the time spent in the open arms of the EPM and the time of active social interaction in the open-field being longer than that of the diazepam group in rats confirming anxiolytic activity [61]. Inhalation of neroli oil (distillate obtained from flowers) at concentration 100µl also showed anxiolytic activity comparable to alprazolam when evaluated using open field test (OFT) and forced swimming test (FST) in gerbils validating its use in aromatherapy [62].

Again, Costa and co-workers proved anxiolytic activity of peel EO using LD box method after acute (5 mg/kg) and repeated administration (1 mg/kg/day for 14 days) in mice. Flumazenil, a competitive antagonist of benzodiazepine binding, and the selective 5-HT1A receptor antagonist WAY100635 were used in the experimental procedures to determine the mechanism of action of the EO. The serotonergic system through 5HT1A receptor was proposed to be involved in anxiolytic effect. The main constituents of EO were found to be limonene (98.66%), β-pinene (0.41%) and β-myrcene (0.53%) by GC-MS analysis [63].

***C. bergamia* (Bergamot):** The effect of EO of peel on the release of amino acid neurotransmitters in rat hippocampus was studied by in vivo microdialysis and by in vitro superfusion of isolated nerve terminals. EO at dose 100 µl/kg, via i.p. route significantly elevated the extracellular concentration of aspartate, glycine and taurine in a Ca^{2+} dependent manner. Also focally injected 1:1 diluted essential oil preferentially caused extracellular increase of glutamate that was strictly Ca^{2+} dependent. These effects were attributed to presence of monoterpene hydrocarbons in bergamot oil [64]. In a further study, EO was found to cause a dose-related sequence of sedative and stimulatory behavioural effects accompanied by increased energy in discrete frequency bands of the EEG spectrum when administered intraperitoneally at doses 100, 250 and 500 µl/kg, in rats. The fast fourier transformation technique was used for analysis of the energy in the total as well in single frequency bands of the EEG spectrum recorded via deep electrodes from discrete regions of the brain. Increase in locomotor activity and exploratory behaviour correlating with a predominant increase in the faster frequency bands recorded from both the hippocampus and the cortex have been reported following treatment with higher doses of oil. These effects were attributed to components of the volatile fraction of the EO other than bergapten [65]. Later on, bergamot peel EO was evaluated for anxiolytic activity using EPM and hole board (HB) test in rats. Acute inhalation of EO at concentration 2.5% w/w showed significant anxiolytic activity comparable to DZP in both the tests without altering motor activity. Also there was a

reduction in stress-induced levels of plasma corticosterone suggesting attenuated hypothalamic-pituitary-adrenal (HPA) activity [66]. In a randomized crossover study, 41 healthy young adult women were given bergamot volatile oil-saturated water vapor to inhale, while their salivary cortisol level was measured repeatedly. Both heart rate variability as an indicator for the activity of the autonomous nervous system and emotional state were monitored. Salivary cortisol level was significantly reduced and the high frequency component of the heart rate variability was significantly increased. These results demonstrated that bergamot oil inhaled together with water vapour exerted psychological and physiological effect leading to stress reduction in a relatively short time. The GC-MS analysis revealed EO to be composed of 45.45% limonene, 23.10% linalyl acetate, 8.05% γ-terpinen, 7.25% β-pinene, 6.50% linalool, 1.35% α-pinene, and 0.35% geranial and a huge variety of unidentified minor compounds constitute the remaining 7.95% [67].

C. latifolia **(Persian Lime):** Peel EO showed anxiolytic and sedative activity in rodents without any motor impairment. In LD test, the experimental procedure that reflected generalised anxiety disorder, EO was active at dose 0.5 g/kg after oral administration and an inverted U shaped dose response curve was obtained. In MB test, that reflects obsessive compulsive disorder, it was found to be active at higher doses of 1.0 and 1.5 g/kg and a remarkable dose dependent effect was observed. EO at dose 1.5 mg/kg orally increased sleep duration induced by ethylic ether inhalation. The GC-MS analysis of oil revealed presence of limonene-58%, β-pinene-13%, γ-terpinene-14%, linalool-0.4%. It was suggested that biological profile of EO resulted from synergism among its constituent compounds [68].

