

# An Experimental Evaluation of Antistress Activity of Tulsi (*Ocimum sanctum* Linn.)

Dr. Agha Parvez Masih<sup>1</sup>, Dr. S. Shabihe Raza Baqri<sup>2</sup>, Dr. Afroz Zareen Athar<sup>3</sup>

Associate Professor, Department of Zoology, Shia PG College, Lucknow, Uttar Pradesh, India<sup>1,2</sup>

Assistant Professor, Department of Zoology, Shia PG College, Lucknow, Uttar Pradesh, India<sup>3</sup>

**ABSTRACT:** Three new compounds, ocimumosides A (1) and B (2) and ocimarin (3), were isolated from an extract of the leaves of holy basil (*Ocimum sanctum*), together with eight known substances, apigenin, apigenin-7-O- $\beta$ -d-glucopyranoside, apigenin-7-O- $\beta$ -d-glucuronic acid (4), apigenin-7-O- $\beta$ -d-glucuronic acid 6'-methyl ester, luteolin-7-O- $\beta$ -d-glucuronic acid 6'-methyl ester, luteolin-7-O- $\beta$ -d-glucopyranoside, luteolin-5-O- $\beta$ -d-glucopyranoside, and 4-allyl-1-O- $\beta$ -d-glucopyranosyl-2-hydroxybenzene (5), and two known cerebrosides. The structures of the new compounds were determined on the basis of extensive 1D and 2D NMR spectroscopic analysis. The new compounds (1–3) and the known compounds 4 and 5 were screened at a dose of 40 mg/kg body weight for acute stress-induced biochemical changes in male Sprague–Dawley rats. Compound 1 displayed promising antistress effects by normalizing hyperglycemia, plasma corticosterone, plasma creatine kinase, and adrenal hypertrophy. Compounds 2 and 5 were also effective in normalizing most of these stress parameters. In contrast, compounds 3 and 4 were ineffective in normalizing any of these effects.

**KEYWORDS:** antistress, experimental, tulsi, *Ocimum sanctum*, compounds, evaluation

## I. INTRODUCTION

The medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day to day practice. In traditional systems of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *Ocimum sanctum* Linn (known as Tulsi in Hindi), a small herb seen throughout India.

*Ocimum sanctum* described as sacred and medicinal plant in ancient literature, Tulsi is known as the “Queen of plants” which is derived from ‘Sanskrit’, which means “the incomparable one” or “matchless one”. This plant belongs to the family Lamiaceae which is native throughout the Old World tropics and cultivated for religious and medicinal purposes. [1,2]

Phytochemicals	Present	in	<i>Ocimum</i>	<i>sanctum</i>	
S/No	Extracts	Phyto	Chemicals	Plant	Parts
1. Fixed oil					
Linoleic acid, Linolenic acid, Oleic acid, Palmitric acid, Stearic acid.					

### Seeds

2. Essential oil

Aromadendrene oxide, Benzaldehyde, Borneol, Bornyl acetate, Camphor, Caryophyllene oxide, cis- $\alpha$ -Terpineol, Cubenol, Cardinene, D-Limonene, Eicosane, Eucalyptol, Eugenol, Farnesene, Farnesol, Furaldehyde, Germacrene,

# International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: [www.ijirset.com](http://www.ijirset.com)

Vol. 8, Issue 11, November 2019

Heptanol, Humulene, Limonene, n-butylbenzoate, Ocimene, Oleic acid, Sabinene, Selinene, Phytol, Veridifloro,  $\alpha$ -Camphene,  $\alpha$ - Myrcene,  $\alpha$ -Pinene,  $\beta$ -Pinene,  $\alpha$ -Thujene,  $\beta$ -Guaiene,  $\beta$ - Gurjunene, methyl chavicol and linalool.



