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Research progress on the basic pharmacodynamic substance and mechanism of Citri Sarcodactylis Fructus in relieving anxiety

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Keywords

Citri Sarcodactylis Fructus, bergamot essential oil, anti-anxiety, anxiety disorder, immunoregulation, neuroendocrine

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Abstract

Anxiety disorder resulting from the interaction of psychological factors-caused anxiety and mood disorder symptoms is one of the clinical mental diseases. Excessive worry, nervousness and fear often bring about several negative outcomes, and seriously impact the physical, mental, and social functioning of patients. What's worse, with poor treatment, anxiety disorder may develop into mental illnesses such as depression and schizophrenia. The treatment of anxiety has mainly recourse to drug therapy and psychotherapy; however, Western drug therapy represented by 5-hydroxytryptamine reuptake inhibitor often causes evident adverse effects, including nausea and constipation, as well as poor long-term medication compliance of patients. Citri Sarcodactylis Fructus can sooth the liver and regulate qi, which can be applied to alleviate anxiety disorder as an ancillary drug. Modern pharmacological research revealed that bergamot volatile oil, also known as bergamot essential oil (BEO), is the main active chemical component of Citri Sarcodactylis Fructus for anti-anxiety. BEO has been confirmed to exert an anti-anxiety effect through modulating neuronendocrine-immune network, including immunoregulation, suppression of hyperactive hypothalamic-pituitary-adrenal (HPA) axis, enhancement of function of hypothalamic-pituitary-thyroid (HPT) axis, modulation of neurotransmitter, and promotion of neurotrophic effect. The study discusses the current research status regarding the application of Citri Sarcodactylis Fructus for anti-anxiety in recent years, and deeply probes into the mechanism of Citri Sarcodactylis Fructus playing an anti-anxiety role via neuronendocrine-immune network, in an effort to provide references to clinical treatment of anxiety disorder and new ideas for developing drugs targeting anxiety disorders.

1 Introduction

At present, the number of patients suffering from psychological and psychiatric disorders remains stubbornly high, and nearly 7.3%~28.0% of global patients develop anxiety disorder. In China, the lifetime prevalence of anxiety disorder is about 7.6% [1]. With socio-economic development, people face more pressure and stressor, resulting in an increasing incidence of anxiety disorder year by year.

Anxiety disorder results from the interaction of psychological factors-caused anxiety and mood disorder symptoms, and mainly features excessive worry, nervousness and fear. This disease can cause restlessness, nervousness, frequent urination, sweating, shaking hands, palpitations, etc., seriously impacting the physical, mental, and social functioning of patients [2]. Hence, how to control and treat anxiety disorder has become a new challenge for medical workers.

Modern medical research revealed that immune dysfunction, hyperactive hypothalamic-pituitary-adrenal (HPA) axis, neurotransmitter changes, etc. are relevant factors that lead to anxiety disorder. The current treatment primarily has recourse to drug therapy and psychotherapy. However, Western drug therapy represented by 5-hydroxytryptamine reuptake inhibitor often causes evident adverse effects, such as nausea, constipation and addictiveness as well as poor long-term medication compliance of patients [3].

In the traditional Chinese medicine (TCM) theoretical system, anxiety disorder is similar to “depression syndrome”, “manic-depressive psychosis”, “hysteria”, “lily disease” and “lantern disease”, which belongs to emotional diseases. TCM attributes anxiety disorder to a combination of internal and external causes. The internal causes mainly include congenital heredity, long illness and weak body, and the external causes contain emotional stimulation and improper diet. Clinically, it is manifested as excess syndrome, deficiency syndrome and combination of both. Anxiety disorder is intimately related to liver, heart and gallbladder, and involves lung, spleen, stomach and kidney, with the main pathogenesis of organism dysfunction, qi stagnation, and orifices confused by phlegm-turbid. TCM in the treatment of anxiety disorder has the advantages of significant therapeutic efficacy, few adverse reactions and easy acceptance by patients [4, 5], which therefore has been gradually emphasized by the medical community.