C. limon **(Lemon):** Olfactory stimulation with peel EO was found to have sedative activity in mice as it shortened sleep time in PST. The sleep time was determined as the time elapsed between i.p. pentobarbital administration and the first time that the animal was able to spontaneously right itself [69]. In another study, lemon oil was administered to mice in vapour form and subsequently evaluated using EPM, FST and OFT for antistress activity. It significantly increased percentage of open arm entries and reduced immobility time in EPM and FST respectively. Furthermore, it significantly reduced locomotor activity and rearing behavior in OFT. The anti-stress effect of lemon oil was significantly blocked by pre-treatment with flumazenil, an antagonist of the benzodiazepine site at the gamma amino butyric acid (GABA)-A receptor and apomorphine, a nonselective dopamine (DA) receptor agonist. In contrast, agonists or antagonists to the 5-hydroxy tryptamine (5-HT) receptor and the alpha-2 adrenaline receptor did not affect the anti-stress effect of lemon oil. Buspirone, DOI, and mianserine blocked the antidepressant-like effect of lemon oil in the FST, but WAY100,635 did not. The findings suggested that the antistress effect of lemon oil is closely related with the serotonergic pathway, especially via 5-HT1A receptor. Moreover, the lemon oil significantly accelerated the metabolic turnover of DA in the hippocampus and of 5-HT in the prefrontal cortex and striatum. Lemon oil possibly reduces distress by modulating GABAergic, serotonergic, and dopaminergic systems in the brain. Suppression of DA activity via enhanced serotonergic neurons under the lemon oil inhalation condition was proposed [70]. In a separate study, it was found that lemon oil components like limonene, γ-terpinene and citral and their metabolites affect monoamine release from rat brain slices. Also, rho-cymene, a gamma-terpinene metabolite, was found to have a stronger effect than gamma-terpinene. These results suggested that the metabolites of monoterpene compounds contained in Citrus EOs have a stronger effect on monoamine release from brain tissue than the monoterpene compounds themselves [71]. Further, constituents of lemon EO were evaluated for antistress activity in rats using acute cold stress and communication box techniques after i.p. administration. It was found that R-limonene, citral and γ-terpinene kept down the concentrations of serum corticosterone and cerebral monoamines. S-limonene (a stereoisomer of R-limonene) had apparently stronger activity compared to other monoterpenes and inhibited monoamines induced elevation of psychological stress. These results showed lemon oil components such as limonene and citral have capability to reduce physical and psychological stress [72]. Methanolic leaf extract of *C. limon* was found to have anxiolytic activity in mice when compared with DZP at dose 100mg/kg in EPM [73]. In a separate study effects of S-limonene on brain neurotransmitter levels and behaviour of rats were studied. S-limonene was administered for one week in different concentrations i.e. 0, 5, 25 and 50 mg/kg to rats. Then neurotransmitters like DA, 5-HT, GABA, glutamic acid as well as some of their metabolites like dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) were detected by HPLC-ECD and amino acid analyzer. Basal HPA activity was determined by corticosterone after one week administration of S-limonene. It was found that S-limonene inhibited HPA activity under physical stress and the stress alleviating effect possibly acted by the $GABA_A$ receptor [74].