*Ocimum sanctum*

## Leaves

3. Mineral Contents<sup>8</sup> Vitamin C, Vitamin A, Calcium, Phosphours, Chromium, Copper, Zink, Iron.

## Whole

4. Alcoholic Extract<sup>2</sup> Aesculectin, Aesculin, Apgenin, Caffiec acid, Chlorogenic Acid, Circineol, Gallic Acid, Galuteolin, Isorientin, Isovitexin, Luteolin, Molludistin, Orientin, Procatechuic acid, Stigmsterol, Urosolic acid, Vallinin, Viceni, Vitexin, Vllinin acid.

## Plant

## II. DISCUSSION

### Antianxiety and Antidepressant Activity:

Chatterjee et al. In 2011 studied the effect of ethanol extract of leaves of *Ocimum sanctum* in Swiss albino mice, against both anxiety and depressive disorder. Depression was studied through tail suspension test and forced swim test. Anxiety experiments included light dark test, elevated plus maze test, and holeboard test. The *Ocimum sanctum* extracts shows antianxiety and antidepressant properties at the same dose and can be a potential therapeutic agent against mixed anxiety and depressive syndrome.

### Neuroprotective Activity:

*Ocimum sanctum* shows ameliorative potential in attenuating vincristineinduced peripheral neuropathic pain in rats, which may be attributed to decrease in oxidative stress and calcium levels. Administration of OS (100 and 200 mg/kg

## International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: [www.ijirset.com](http://www.ijirset.com)

Vol. 8, Issue 11, November 2019

p.o.) and its saponin rich fraction (100 and 200 mg/kg p.o.) for 14 days significantly attenuated vincristine-induced neuropathic pain along with decrease in oxidative stress and calcium levels.[3,4]

### PHARMACOLOGICAL ACTIVITIES

	<b>Therapeutic activity</b>	<b>Extract used</b>	<b>Part used</b>
1	Anti-stress	Ethanolic	Whole plant (dried)
2	Anti-inflammatory	Methanolic/aqueous	Leaves
3	Anti-fungal	Methanolic/Ethanolic	Leaves
4	Anti-fertility	Benzene	Leaves
5	Hepatoprotective	Ethanolic/aqueous	Whole plant (aerial)
6	Anti-diabetic	Ethanolic/aqueous	Leaves
7	Anti-ulcer	Ethanolic/aqueous	Leaves
8	Anti-microbial	Ethanolic	Leaves
9	Anti-psychotic	Methanolic/ leaves paste	Leaves
10	Anti-cancer	Ethanolic	Root

#### Antifatigue activity

*Ocimum sanctum* L. is known for its medicinal properties and also has been considered sacred in India since Vedic times. The aqueous suspension of 70% alcoholic extract of *Ocimum sanctum* L. was investigated for antifatigue activity. Rats were subjected to Weight-loaded Forced Swim Test (WFST) every alternate day for 2 weeks. *O. sanctum* at a dosage of 150, 300 and 450 mg kg<sup>-1</sup> b.wt. was administered to rats every day. Swimming time, change in body weight, lipid peroxidation, Lactic Acid (LA), glycogen and blood biochemical parameters namely hemoglobin (Hb%), Blood Urea Nitrogen (BUN) and Creatine Kinase (CK) were evaluated as biomarkers of physical fatigue. The *O. sanctum* treatment was found to lower malondialdehyde (MDA) and lactic acid levels in liver and muscle tissues compared with control exercise group (p<0.05). Serum biochemical parameters viz., BUN and CK activity were also reduced as compared with control exercise group (p<0.05). The present study thus indicates that out of three studied treatments of *O. sanctum* (*O. sanctum* -150, 300 and 450); the *O. sanctum* at the dose of 300 mg kg<sup>-1</sup> b.wt. showed better performance against fatigue when compared with other two treatments. As treatment at 300 mg kg<sup>-1</sup> b.wt.