Citri Sarcodactylis Fructus has been commonly used to treat anxiety disorder, with pungent, bitter and sour taste, and warm nature, which has therapeutic effects in the liver, spleen, stomach and lung. Citri Sarcodactylis Fructus enters into the liver meridian, and warms channel for dispelling cold with pungent taste, the aroma of which can migrate throughout the body, so as to regulate the activities of qi, relieve the depressed liver and remove obstruction in the collateral [6]. Citri Sarcodactylis Fructus also impacts spleen meridian with aroma, pungent taste and warm nature, which can resolve turbidity, activate spleen and stomach, restore the function of spleen in transportation, remove dampness to reduce phlegm and induce resuscitation. Hence, it can be applied to treat anxiety disorders arising from liver qi stagnation, liver-stomach disharmony, and orifices confused by phlegm-turbid.

This study discusses the current research status regarding the application of Citri Sarcodactylis Fructus for anti-anxiety in recent years, and deeply probes into the pharmacodynamics substance foundation and mechanism of Citri Sarcodactylis Fructus playing an anti-anxiety role via neuronendocrine-immune network, so as to provide references for its clinical application in treating anxiety disorder and for comprehensively and completely exploring corresponding mechanism. There is a paucity of clinical trials with abundant samples and precise targets for clinical research of Citri Sarcodactylis Fructus. Thus, the clinical research on the anti-anxiety effect of Citri Sarcodactylis Fructus and the mechanism of action are still the main direction in the future.

2 The clinical research on the anti-anxiety effect of Citri Sarcodactylis Fructus

Anxiety and pressure are a kind of physical or psychological reactions that trigger severe mental and

physical problems and bring about significant social costs. Aromatherapy is a widely used alternative treatment method, and is commonly applied for alleviating anxiety and pressure due to its high safety and accessibility. The aromatic product of Citri Sarcodactylis Fructus mainly refers to bergamot essential oil (BEO), which is a commonly-used natural drug in aromatherapy, owing to its anti-anxiety and emotion-relieving properties.

It has been documented [7] that inhalation of BEO for 15 min can notably reduce the levels of anxiety and pressure. Meanwhile, BEO is more effective at relieving anxiety and stress and promoting positive emotions than artificial aroma with similar odors, and its efficacy is free from the effect of the recognition ability of the participants. Therefore, the efficacy of BEO is more attributed to its inherent pharmacological properties than to its induced psychological effects. Hung et al. [8] treated 26 nursing staff with 1.5% bergamot essential oil via aromatherapy for 4 weeks, and asked them to fill in a Nurse Job Stressors Scale and Copenhagen Burnout Inventory. The results indicated that this treatment can evidently relieve anxiety, and the efficacy is more significant for subjects with less workload. Yuanyunzi Wang [9] pointed out that through the State Anxiety Inventory Scale, 1 h inhalation of BEO has been confirmed to mitigate post-operative anxiety and pain of patients undergoing laparoscopic cholecystectomy after 3 h. Pasyar et al. [10] also found that BEO can reduce anxiety of patients before laparoscopic cholecystectomy. Qi et al. [11] identified that Citri Sarcodactylis Fructus and Cyperi Rhizoma combined aromatherapy accompanied with Horn tune music appreciation and Baduanjin(eight brocades) exercise can improve anxiety symptoms of patients with hyperplasia of mammary glands with stagnation of liver depression and phlegm coagulation after treatment of Xiaoyao Loubei powder, mitigate insomnia and dreamful sleep, and ameliorate unhealthy emotions. Scuteri et al. [12] proved that BEO has a non-sedative anti-anxiety effect, which is beneficial for treating dementia. In addition, many studies have found that BEO can play an anti-anxiety role [13-16].

Collectively, BEO has an apparent anti-anxiety effect and widely applied in aromatherapy. Also , it has advantages of no evident side effects, easy application, and easy acceptance as well as good compliance of patients, which can reduce the levels of anxiety, fatigue and pressure, improve negative emotions and relieve pain.