EO of leaves of *C. limon* after oral administration for 30 days, showed significant sedative and anxiolytic activities comparable to DZP at doses of 50, 100 and 150 mg/kg without causing any muscle relaxant effect. These activities were demonstrated by OFT, EPM, rota rod and PST tests in mice and possible role of benzodiazepine-type receptors was proposed to be responsible for sedative and anxiolytic activities. GC-MS analysis showed a mixture of monoterpenes among which limonene (52.77%), geranyl acetate (9.92%) and trans limonene oxide (7.13%) were the main compounds in the EO [75]. Ethanolic leaf extracts at doses 100 and 150mg/kg were found to have significant CNS depressant activity comparable to DZP as demonstrated by OFT and rota rod test in mice after oral administration. Phytochemical screening of the ethanolic extracts indicated the presence of coumarin and triterpenoids/steroids and the absence of alkaloids, flavonoids, tannins and saponins. Behavioral changes in animals during 30 days of treatment like decrease of spontaneous activity, palpebral ptosis, ataxia, analgesia, and sedation were observed. Also, it was suggested that ethanolic extract interacts with the $GABA_A$ receptor, probably at the receptor subtypes that mediate benzodiazepine effects, to produce sedative and hypnotic activities [76]. Chronic administration of lemon oil at dose of 0.4ml/kg produced anxiolytic effect when evaluated using OFT and EPM in rats [77]. Lemon juice was found to have anxiolytic activity comparable to lorazepam using head dip method in experimental mice when administered for 7 consecutive days [78].

C. macroptera **(Wild Orange):** Treatment with ethanolic extract of fruit peel at dose 250 and 500 mg/kg, administered for 7 consecutive days was found to have significant

anxiolytic activity comparable to DZP in EPM and LD model in mice. Acute oral toxicity studies of ethanolic peel extract were carried out according to OECD-423 guidelines in mice and found to be non toxic [79].

C. maxima **(Pomelo):** Ethanolic leaf extract at dose 200 and 400 mg/kg, administered orally was found to have anxiolytic and hypnotic activity in rats. Treatment with leaf extract significantly increased time spent in light arena comparable to DZP in LD test. There was a significant increase in frequency of open arm entries and a dose dependent increase in duration of time spent in open arms of EPM. In HB test, there was a significant decrease in number of head dips comparable to DZP at dose of 400 mg/kg, further confirming anxiolytic activity. Treatment with both the doses significantly increased duration of hypnosis when evaluated using PST. There was a reduction in locomotor activity as measured by actophotometer. Leaf extract at dose 400 mg/kg showed skeletal muscle relaxant activity as it reduced time spent in rota rod test, delayed time taken to climb the chain in climbing test and decreased sliding time in inclined screen test. The leaf extracts did not produce any mortality orally up to 2000mg/kg, as observed for 5h after administration. There were not any visible signs of delayed toxicity and mortality observed for 14 days according to OECD-423 guidelines [80].

C. paradisi **(Grapefruit):** Various leaf extracts (petroleum ether, chloroform, methanol and aqueous extracts at 3 doses i.e. 100, 200 and 400 mg/kg) of *C. paradisi* var. *foster* and *C. paradisi* var. *duncan* were evaluated for anxiolytic activity using EPM in mice. Methanol extract in both the cases, at dose 100 mg/kg administered orally increased time spent in open arm of EPM comparable to DZP [81, 82]. Different leaf extracts of *C. paradisi* var. *marsh seedless* at doses 50, 100, 200 and 400 mg/kg orally were evaluated for anxiolytic activity using LD and HB tests in mice. All doses of methanol leaf extract increased number of line crossing and head dipping comparable to DZP in HB test. Same extract at dose of 100 and 200 mg/kg significantly increased time spent in lit box comparable to DZP in LD test. The extract was administered orally at a dose 2000 mg/kg body weight to different groups of mice and observed for signs of behavioural, neurological abnormality and mortality for 14 days as per OECD-423 guidelines [83].