## International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

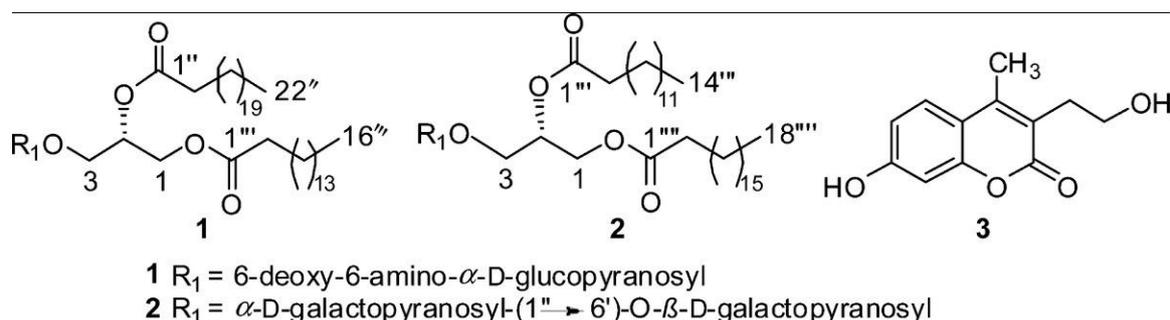
Visit: [www.ijirset.com](http://www.ijirset.com)

Vol. 8, Issue 11, November 2019

facilitated the aerobic glucose metabolism and promote swimming time, suggesting that *O. sanctum* ameliorates the various impairments associated with physical fatigue.

### Antistress activity

Environmental factors like stress can influence the neurobehavioral profile of the organism and precipitate an anxiety-like syndrome. Behavioral factors, such as emotionality, are useful predictors of stress susceptibility.



### Anti-stress constituents of *Ocimum sanctum*

The present study was aimed at investigating the anti-stress effects of *Ocimum sanctum* and compare it with Alprazolam in mice and rats. *Ocimum sanctum* is a medicinal plant distributed mainly in the tropical and subtropical regions including India. Besides being widely used as a religion plant, it has also been used as indigenous medicine in several other countries in Asia and Africa. Alprazolam is a drug belonging to newer benzodiazepine group. It is an antianxiety benzodiazepine derivative chemically and pharmacologically related to the other drugs in this class. They preferentially act on the mid brain, ascending reticular formation, limbic system by enhancing the presynaptic/postsynaptic inhibition through a specific BZD receptor which is an integral part of the GABA<sub>A</sub> receptor chloride channel complex. When habituated mice were exposed to forced swim test (FST) which induces stress, the swimming performance of mice will be impaired leading to immobility period. The forced swim test is used to evaluate the effects of the drugs like antidepressants in the central nervous system. In the present study, normal control mice were allowed to swim for six minutes in order to induce stress. [5,6]

## International Journal of Innovative Research in Science, Engineering and Technology

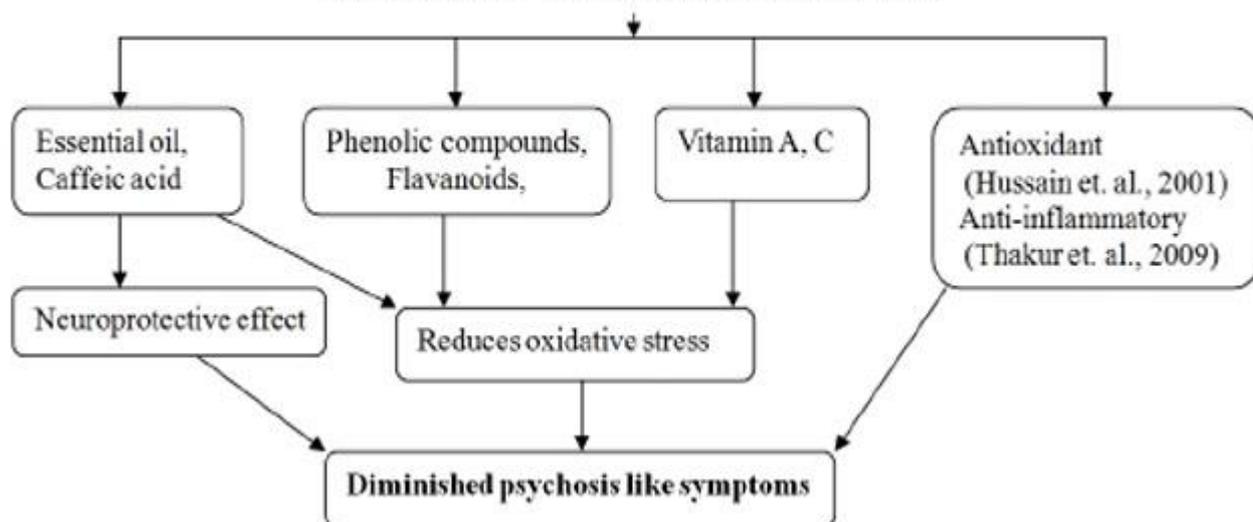
(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: [www.ijirset.com](http://www.ijirset.com)