Nevertheless, there exists controversy with regards to the anti-anxiety effect of Citri Sarcodactylis Fructus. A previous report [17] indicated that compared to a placebo (hair conditioner), 10 min of BEO aromatherapy cannot effectively alleviate anxiety, and it was even found that in pediatric patients post stem cell transplantation, the BEO aromatherapy causes more anxiety and nausea after inhalation. Under certain circumstances, BEO is less effective in reducing anxiety than non-fragrant oils. Accordingly, the anti-anxiety effect of Citri Sarcodactylis Fructus may vary from person to person, which can be further explored.

3 The components with anti-anxiety properties in Citri Sarcodactylis Fructus

Modern pharmacological research revealed that BEO is the main active component of Citri Sarcodactylis Fructus with anti-anxiety properties [18], composed of monoterpenes, sesquiterpenes, coumarins and flavonoids. Of them, monoterpenes and sesquiterpenes are volatile components, accounting for over 93% in BEO, while coumarins and flavonoids are non-volatile components. The current pharmacological study has confirmed the pharmacological activities of Citri Sarcodactylis Fructus, such as anti-anxiety, immunoregulation, anti-oxidation, anti-inflammation, anti-tumor, blood glucose regulation and blood lipid reduction [6,19]. Based on the network pharmacology, through limonene, hesperidin, polysaccharide, bergapten, flavone, coumarin, and other active components, Citri Sarcodactylis Fructus can exert an anti-anxiety effect by regulating Nuclear Factor Kappa B (NF-κB), Tumor necrosis factor (TNF) and Mitogen-activated protein kinase (MAPK) signaling pathways, and

impacting TNF, interleukin-1β (IL-1β), dopamine (DA), and γ-aminobutyric acid (GABA).

Specifically, limonene, one of the main components of BEO, is a monoterpenoid substance [20], which plays an anti-anxiety role via inhibiting the protein expressions of nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) to dampen the production of nitric oxide (NO), prostaglandin E2 (PGE2), TNF-α, IL-1β and IL-6, and controlling lipopolysaccharide (LPS)-stimulated neuroinflammatory responses dependent on NF-κB, stress activated protein kinase (SAPK) pathway and extracellular regulatory protein kinase (ERK) pathway [21,22]. The monoterpenoid substances, nerolyl acetate, nerol and linalool, participate in neuroactive ligand receptor interactions to combat anxiety via mediating key targets, including serotonin transporter gene (SLC6A4), N-methyl-D-aspartate receptor 2B subunit gene (GRIN2B), and D2 dopamine receptor (DRD2) [23]. Hesperidin, the main active monomer of flavones plays anti-inflammatory and anti-oxidant roles via suppression of NF-κB signaling pathway, and protects nerve cells and improves anxiety by ameliorating neuronal injury and repressing neuronal apoptosis [6]. Coumarin with the main active monomers of bergapten and 5, 7-dimethoxycoumarin exerts anti-anxiety effects through blocking NF-κB signaling pathway to dampen the upregulation of enzyme-mediated COX-2 and iNOS inflammatory proteins as well as IL-6 and TNF-α inflammatory cytokines [24]. The anti-anxiety effect of Citri Sarcodactylis Fructus-derived crude polysaccharide is mainly achieved by scavenging free radicals and promoting the transformation of lymphocytes [6].

However, the above components do not play a single role, and the anti-anxiety effect of Citri Sarcodactylis Fructus is the result of the combined action of multiple active substances. Accordingly, investigating the interplay and interaction of various active substances is the direction of research in the future.