C. reticulata **(Mandarin):** EO from peel was found to be anxiolytic and sedative when evaluated using MB test and sleep induced by ethylic ether test in mice respectively. Treatment with EO at dose 1.5 g/kg orally significantly decreased the number of marbles buried as compared to imipramine (IMP) and increased duration of sleep induced by ethylic ether inhalation without impairing motor activity. The GC-MS analysis of oil revealed presence of limonene-90%, β-pinene-0.35%, γ-terpinene-4.0%, linalool-1.0% as main components [68]. In another study, peel extracts of *C. reticulata* and *C. unshiu* (commonly known as chimpi in Japan) and their major chemical constituent hesperidin and its aglycone hesperetin were found to have anxiolytic activity comparable to fluoxetine (FXT) when evaluated using elevated open-platform test using mice. Peel extracts of both the plant (100 and 500 mg/kg orally) as well as hesperidin and hesperetin (50 mg/kg orally) significantly decreased duration of freezing comparable to FXT without altering locomotor activity. It was suggested that pharmacological action may be associated with serotonergic neurotransmission rather than GABAergic neurotransmission pathway as it was comparable to FXT which is a selective serotonin reuptake inhibitor [84].

C. sinensis **(Sweet Orange):** Ambient odour of *Citrus sinensis* was found to reduce state anxiety and improve mood in patients, particularly in females, waiting for dental treatment. Patients were assessed by means of self-report demographic and cognitive variables, trait and state anxiety, current pain, mood, alertness, and calmness [85]. The methanol and dichloromethane extracts, obtained from the flowers, showed a dose-dependent sedative effect in the exploratory cylinder model in mice, with ED50 (i. p.) values of 47.04 ± 12.03 mg/kg and 129.15 ± 21.25 mg/kg, respectively. Hesperidin (ED50 = 11.34 ± 2.48 mg/kg) was identified in the methanol extract as the sedative active principle of this plant. The pre-treatment with atropine (1 mg/kg i.p.), flumazenil (2 mg/kg i.p.), clonidine (0.01 mg/kg i.p.), isoproterenol (0.3 mg/kg i.p.), haloperidol (0.3 mg/kg i.p.), WAY 100 635 (3 mg/kg i.p.), p-chlorophenylalanine (250 mg/kg i.p., twice per day for 2 days), forskolin (3 mg/kg i.p.) and rolipram (0.173 mg/kg i.p.) did not modify the sedative effect of 30 mg/kg hesperidin. However, the sedative effect of this compound was potentiated by yohimbine (1.25 mg/kg i. p.) and buspirone (1 mg/kg i.p.), and reverted by pretreatment with aminophylline (30 mg/kg i.p.), caffeine (30 mg/kg i.p.) and several doses of 1,3-dimethyl-8-phenylxanthine (10, 30 and 54.7 mg/kg i.p.). These results suggested that sedative action of hesperidin, identified as the active principle of the flowers of *Citrus sinensis* may be mediated through adenosine receptors [51]. EO of peel was evaluated for anxiolytic-like activity using EPM and LD paradigm in rats. Inhalation of orange aroma at doses 100, 200 or 400 µl was found to have significant anxiolytic activity comparable to DZP at least in one test. Highest dose of 400 µl was found to be effective in both the tests as indicated by increased exploration of the open arms of the EPM and increased stay in the lit chamber of the LD paradigm. Further tests with the EO of *Melaleuca alternifolia (Myrtaceae)* showed that these results were not influenced by any other odour exposure confirming anxiolytic-like effect of sweet orange aroma. The GC/MS analysis determined the main component of orange oil sample to be limonene (97.66%), so this may probably be the responsible constituent for the observed anxiolytic-like effect [86].

Antidepressant activity

C. limon **(Lemon):** EO of leaves was found to have antidepressant effect at different doses 50, 100 and 150 mg/kg administered orally, comparable to IMP in FST in mice. Since EO at these doses did not show any sedative effect in the OFT, these three doses were used in the FST. In the rota rod, EO at a dose of 150 mg/kg orally, decreased the time of permanence on the bar related to control. The antidepressant effect of EO was not altered by the previous administration of paroxetine but totally blocked by reserpine pretreatment. So, it was suggested that observed antidepressant effect of oil may probably be accomplished through noradrenergic and serotonergic mechanisms [87]. Ethanolic leaf extracts at doses 50, 100 and 150 mg/kg, administered orally for 30 days were evaluated for antidepressant activity using FST in mice. Treatment with all the three doses significantly reduced time spent immobile by mice as compared to IMP in FST. The association of ethanol extract at dose of 150 mg/kg with IMP showed a reduction of 6% and 15% in the immobility time, as related to the groups treated with ethanol extract alone or IMP