Vol. 8, Issue 11, November 2019

When *Ocimum sanctum* and Alprazolam were administered to stress induced mice, these drugs significantly decreased the immobility period. Administration of *Ocimum sanctum* and Alprazolam has altered the behavioral performance of stress induced mice in forced swim test.

### Possible protective mechanism of action of *Ocimum sanctum*



The change in mobility period induced by stress was significantly ameliorated by a single oral administration of Alprazolam and *Ocimum sanctum* as these drugs significantly decreases the immobility period because of its anti-stress effect. The change in the mobility period after administration of *Ocimum sanctum* is in according to the study conducted by Bhargva and Singh (1981) who have reported *Ocimum sanctum* to posses adaptogenic (anti-stress) properties. *O. sanctum* leaf extract was found to produce an effect which was similar to those produced by low doses of barbiturates in pharmacological studies but, at the same time, produced some effects which were reminiscent of amphetamines. These results suggest that the effects of *O. sanctum* on the central nervous system might involve dopaminergic neurons. The drug increased the physical endurance (increased survival time) of swimming mice, prevented stress-induced ulcers in rats: Protected mice and rats. A 70% ethanolic extract of *O. sanctum* leaves administered to Wistar strain albino rats had a normalizing action on discrete regions of the brain and controlled the alteration in neurotransmitter levels due to noise stress, emphasizing the anti-stressor potential of this herb. However, *Ocimum sanctum* has less magnitude of anti stress activity when compared to Alprazolam in FST. [15]