4 Study on the mechanism of the anti-anxiety of Citri Sarcodactylis Fructus

4.1 Immunoregulation

4.1.1 Inflammatory cytokines

Uzunova et al. [25] revealed that the action mechanism of COVID-19 causing anxiety is to induce neuroinflammatory response by the increment of proinflammatory factors after immune system activation. Wang et al. [26] found that the levels of serum inflammatory factors are positively correlated with anxiety-like behavior in alcohol withdrawal rats. Deng et al. [27] suggested that levels of proinflammatory factors (IL-1β, IL-6 and TNF-α) are higher in anxiety disorder patients than in normal controls, and are positively correlated to the degree of anxiety. However, some studies [28,29] revealed that IL-6 level is lower in anxiety disorder patients than normal controls. In most studies, upregulation of proinflammatory factors (IL-1β, IL-6 and TNF-α) are commonly present in patients with anxiety disorders, but in some experiments, these levels are downregulated, which may be related to individual conditions such as age, gender, and the type of anxiety disorders [29].

Trombetta et al. [30] evaluated the effects of two extracts from Citri Sarcodactylis Fructus on the antioxidant/anti-inflammatory activity of human vascular endothelial cells exposed to the pleiotropic inflammatory cytokine TNF-α, and demonstrated that the two extracts both prevent TNF-α-induced oxidative stress and modulate the activation of NF-κB, a redox-sensitive transcription factor. It has also been evidenced that BEO inhibits the expressions and secretion of LPS-induced proinflammatory factors (IL-6, IL-1β, TNF-α) by blocking NF-κB activation [31,32]. Lombardo et al. [33] indicated that BEO signally decreases the levels of IL-6, IL-1β and TNF-α in homogenate of rat claw. In addition, Cui et al. [34] unveiled that intraperitoneal injection of 200 mg/kg BEO into aluminum-induced anxiety-like rats for 60 days can decline IL-6, IL-1β and TNF-α levels in the hippocampus and frontal cortex of rats. Impellizzeri et al. [35, 36] proved that BEO lessens IL-1β and TNF-α levels in rats through multiple research. In view of the above information, it can be inferred that Citri Sarcodactylis Fructus can play an anti-anxiety role by decreasing levels of proinflammatory factors (IL-6,

IL-1β, TNF-α).

4.1.2 T lymphocytes

T lymphocytes play a pivotal role in cellular immunity and immune regulation. Zhou et al. [37] suggested that $CD3^+$ level is apparently diminished in the peripheral blood of anxiety disorder model rats, while after anti-anxiety treatment, $CD3^+$ level is increased and $CD4^+$ level has no evident changes. Fan et al. [38] found upregulation of $CD4^+$ in the peripheral blood of anxiety disorder patients. Notably, Bao et al. [39] demonstrated that Suanzaoren (Ziziphi Spinosae Semen) decoction dampens the proliferation of T lymphocytes to modulate immune function, and thus relieves anxiety. Thus, there are different opinions about anti-anxiety by regulating the immune mechanism of T lymphocytes. Fang et al. [38] put forward that acute stress results in activation of mouse immune system and increment of peripheral T lymphocytes, while chronic stress leads to inhibition of mouse immune system and decrement of peripheral T lymphocytes, and anxiety is caused by excessive production of xanthine after the mitochondrial fragmentation of $CD4^+$T cells.

Song et al. [40] treated chronic unpredictable mild stress (CUMS) combined with solitary rearing model rats with the aqueous extracts and ethanol extracts of Citri Sarcodactylis Fructus and Citri fructus, respectively, by gavage, and found that aqueous extracts can remarkably elevate the proportion of $CD3^+$T cells in blood, and ethanol extracts can markedly augment the proportion of $CD3^+$T cells and $CD4^+$T on the anti-anxiety of Citri Sarcodactylis Fructus. Moreover, a dearth of study focuses on the mechanism of anti-anxiety effect of Citri Sarcodactylis Fructus by regulating the immune function of T lymphocytes, so in-depth investigation on this aspect can be a research direction.

4.1.3 Regulation of PGE2 level

PGE2 is secreted from vascular endothelial cells and macrophages, and anxiety can trigger excessive secretion of PGE2 from nerve terminal, which also cause hyperactive immune system. After treatment of anxiety disorder, PGE2 level is apparently reduced [41].