alone, respectively. Also, the highest dose of 150 mg/kg produced motor in coordination in rota rod test. The antidepressant effect was enhanced by paroxetine. On the contrary, the antidepressant activity of extract was totally blocked by the previous administration of reserpine. These data suggested that the noradrenergic and serotoninergic system may be responsible for antidepressant action [76]. In another study behavioural effects of *C. limon* in rats at three different doses i.e. 0.2, 0.4 and 0.6 ml/kg were evaluated twice during 15 days. In OFT, treatment with moderate dose showed increase in distance travelled, number of central entries and number of rearings. Whereas in FST, there was decrease in duration of immobility and increase in duration of climbing suggesting antidepressant effect [77].

***C. macroptera* (Wild Orange):** Treatment with ethanolic extract of fruit peel at dose 250 and 500 mg/kg, for 7 consecutive days was found to have significant antidepressant activity comparable to IMP in FST and tail suspension test (TST) as it decreased duration of immobility in mice. Acute oral toxicity studies of ethanolic peel extract were carried out according to OECD-423 guidelines in mice and found to be non toxic [79].

***C. maxima* (Pomelo):** Three doses of aqueous leaf extract (100, 200 and 300 mg/kg, orally) were evaluated for locomotor and antidepressant activity using actophotometer test, modified FST and TST in mice. The dose of 300 mg/kg showed significant increase in locomotor activity comparable to FXT. No sedative effects were observed at any of the doses. Extract was found to be safe as no mortality was observed following treatment with doses as high as 2000 mg/kg. All the doses of leaf extract significantly reduced the immobility time in dose dependent manner in both the tests. The results also showed that accompanied with the decrease in immobility time, leaf extract increased climbing time without significant change in swimming time similar to the effect of IMP. The reductions of the immobility time were 37%, 56.56% and 69.69% for the extract at 100, 200 and 300 mg/kg, respectively. The pattern of behaviour exerted by the extract in the FST was similar to that of IMP which suggested that this plant extract acts probably by enhancement of norepinephrine neurotransmission [88]. In another study, ethanolic leaf extract at doses 200 and 400 mg/kg was evaluated for antidepressant activity using FST and TST in rodents. Oral treatment with 400 mg/kg of leaf extract significantly reduced immobility time and increased climbing behaviour in FST, decreased duration of immobility in TST comparable to IMP. There was a reduction in locomotor activity as measured by actophotometer. Leaf extract at dose 400 mg/kg showed skeletal muscle relaxant activity as it reduced time spent in rota rod test, delayed time taken to climb the chain in climbing test and decreased sliding time in inclined screen test [80].

***C. paradisi* (Grapefruit):** Different leaf extracts (petroleum ether, chloroform, methanol and aqueous) of *C. paradisi* var. *foster* and *C. paradisi* var. *duncan* were evaluated for antidepressant activity at three doses i.e. 100, 200 and 400 mg/kg orally using FST in mice. Methanol leaf extracts of both the varieties at dose of 400 mg/kg significantly decreased time spent immobile by mice as compared to IMP showing antidepressant activity [81, 82].

***C. sinensis* (Sweet Orange):** Aqueous peel extract of *C. sinensis* and a combination of peel extracts of *C. sinensis and C. limon* was evaluated for antidepressant, locomotor and skeletal muscle relaxant activities using a series of tests like FST, TST, actophotometer, rota rod test and muscle grip strength in mice. There was a significant dose dependent decrease in locomotor activity, duration of time of fall and decrease in immobility time comparable to DZP in rodents treated orally with 20mg/kg doses of both the extracts. It was suggested that these effects may be produced through $GABA_A$ receptors as the observed effects were similar to DZP [89].