### III. RESULTS

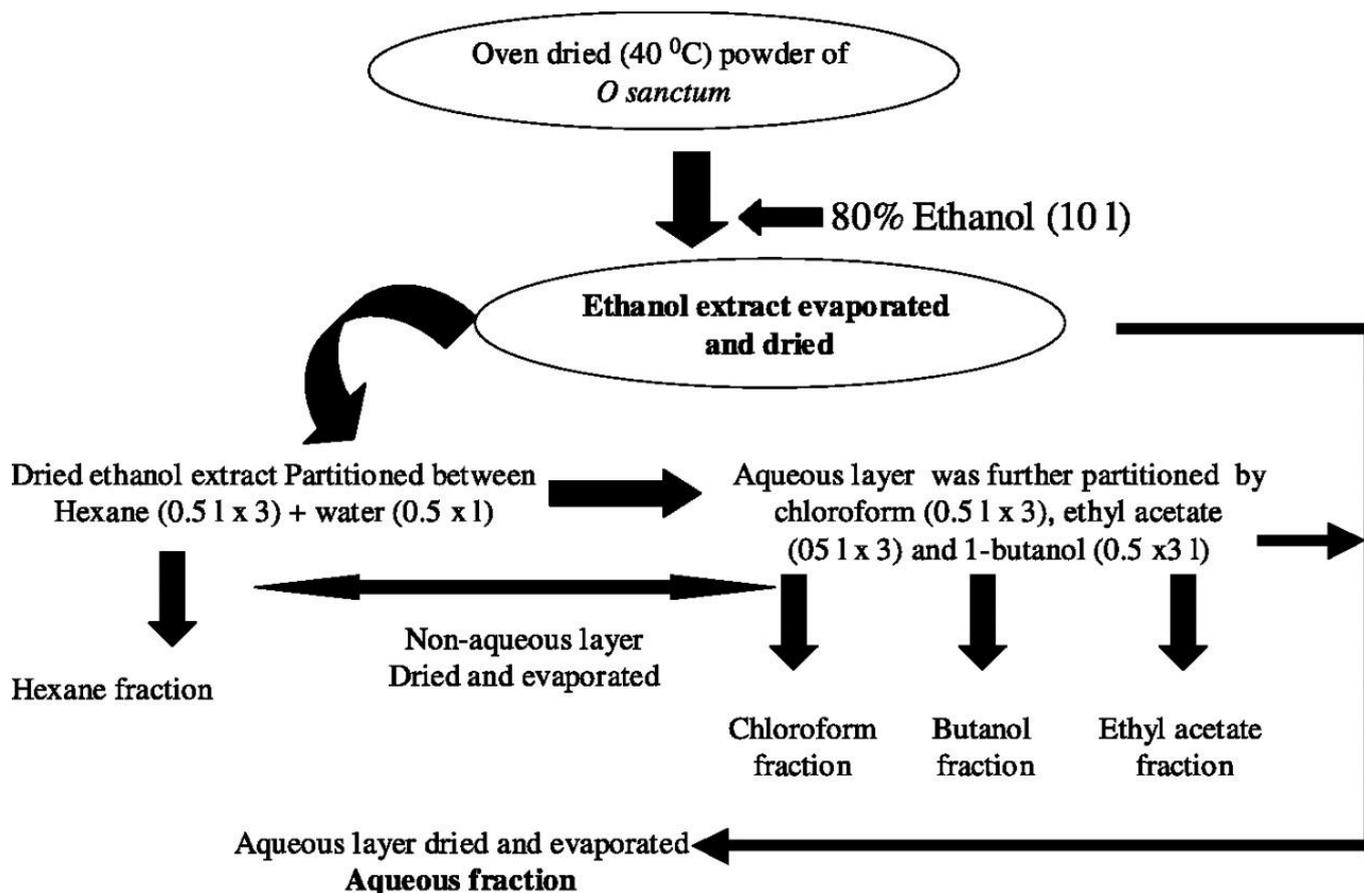
The impairment of performance on rotarod appears to be a cumulative consequence of stress. Our results revealed that stress impaired performance on the rotarod. The stress induced impairment of rotarod performance was significantly ameliorated by both Alprazolam and *Ocimum sanctum* after administration of single dose.

## International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: [www.ijirset.com](http://www.ijirset.com)

Vol. 8, Issue 11, November 2019



### Tulsi leaf extracts are antistress

However, *Ocimum sanctum* has less magnitude of anti-stress activity when compared to Alprazolam. Ameliorating effect of Alprazolam and *Ocimum sanctum* on stress-induced impairment of rotarod performance is thought to be based on the anti-stress effect (Fig. 2). This effect could be due to sedative action as well as ataxic action of Alprazolam and *Ocimum sanctum* and might be acting through the serotonergic system or it could be due to the change in the humoral response as reported by PK Mediratta et al (1988) have reported that *Ocimum sanctum* modulates the humoral response by acting at various levels in the immune mechanism, such as anti body production, release of mediators of hypersensitivity[13,14]

