ORIGINAL ARTICLE

Screening of antioxidant and antidepressant activity of *Vanda tessellata* leaves extract

Bindiya Prakash[1]*, Ritu Thakur Bais[2], Ram Kumar Sahu[3]

[1]Department of Botany, Sarojini Naidu Government Girls Post Graduate (Autonomous) College, Shivaji Nagar, Bhopal, Madhya Pradesh, India, [2]Department of Botany, Government Maharani Laxmi Bai Girls Post Graduate (Autonomous), College, Bhopal, Madhya Pradesh, India, [3]University College of Pharmacy, Pt. Deendayal Upadhyay Memorial Health Sciences and Ayush University of Chhattisgarh, Raipur, Chhattisgarh, India

Abstract

Aim: *Vanda tessellata* is an endangered orchid of high medicinal value plant containing flavonoid and polyphenol components. The present study was aimed to evaluate the antioxidant and antidepressant activity of leaves extract of *V. tessellata*. **Materials and Methods:** The chloroform and ethanol extracts were prepared from *V. tessellata* leaves. The total phenolic content and total flavonoid content were determined for antioxidant activity of extracts. Antidepressant activity was identified using a modified forced swimming test (FST) and tail suspension test (TST). **Results and Discussion:** The total phenolic content of chloroform and ethanol extracts of *V. tessellate* were 98.25 ± 0.11 and 202.00 ± 0.21 gallic acid equivalent mg/gm, respectively. The concentrations of flavonoids in chloroform and ethanol extracts of *V. tessellate* were 23.57 ± 0.47 and 114.48 ± 0.12 quercetin equivalent mg/gm, respectively. The ethanol extract demonstrated significant antidepressant activity by a reduction in immobility times of rats in TST and FST. **Conclusion:** The findings concluded that the antidepressant activity of *V. tessellate* extracts may be due to the presence of polyphenol and flavonoids.

Key words: Total flavonoid content, total phenolic content, *vanda tessellata*

INTRODUCTION

Reactive oxygen species (ROS)/free radicals are a vital risk factor in the development of various chronic diseases. ROS are renowned as agents involved in the pathogenesis of >100 diseases including asthma, diabetes, inflammation, cancer, depression disorders, atherosclerosis, liver cirrhosis, immunosuppression, and nephrotoxicity. The numerous previous studies demonstrated that ROS are also responsible for human aging. ROS are innumerable forms of activated oxygen such as superoxide anion radicals and hydroxyl radicals.

An imbalance between ROS and the inherent antioxidant capacity of the body suggested using of dietary and/or medicinal supplements. Previous studies indicated that fruits, vegetables, and plants are the main source of antioxidant in the diet. The antioxidants compound such as phenolics, flavonoids, tannins, and proanthocyanidins scavenge free radicals such as peroxide, hydroperoxide, or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Natural antioxidants may have free-radical scavengers, reducing agents, complexes of pro-oxidant metals, quenchers of singlet oxygen, etc. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress. It has been documented by researchers that medicinal plants are considered as a good antioxidant since prehistoric times.[1-3]

Depression is a neurological disorder that leads to changes in mood, thoughts, behavior, and physical health. It is reported that 21% of the global population affects from depression. Depression can happen at any age groups from

Address for correspondence:
Bindiya Prakash, Department of Botany, Sarojini Naidu Government Girls Post Graduate (Autonomous) College, Shivaji Nagar, Bhopal, Madhya Pradesh, India.
Phone: +91-8120718626.
E-mail: prakashbindiya32@gmail.com

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childhood to later life. It is estimated that >20% of the adult population undergoes from these conditions at least some time in their lifetime. The world health organization expects that depression will become the second leading cause of premature death or disability all over the world by the year 2020. At present, synthetic drug prescribed for depression is expensive and associated with various side effects, namely body weight gain, cardiac toxicity, and sexual dysfunction. There is emergent interest in the use of herbal medicines for the management of depressive disorders due to the minimum side effect and economic.[4-7]

Among the various medicinal plants, *Vanda tessellata* is one such orchid having a number of medicinal properties. It is an epiphytic orchid. *Vanda tessellate* plants have been used in indigenous medicine such as Ayurveda and local traditional medical practices. The different pharmacological properties such as anti-inflammatory, anticonvulsant, hepatoprotective, antidiarrheal, and aphrodisiac properties of *V. tessellate* have been reported. The phytochemical screening of *V. tessellata* has exhibited an alkaloid, sitosterol, resin, saponin, polyphenol, flavonoids, tannins, fatty acids, coloring agents, etc.[8-11] Hence, the present study was designed to evaluate the antioxidant and antidepressant activity of leaves extract of *V. tessellata*.

MATERIALS AND METHODS

Plant Material

The leaves of *V. tessellata* were selected for the proposed study. The leaves were shade dried, reduced to coarse powder and stored in an airtight container until further use.

Preparation of Extract

The powdered leaves of *V. tessellate* about 500 g were packed in Soxhlet apparatus and extracted with successively with chloroform and ethanol, until the completion of the extraction. The extract was filtered while hot, and the resultant extract was distilled in vacuum under reduced pressure to remove the solvent completely, and later dried in a desiccator.