Anticonvulsant Activity

***C. aurantium* (Sour Orange):** EO (0.5 and 1.0 g/kg), extracts and fractions (hydroethanolic, hexanic, dichloromethanic and aqueous – 1.0 g/kg), were evaluated for their potential to interfere in convulsive processes using pentylenetetrazole (PTZ) induced seizures and maximal electroshock seizures (MES) in mice and compared with valproic acid as standard drug. Treatment with EO at dose 0.5 g/kg did not alter the percentage of occurrence of clonic or tonic episodes or lethality, but increased the latency period until the first tonic convulsion in both tests. However, this effect was not observed with 1.0 g/kg dose of oil, confirming the absence of dose dependency [40]. In another study the effect of hydro-alcoholic extract (100, 500 and 1000 mg/kg i.p) of flowers on PTZ induced seizures and MES was investigated in mice. Behavioural responses of the animals such as latency to first minimal clonic seizure and latency to the first generalized tonic-clonic seizures were recorded. In electroshock model, duration of tonic convulsion (a tonic extension of the hind limb) was recorded. Treatment with 500 and 1000 mg/kg of extract significantly increased latency to first minimal clonic seizure while first generalized tonic-clonic seizures latency was increased by all the three doses of extract comparable to DZP. In electroshock model, there was no significant activity observed [40].

***C. bigaradia* (Seville Orange):** Aqueous and ethanolic extracts of leaves of were found to have moderate anticonvulsant activity when evaluated using PTZ and MES model in rodents. In the PTZ-induced seizure, the administration of all doses (0.35, 1.4, 2.1, 3 g/kg i.p) of aqueous extract 30 min before the injection of PTZ prolonged the latency of myoclonic seizures comparable to phenobarbital. Also, ethanolic extract at doses 2.1 and 3 g/kg, 40 min before the injection of PTZ reduced the duration of myoclonic seizures. Both extracts exhibited its anticonvulsant activity through a dose-dependent manner. The protective effect against lethality was 75% and 25% in the highest dose of aqueous and ethanolic extracts, respectively. It was found that doses 2.45 and 3.5 g/kg of aqueous extract indicated moderate effect on tonic seizure in maximal electroshock seizure test. As this plant showed more anticonvulsant effect in the PTZ-induced seizure model than MES test, thus it may be more effective against petit mal epilepsy. Phytochemical screening indicated the presence of alkaloids, flavonoids, tannins and saponins in aqueous extract while tannins in ethanolic extract. Acute toxicity studies were conducted for both the extracts. Different doses of extracts were injected intraperitoneally into groups of six mice. The number of deaths were counted at 48 h after treatment. LD50 values and corresponding confidence limits (CL) were determined by the

Litchfield and Wilcoxon method. LD50 values of the aqueous and ethanolic extracts were 5.74 g/kg body wt. (95% CL: 5.05, 6.53) and 6.59 g/kg body wt. (95% CL: 5.54, 7.84), and the maximum non-fatal doses were 3.5 g/kg body weight and 3 g/kg body weight, respectively [90].

***C. limon* (Lemon):** EO of leaves (50, 100 and 150 mg/kg, *p.o.*) was evaluated for anticonvulsant activity against PTZ and picrotoxin induced convulsions in mice. Treatment with all doses for 30 days delayed the onset of PTZ-induced tonic convulsion significantly as compared to DZP. EO (150 mg/kg, *p.o.*) protected 85% of mice against the convulsion and reduced the mortality rate by 60% induced by PTZ. Only the highest dose of EO (150 mg/kg, *p.o.*) increased the latency for convulsions and reduced mortality rate induced by picrotoxin when compared to the negative control. The anticonvulsant effect of EO was antagonised by pretreatment with flumazenil, a selective antagonist of benzodiazepine site of $GABA_A$ receptor. GC-MS analysis showed limonene (52.77%), geranyl acetate (9.92%) and trans-limonene-oxide (7.13%) as the main components of EO [87].