Reactions and tissue response to these mediators. Bhattacharya et al stated *Ocimum sanctum* potentially regulates the HPA axis in stress-related disorders. *O. sanctum* seemed to possess sedative properties for they strongly increased and, in a dose-dependent manner, the sleeping time induced by diazepam. Although mechanism of action of antistress activity of Alprazolam is well known but the exact mechanism of action of *O. sanctum* is yet to be ascertained. But it could be due to because *O. sanctum* plant contained alkaloids, glycosides, tannins and saponins. The leaves contain ascorbic acid (85/100 mg) and carotene (2.5/100 mg). the overall activity of *O. sanctum* may be due to the numerous constituents that is gas liquid chromatography of the essential oil also revealed the presence of eugenol (70%) as major

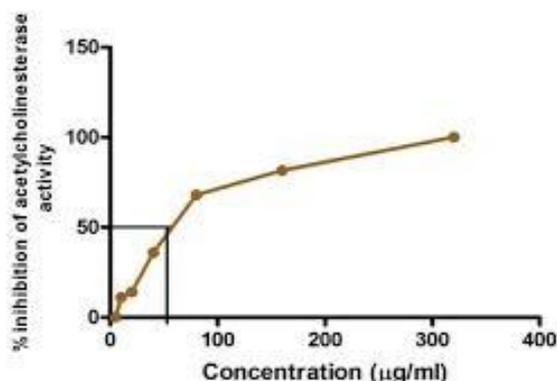
# International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: [www.ijirset.com](http://www.ijirset.com)

Vol. 8, Issue 11, November 2019

constituent; other components identified were nerol, eugenol methyl ether, caryophyllene, terpinene, decylaldehyde, gammaselinene, alpha-pinene, beta-pinene, camphor and carvacrol.



Gunasegaran R Ramachandhra NAG have reported that the leaves yield ursolic acid, apigenin, luteolin, apigenin-7-0-glucuronide, luteolin-7 – 0 glucuronide and molludistin.<sup>34</sup> The mechanism of action of *O. sanctum* may be due to<sup>36</sup> exhibition of cortisol sparing effect, they act on the central nervous system as a stimulant. They may act as immune stimulant, It scavenges free radicals in the brain, induces a state of nonspecific increased resistance (SNIR). The study revealed that *O. sanctum* possess maximal antistress activity but the efficacy and magnitude of antistress activity of *O. sanctum* is less, when compared to the standard anxiolytic agent. However, the exact mechanism of action needs to be evaluated and, hence, further studies are needed on human beings, on stress.[7,8]

## IV. CONCLUSIONS

Plants have been used for the treatment of diseases throughout the world since the beginning of civilization. Tulsi is cultivated for religious and medicinal purposes. It is widely known across South Asia as a medicinal plant and an herbal tea. Several medicinal properties have been attributed to the plant not only in Ayurveda and Siddha but also in Greek, Roman and Unani. The vast survey of literature showed that *Ocimum sanctum* has a huge spectrum of pharmacological activities. It has an esteemed status in herbs with diverse biological potentials and has a great scope for further new area of investigations. Traditionally crude extracts of various parts of plants have been used for their antidiabetic, antioxidant, antistress, antihyperlipidemic and antibacterial properties. Future research on sacred basil should be emphasized for control of various diseases especially it should be explore as a significant remedy regarding neuropsychological disorders for the welfare & service of mankind.[9,10]

## International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: [www.ijirset.com](http://www.ijirset.com)

Vol. 8, Issue 11, November 2019



Stress is the physiological, psychological and behavioral response by individuals when they perceive a lack of equilibrium between the demands placed upon them and their ability to meet those demands, which, over a period of time, leads to ill health. Symptoms of stress include fatigue, tenseness, irritability, apathy, sleeping disorders, emotional instability, thoughts and concentration problems. Stress induces a variety of autonomic, visceral, immunological, and neurobehavioral responses, such as anxiety, depression, anorexia, syncope and activation of the hypothalamic-pituitary-adrenal axis resulting in elevated corticosterone levels, in animals and humans. There are several ways of coping with stress. Stress revealed to be high risk factor for cancer and heart disease than either smoking or intake of high cholesterol diet. Some techniques of time management may help a person to control stress.