According to the findings of Lombardo et al. [33], pre-treatment of BEO can lessen PGE2 level in homogenate of rat paw, suppress the resultant immunoreaction and further relieve anxiety. Therefore, it can be deduced that Citri Sarcodactylis Fructus can play an anti-anxiety role via reducing PGE2 level. However, the mechanism of BEO mitigating anxiety by diminishing PGE2 level has been less studied, so that anti-anxiety by downregulation of PGE2 may be a novel research direction.

4.2 Oxidative stress

The stimulation-induced excessive accumulation of reactive oxygen species (ROS) and other oxides can activate cellular antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), which are beneficial to scavenge excess free radicals and thus protect the body from oxidative stress-caused damage [42,43]. In case that the balance between ROS and antioxidase is disrupted, oxidative stress injury is triggered, the peroxidization of lipids in the brain tissue is transformed into malondialdehyde (MDA), and neurocytes are damaged, ultimately inducing anxiety disorder. Reportedly, suppressing oxidative stress response, elevating SOD, CAT and GSH-Px levels and diminishing MDA level contribute to improving anxious behaviors [44], implying that the regulation of anxiety level by the body has a close association with oxidative metabolism system [45].

In a previous report [46], BEO was smeared to the skin of mice with hair removal once a day for 42 days, and later BEO was confirmed to enhance the activity of SOD and decrease MDA content in the skin. Wang et al. [47] discovered that feeding BEO and fermented Citri Sarcodactylis Fructus extract can significantly reduce MDA level in the liver of mice with glucose and lipid metabolism disorder, and augment SOD, CAT and GSH-Px levels. Importantly, the fermented Citri Sarcodactylis Fructus extract with higher content of active components is more effective than BEO, so the fermentation technology provides a new insight into the clinical research on the anti-anxiety effect of

Citri Sarcodactylis Fructus. Cui et al. [29] revealed that intraperitoneal injection of 200 mg/kg BEO into aluminum-induced anxiety-like rats for 60 days can decline MDA level in the hippocampus and frontal cortex of rats and increase SOD, CAT and GSH-Px levels. Due to the strong antioxidant effect in the body, BEO may decrease the formation of free radicals in the liver [34], accompanied by downregulation of proprotein convertase subtilisin/kexin type 9 (PCSK9) [48], a gene related to oxidative stress [49,50]. Accordingly, Citri Sarcodactylis Fructus may ameliorate anxiety through mitigating oxidative stress, increasing SOD, CAT and GSH-Px levels and reducing MDA level.

4.3 Regulation of neurotransmitter and trophic factor levels

Central neurotransmitters mainly consist of monoamine neurotransmitters and amino acid neurotransmitters, abnormal contents or concentrations of which may bring about anxiety. Recently, research on the mechanism of Chinese medicine against anxiety via central neurotransmitters is in full swing.

4.3.1 Upregulation of 5-HT and DA

5-hydroxytryptamine and DA belong to monoamine neurotransmitters in synaptic cleft, the dwindled contents of which cause anxiety disorder, but the two expression levels can be increased with the improvement of clinical symptoms after treatment.

Xu et al. [51] demonstrated that gavage of BEO for 12 days can upregulate 5-HT and DA levels. Huang et al. [52] unraveled that gavage of Foshouningshen (the application of Citri Sarcodactylis Fructus with calming effect) decoction for a week increases the contents of 5-HT and its receptor in rat hippocampus. Further, it has been proved [53] that 5-HT receptor can indirectly interfere with BEO activity, the anti-anxiety and relaxant effects of which are exerted by means of multiple complex mechanisms. Hence, Citri Sarcodactylis Fructus may alleviate anxiety through upregulation of 5-HT and DA levels. Additionally, anti-anxiety effect of Citri Sarcodactylis Fructus through elevating DA level is few studied , which can be the research direction in the future.