***C. maxima* (Pomelo):** Ethanolic extract of leaves at dose 200 and 400 mg/kg, orally was found to have anticonvulsive activity in rodents. It significantly increased the latency of the seizures in PTZ induced seizures test comparable to DZP and protected 22.84 ±0.41 and 20.30 ±0.17 of the mice against seizures at dose 200 and 400 mg/kg, respectively. Also, dose dependent anticonvulsant activity was observed in strychnine induced convulsions test. In MES model, administration of leaf extract showed a dose dependent increase in the delay of the onset time of seizures induced by MES and also decreased duration of tonic hind limb extension [80].

Neuroprotective activity

***C. aurantium* (Sour Orange):** The effect of flower extract on scopolamine-induced learning and memory deficit in rats was investigated. Learning and memory impairment as the most characteristic manifestation of dementia was chemically induced by scopolamine, a cholinergic antagonist. Treatment with flower extract at doses 300 and 600 mg/kg per day for 15 days, significantly restored memory and learning impairments induced by scopolamine in the passive avoidance test and also reduced escape latency during trial sessions in the Morris water maze test. Also serum malondialdehyde (MDA) levels were significantly decreased. So, it was found to have a repairing effect on memory and may have beneficial effects in the treatment of AD [91].

***C. bergamia* (Bergamot):** In the human SH-SY5Y neuroblastoma cell line exposed to N-methyl-D-aspartate (NMDA), bergamot oil (0.0005–0.01%) reduced the death of SH-SY5Y cells caused by 1 mM NMDA in a concentration dependent manner. In addition, 0.01% oil counteracted the deactivation of Akt (a serine/threonine-specific protein kinase) and the consequent activation of glycogen synthase kinase 3 beta induced by NMDA. Results obtained with specific fractions of bergamot oil, suggested that monoterpene hydrocarbons could be important for neuroprotection [92]. In another study, EO from peel (0.5 ml/kg, i.p) given 1 h before experimental occlusion of the middle cerebral artery, significantly reduced infarct size after 24 h, especially in the medial striatum and the motor cortex, as revealed by 2,3,5-triphenyl-2H-tetrazolium chloride staining of tissue slices [93].

***C. junos* (Yuzu):** The protective effect of naringenin, a major flavanone constituent isolated from *C. junos*, against Amyloid β protein-induced neurotoxicity was investigated using PC12 cells and assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Pretreatment with isolated naringenin and vitamin C prevented the generation of the Amyloid β-induced reactive oxygen species and inhibited the Amyloid β-induced neurotoxic effect. Naringenin, at dose 4.5 mg/kg, significantly ameliorated scopolamine-induced amnesia as measured in the passive avoidance test in mice. Therefore, these results indicate that micromolecular Amyloid β-induced in vitro oxidative cell stress is reduced by naringenin and it may prove useful against neurodegenerative disease such as AD [94].

***C. limon* (Lemon):** The activity of EO components, namely S-limonene and S-perillyl alcohol, on dementia was evaluated by dementia induced by scopolamine test, passive avoidance test as well as the open field habituation. In these tests, both the components presented a strong improvement in memory. More precisely, S-perillyl alcohol could only improve associative memory in passive avoidance, but was not able to improve non-associative memory in open field habituation. The neurotransmitter concentration in some cerebral regions was analysed and showed that the concentration of DA was lower when scopolamine was applied, but this could be reversed by S-limonene or S-perillyl alcohol administration before the scopolamine injection. With help of the Ellman method, it even could be demonstrated that these EO components inhibited acetylcholinesterase activity *in vitro* [95].

***C. macroptera* (Wild Orange):** Ethanolic extract of fruit peel at dose 250 and 500 mg/kg, administered for 7 consecutive days was found to have neuroprotective activity. Superoxide dismutase activity, catalase activity and lipid peroxidases were estimated in isolated brain tissue from mice using method of Misra and Fridovich, method of Aebi and thiobarbituric acid reaction method respectively and levels were found to be improved. The total phenolic content of extract of was estimated using standard gallic acid equivalent of phenols and found to be 142.5mg/g equivalent of gallic acid and total flavonoid content 333.00 mg/g equivalent of quercetin. So, the extract was found to protect brain tissue from oxidative stress that could be related to high flavonoid content [79].