Techniques of stress management may include some of the following like autogenic therapy, cognitive therapy, exercise, meditation, deep breathing, natural medicine and pharmacotherapy. Use of medicinal plants is very old, this indicates that therapeutic use of plants is as old as 4000-5000 BC earliest use of plants as medicine appear in Rigveda which is said to be written between 3500 and 1600 BC which is basic foundation of ancient medical science in India. Millions of people around the world use traditional medicines to relieve the symptoms of the stress or to cure it. Indian system of traditional medicine describes remedies based on herbal supplementation, minerals and other therapeutic procedures for enhancing physical and mental performance to evade stress levels. Among all medicinal plants, *Ocimum sanctum* is a traditional medicinal plant belongs to Labiatae family which grows different parts of world. Different parts of *Ocimum sanctum* like roots, stem, leaves, flowers and seeds possess medicinal properties and are used for treatment for many pathologic conditions as analgesic, antipyretic, anticancer, expectorant, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidemic and also been used in arthritis, bronchitis, convulsions and antistress agent. The main factors that make natural products attractive candidates for human use include their ease of availability, cost effectiveness and presumed safety. [11,12]

# International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: [www.ijrset.com](http://www.ijrset.com)

Vol. 8, Issue 11, November 2019

## REFERENCES

1. Woo-Ri Kim, Eun Ok Kim, Kyungsu Kang, Sarangerel Oidovsambuu, Sang Hoon Jung, Byung Sup Kim, Chu Won Nho, and Byung-Hun Um . Antioxidant Activity of Phenolics in Leaves of Three Red Pepper (*Capsicum annum*) Cultivars. *Journal of Agricultural and Food Chemistry* **2014**, 62 (4) , 850-859. <https://doi.org/10.1021/jf403006c>
2. Eric H. Andrianasolo, Liti Haramaty, Assaf Vardi, Eileen White, Richard Lutz and Paul Falkowski. Apoptosis-Inducing Galactolipids from a Cultured Marine Diatom, *Phaeodactylum tricornutum*. *Journal of Natural Products* **2008**, 71 (7) , 1197-1201. <https://doi.org/10.1021/np800124k>
3. L. d'Aquino, B. Lanza, E. Gambale, M. Sighicelli, P. Menegoni, G.C. Modarelli, J. Rimauro, E. Chianese, G. Nenna, T. Fasolino, G. D'Urso, S. Piacente, P. Montoro. Growth and metabolism of basil grown in a new-concept microcosm under different lighting conditions. *Scientia Horticulturae* **2011**, 299 , 111035. <https://doi.org/10.1016/j.scienta.2022.111035>
4. Hien Thu Nguyen, Xoan Thi Le, Tai Van Nguyen, Hoa Nhu Phung, Hang Thi Nguyet Pham, Khoi Minh Nguyen, Kinzo Matsumoto. Ursolic acid and its isomer oleanolic acid are responsible for the anti-dementia effects of *Ocimum sanctum* in olfactory bulbectomized mice. *Journal of Natural Medicines* **2015**, 4 <https://doi.org/10.1007/s11418-022-01609-2>
5. Kakali Baruah, Mahabul Haque, Leader Langbang, Sourav Das, Kripamoy Aguan, Atanu Singha Roy. *Ocimum sanctum* mediated green synthesis of silver nanoparticles: A biophysical study towards lysozyme binding and anti-bacterial activity. *Journal of Molecular Liquids* **2013**, 337 , 116422. <https://doi.org/10.1016/j.molliq.2021.116422>