4.3.2 Upregulation of GABA and downregulation of Glu

Inhibitory neurotransmitter GABA and excitatory neurotransmitter glutamate (Glu) are amino acid neurotransmitters. Reportedly, anxiety disorder is ascribed to poor information transmission in neural circuits caused by the imbalance of excitatory and inhibitory signal transmission among neurons [54,55]. When GABA synthesis is reduced and GABA transmission is damaged, sympathetic nerves will be activated and anxiety can occur. On this basis, selecting appropriate drugs to elevate GABA level and decrease Glu level in hippocampus may be contributive to treating anxiety disorder.

Rombolà et al. [56] unraveled that systemic BEO administration induces appropriate release of GABA in the hippocampus of freely moving rats. Amantea et al. [57] corroborated that intraperitoneal administration of BEO 1 h before occlusion of the middle cerebral artery prominently declines excitatory amino acids, namely aspartate (Asp) and Glu, in orbitofrontal cortex, manifesting that Citri Sarcodactylis Fructus may mitigate anxiety disorder by increment of GABA level and decrement of Glu level. Nonetheless, Morrone et al. [58] found that it was dose-dependent. When perfused into the hippocampus of rat via the dialysis probe, BEO produced a significant increase of extracellular Asp, GABA as well as glutamate. At relatively high concentrations, the BEO decreased Asp and Glu levels,while Relatively low concentrations of beo increase Asp and Glu levels, which was dependent on extracellular Ca(2+). Therefore, the related mechanism can be further probed.

4.4 Upregulation of brain-derived neurotrophic factor (BDNF) levels

BDNF can modulate synaptic plasticity and promote the growth, development and regeneration of neurons [59]. Xie et al. [60] found that CUMS anxiety disorder model mice have decreased BDNF level. Li et al. [61]

also confirmed that BDNF level is evidently reduced in anxiety disorder patients, suggesting a decline in neurotrophy, and that BDNF expression is lessened as anxiety level is increased, hinting that aberrant expression of BDNF is intimately related to anxiety behaviors.

Of note, Gao et al. [62] established that continuous administration of BEO for 21 days dose-dependently upregulates BDNF level in hippocampus of CUMS model rats, which is consistent with the findings of Saiyudthong et al. [63]. These data signified that Citri Sarcodactylis Fructus may play an anti-anxiety role via increasing BDNF level in hippocampus and boosting neurotrophic action.

4.5 Regulation of neuroendocrine axis

4.5.1 Suppression of hyperactive HPA axis

Dysfunction of HPA axis is a vital neuroendocrine mechanism for the occurrence and development of anxiety disorder.Under the state of stress, the hypothalamus of patients with anxiety disorder will secrete excess corticotrophin-releasing hormone (CRH), which stimulates the secretion of adrenocorticotropic hormone (ACTH) and leads to excessive glucocorticoid (GC) produced by adrenal gland. GC primarily contains human- and animal-secreted cortisol (CORT). Hyperactive HPA axis, increasing CORT level and hindered negative feedback mechanism of HPA axis form a vicious circle and aggravate anxiety condition [60]. Xiao et al. [64] found that suppressing HPA axis hyperactivity can relieve anxiety degree in anxiety-like rats.

Gao et al. [62] verified that BEO treatment for 21 days dramatically dwindles serum CORT content in CUMS model rats in a dose-dependent manner. Saiyudthong et al. [63] discovered that intraperitoneal injection of 2.5% BEO can reduce GC level and dampen hyperactive HPA axis to alleviate anxiety. A study [65] identified that BEO can distinctly lower CORT level in mouse plasma, weaken the activity of HPA axis, and thereby alleviate the stress anxiety caused by the elevated plus maze. Watanabe et al. [62] instructed 41 healthy women to inhale BEO vapor for 15 min, and found that compared to control group (inhalation of steam or rest), BEO vapor group has lower CORT level in saliva, reflecting that suppressing hyperactive HPA axis can ameliorate anxiety. Saiyudthong et al. [13] revealed that BEO exhibit anxiolytic-like behaviours and attenuated HPA axis activity by reducing the CORT response to pressure.