***C. sinensis* (Sweet Orange):** In an open-label trial, effect of ten essential oils (vaporised mixture of volatile oil of rosemary, orange, ylang ylang, patchouli, basil, rosemary, peppermint, rosewood, geranium, bergamot, chamomile and jasmine) on cognition and mood in 144 patients with dementia was evaluated. There was a marked decrease in disturbed behaviour in the majority of patients, leading to reductions in psychotropic medications [96].

Conclusion and future prospects

The present review on neuropharmacological potential of the genus *Citrus* has revealed that various *Citrus* species have been used in traditional medicinal systems of one region or another throughout the globe for neurological conditions like insomnia, nervousness, mental exhaustion, headache, hysteria, epilepsy and depression. The psychotherapeutic potential of essential oils of many *Citrus* species which have been used in aromatherapy for management of mind related disorders for

centuries, has been scientifically validated at the level of animal as well as subclinical studies. Many studies have established that limonene is the principal constituent of essential oils of *Citrus* and it is responsible for central nervous system related effects. So, it is expected that aromatherapy with *Citrus* oils for management of stress, anxiety, insomnia, depression and cognitive impairment, will continue to be used and with scientific evidence shall become reliable complementary therapy in future.

While reviewing the available literature, it has come to light that most of the researchers have attempted to evaluate fruit peel extracts and essential oils of fruit peel for neurobiological activity while lesser body of research is available for leaf extracts, essential oil from leaves, flower extracts and essential oil from flowers. Maximum number of studies have reported anxiolytic activity of *Citrus* species followed by antidepressant, sedative, antistress, anticonvulsant, neuroprotective and memory enhancing activities in descending order. *C. limon* has been found to be the most explored species for neurological activities followed by *C. aurantium, C. bergamia, C. sinensis, C. maxima, C. reticulata, C. paradisi, C. aurantifolia, C. junos, C. macroptera, C. latifolia, C. bigaradia* in descending order in terms of number of studies conducted. As all of these experimental reports are based on animal level studies, clinical efficacy and safety profile of these plants need to be demonstrated at the level of controlled clinical trials. Some of the studies have attempted to explore the possible mechanism of action responsible for the observed biological activity and have suggested that changes at the level of HPA axis, GABAergic or monoaminergic neurotransmission may be responsible for observed effects. However, further studies are needed so that detailed mechanism of action of these plants can be elucidated. For some of the *Citrus* species, preliminary phytochemical screening of the bioactive leaf and peel extracts has been carried out and found to contain terpenoids, steroids, flavonoids and phenolic compounds. A study has reported hesperidin to be active principle responsible for observed sedative activity of *C. sinensis* flower extract. In other cases, the bioactive extracts have not been standardised with respect to marker compounds and phytoconstituents have not been correlated with observed biological activities. *Citrus* species are a rich source of flavonoids and polyphenolic compounds. Flavonoids like apigenin and chrysin are established as ligands for $GABA_A$ receptors and have shown anxiolytic and sedative activities in various in-vivo studies [97, 98, 99]. Hesperidin has shown CNS depressant and neuroprotective effects [101, 102]. Apigenin, rutin, hesperidin and nobiletin are found to have antidepressant like effects in different studies [103, 104, 105, 106]. In addition, polyphenolic compounds found in *Citrus* species are established to have antioxidant, free radical scavenging and antiinflammatory properties which may prove beneficial for neurodegenerative disorders [108, 109, 110, 111].

On the basis of available body of research, it can be concluded that genus *Citrus* is very promising in terms of its neuropharmacological potential and still remains to be explored systematically. Hence, detailed pharmacognostic, phytochemical and biological studies are required to locate and isolate the neuroactive compounds that could be introduced into clinical practice after suitable toxicological investigation.

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