6. Xoan Thi Le, Hien Thu Nguyen, Tai Van Nguyen, Hang Thi Nguyet Pham, Phuong Thi Nguyen, Khoi Minh Nguyen, Ba Van Nguyen, Kinzo Matsumoto, . *Ocimum sanctum* Linn. Extract Improves Cognitive Deficits in Olfactory Bulbectomized Mice via the Enhancement of Central Cholinergic Systems and VEGF Expression. *Evidence-Based Complementary and Alternative Medicine* **2010**, 2021 , 1-12. <https://doi.org/10.1155/2021/6627648>
7. Lata Rana, Geeta Tewari, Chitra Pande. Aroma Profile of the Aerial Parts of *Ocimum sanctum* L. Harvested at Vegetative and Full Blooming Stages from Three Atitudes of North India. *Journal of Essential Oil Bearing Plants* **2008**, 24 (3) , 408-420. <https://doi.org/10.1080/0972060X.2021.1939168>
8. Sachin Neekhara, Himani Awasthi, Dharmchand Prasad Singh. *Streblus asper* attenuates stress-induced physical and Biochemical changes in Rat Model. *Research Journal of Pharmacy and Technology* **2001**, , 1910-1914. <https://doi.org/10.52711/0974-360X.2021.00337>
9. Venugopal Singamaneni, Bashir Lone, Jasvinder Singh, Pankaj Kumar, Sumeet Gairola, Shashank Singh, Prasoon Gupta. Coronarin K and L: Two Novel Labdane Diterpenes From *Roscoea purpurea*: An Ayurvedic Crude Drug. *Frontiers in Chemistry* **2012**, 9 <https://doi.org/10.3389/fchem.2021.642073>
10. Hyemi Lee, Xiangyu Zhao, Young-Ok Son, Siyoung Yang. Therapeutic Single Compounds for Osteoarthritis Treatment. *Pharmaceuticals* **2010**, 14 (2) , 131. <https://doi.org/10.3390/ph14020131>
11. Eman Maher Zahran, Usama Ramadan Abdelmohsen, Hany Ezzat Khalil, Samar Yehia Desoukey, Mostafa Ahmed Fouad, Mohamed Salah Kamel. Diversity, phytochemical and medicinal potential of the genus *Ocimum* L. (Lamiaceae). *Phytochemistry Reviews* **2003**, 19 (4) , 907-953. <https://doi.org/10.1007/s11101-020-09690-9>
12. Kyung Jo Jo, Gun He Nam, Ye Seul Park, Hye Won Kawk, Jong Tae Kim, Won Hee Choi, Seung Hee Jang, Min Jeong Kim, Young Min Kim. Evaluation of Stress-related Behavioral and Biological Activity of *Ocimum sanctum* Extract in Rats. *Biotechnology and Bioprocess Engineering* **2008**, 25 (2) , 170-180. <https://doi.org/10.1007/s12257-019-0365-2>
13. Sudhir Chandra Sarangi, Soumya S. Pattnaik, Jatinder Katyal, Thomas Kaleekal, A.K. Dinda. An interaction study of *Ocimum sanctum* L. and levetiracetam in pentylenetetrazole kindling model of epilepsy. *Journal of Ethnopharmacology* **2002**, 249 , 112389. <https://doi.org/10.1016/j.jep.2019.112389>
14. Shahid Akbar. *Ocimum tenuiflorum* L. (Lamiaceae). **2007**,,, 1339-1357. [https://doi.org/10.1007/978-3-030-16807-0\\_138](https://doi.org/10.1007/978-3-030-16807-0_138)
15. Madhumita Srivastava, Manju Singh, Priyanka Maurya, Nupur Srivastava, Namita Gupta, Karuna Shanker. Simultaneous quantification of five bioactive phenylethanoid, iridoid, and flavonol glycosides in *Duranta erecta* L.: Ultra performance liquid chromatography method validation and uncertainty measurement. *Journal of Pharmaceutical and Biomedical Analysis* **2002**, 174 , 711-717. <https://doi.org/10.1016/j.jpba.2019.06.044>