4.5.2 Enhancement of HPT function

HPT axis is another neuroendocrine axis composed of hypothalamus, pituitary and thyroid gland. Hypothalamus secretes thyrotropin-releasing hormone (TRH) that stimulates the secretion of thyroid-stimulating hormone (TSH) by pituitary, and TSH further promotes the secretion of thyroid hormone (TH) by thyroid gland, including thyroxine (T4) and triiodothyronine (T3) [29]. An existing report [66] unveiled that in anxiety disorder patients, the response of HPT axis is weakened, that is, hypothyroidism can induce anxiety. T3, T4 and free triiodothyroxine (FT3) levels have been confirmed to be lessened in serum of anxiety-like rats.

Song et al. [67] revealed that the aqueous extracts of Citri Sarcodactylis Fructus and Citri fructus visibly elevate serum T3, T4 and FT3 levels, decline CORT and TRH contents, and attenuate the hypofunction of HPT axis and hyperactivity of HPA axis; notably, the effects on HPT axis is stronger than that on HPA axis. Nonetheless, rare research has emphasized the anti-anxiety of Citri Sarcodactylis Fructus by enhancing the function of HPT axis, which, therefore, is a new direction worth investigating.

Table 1. The anti-anxiety mechanism of BEO

Main mechanism	Target	Trend	Possibly relevant chemical component

Immunoregulation	Inflammatory cytokines	TNF-α	↓	Limonene, hesperidin
		IL-1ß	↓	Limonene, hesperidin
		IL-6	↓	Limonene
	T lymphocytes	$CD3^+T$	↑	Polysaccharide
		$CD4^+T$	↑	Polysaccharide
	/	PGE2	↓	Limonene
Oxidative stress	Antioxidase	SOD	↑	Polysaccharide, flavone, hesperidin
		CAT	↑	Polysaccharide, flavone, hesperidin
		GSH-Px	↑	Polysaccharide, flavone, hesperidin, bergapten
	Peroxide	MDA	↓	Polysaccharide, flavone, hesperidin
	/	PCSK9	↓	Polysaccharide, flavone
Regulation of neurotransmitters	Monoamine neurotransmitter	5-HT	↑	Bergapten
		DA	↑	Bergapten
	Amino acid neurotransmitter	GABA	↑	Bergapten
		Glu	↓	Bergapten
Promotion of neurotrophic action		BDNF	↑	Bergapten
Inhibition of hyperactive HPA axis		GC/CORT	↓	Coumarins
Enhancement of HPT functions		T3	↑	/

	FT3	↑
	T4	↑
	TRH	↑

5 Conclusion and prospect

Anxiety disorder is one of the mental diseases endangering human physical and mental health globally. Citri Sarcodactylis Fructus, as a common Chinese medicine against anxiety disorder, has an active component BEO that can regulate networks through neuroendocrine immunity system, including immunoregulation, modulation of neurotransmitter levels, improvement of neurotrophy, enhancement of the function of HPT axis, and suppression of hyperactive HPA axis, so as to alleviate anxiety symptoms. Anti-anxiety with Citri Sarcodactylis Fructus has a satisfactory clinical effect, with good drug compliance, no obvious side effects and easy application. Although the current research on the anti-anxiety mechanism of Citri Sarcodactylis Fructus has preliminarily reached the molecular stage, there still exists controversy on its anti-anxiety outcome and mechanism, due to complex components and multiple functions and targets. In the future, the mechanism should be deeply explored through enriching sample sources, increasing observation indicators, extending observation time, conducting experiments, and utilizing methods such as genomics, proteomics, epigenetics, and network pharmacology.

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Conflict of Interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization, Ting Chen; Data curation,Ting Chen; Formal analysis, Ting Chen; Methodology, Ting Chen; Writing-Original draft, Ting Chen; Writing-review and editing, Ting Chen; All authors have read and agreed to the published version of the manuscript.

Ethics Approval and Consent to Participate

The study was approved by the Medical Ethics Committee, and the patients were informed and consented.

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Availability of Data and Materials

The data presented in this study are available on request from the corresponding author.

Supplementary Material

Not applicable

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