Total Polyphenol Content

Total polyphenol content was determined using the colorimetric method. 1.0 ml of the prepared extract was oxidized using 2.5 ml of Folin–Ciocalteu reagent, and 2.0 ml of sodium carbonate solution (75 g/l) was then added to the reaction mixture. The absorbance readings were taken at 760 nm after incubation at room temperature for 2 h. The amount was calculated using the gallic acid calibration curve. The results were expressed as gallic acid equivalent (GAE) mg per 100 ml of the sample (extract).

Calibration curves of gallic acid

Accurately weighed 100 mg of gallic acid was dissolved in 100 ml of distilled water which gives the concentration of 1000 µg/ml. 10 ml of this solution was taken and made up to 100 ml with gallic acid which contains the concentration of 100 µg/ml. Further, 25 ml of this solution was taken and made up to 100 ml with quercetin which contains the concentration of 25 µg/ml. Similarly, 50, 75, 100, 125, and 150 µg/ml concentrations of gallic solution were prepared. Calibration curve was plotted by mixing 1 ml aliquots of gallic acid solutions with 2.5 ml of Folin–Ciocalteu reagent and 2.0 ml of sodium carbonate solution (75 g/l). The absorbance was measured after incubation at room temperature for 2 h at 760 nm using Ultraviolet (UV) spectrophotometer, against the blank solution.

Total Flavonol Content

Flavones and flavonols contents were analyzed by the colorimetric method. 9.8 ml of the prepared extract was mixed with a 10% solution of aluminum chloride (200 µl). After 30 min, absorption was measured at a 425 nm wavelength. The amount was calculated using quercetin calibration curve. The results were expressed as the quercetin equivalent (QE) mg per 100 ml of the sample.

Calibration curves of Quercetin

Accurately weighed 100 mg of quercetin was dissolved in 100 ml of distilled water which gives the concentration of 1000 µg/ml. 10 ml of this solution was taken and made up to 100 ml with quercetin which contains the concentration of 100 µg/ml. Further, 25 ml of this solution was taken and made up to 100 ml with quercetin which contains the concentration of 25 µg/ml. Similarly, 50, 75, 100, 125, and 150 µg/ml concentrations of quercetin solution were prepared. Calibration curve was plotted by mixing 9.8 ml aliquots of quercetin solutions with a 10% solution of aluminum chloride (200 µl). The absorbance was measured 30 min at 425 nm using UV spectrophotometer, against the blank solution.[12-15]

Antidepressant Activity

All the rats were divided into the four groups; each group consisted of 6 animals. Group I received saline 2 ml/kg orally; Group II received imipramine (10 mg/kg) i.p.; and Group III and Group IV administered ethanol extracts of *V. tessellate* 200 mg/kg and 400 mg/kg, respectively orally.

Forced swim test (FST)

For the FST, rats of either sex were individually forced to swim in an open cylindrical container (diameter 10 cm and height 25 cm) containing 19 cm of water at 25 ± 1°C. Treatment was given 60 min before study as described by

study design. All animals were forced to swim for 6 min, and the duration of immobility was observed and measured during the final 4 min interval of the test. Each animal was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect.

Tail suspension test (TST)

Treatment was given 60 min before study as described by study design. Animals were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a 6 min of the 10 min period. Animal was considered to be immobile when it did not show any movement of the body, hanged passively, and completely motionless.[4]

Statistical Analysis

All the values were expressed as Mean ± structural equation modeling; the results were analyzed statistically by one-way ANOVA followed by Dunnett Multiple comparison test, $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The plants consisting numerous biologically active compounds and are responsible for revealing various pharmacological activities. The active chemical constituents exhibiting antioxidant activity compounds are preferred more over synthetic ones due to safety concerns. The natural antioxidants have been shown to reduce the risk and progression of varied diseases such as cancer, depression disorders, liver cirrhosis, renal impairment, cardiovascular, hypertension, diabetes, and neurodegenerative diseases by scavenging free radicals through various biological mechanisms

In the present study, *V. tessellate* leaves were selected to evaluate the quantitative estimation of TPC and TFC and antidepressant activity of extracts.

Antioxidant Activity

TPC of V. tessellata

The chloroform and ethanol extracts of *V. tessellate* were assessed for exploration of the total phenolic content concentrations in extracts. Standard curve of gallic acid was calculated and plotted in distilled water for determining absorption data [Table 1]. The linear equation of gallic acid was found to be y = 0.0042x−0.0029 [Figure 1]. The total phenolic content of chloroform and ethanol extracts of *V. tessellate* were 98.25 ± 0.11 and 202.00 ± 0.21 GAE mg/gm, respectively [Table 2]. The ethanol extracts exhibited the highest amount of total polyphenol content compared to chloroform extracts.

The findings of TPC are similar to the data presented by Stanković (2011) for *Marrubium peregrinum*. It is reported that phenol components are highly soluble in polar solvent, namely ethanol and results of TPC justified it.[16] Phenolic compounds are large and assorted group of plant metabolites that are known to display diverse biological activities, most of which are attributed to antioxidant activity.

TFC of V. tessellata

The content of flavonoids for chloroform and ethanol extracts of *V. tessellate* was expressed in terms of quercetin equivalents. Standard curve of quercetin was calculated and

Table 1: Absorbance by gallic acid in different concentration

Concentration	Absorbance
0	0
25	0.101
50	0.216
75	0.315
100	0.415
125	0.512
150	0.651

Table 2: Estimation of total phenolic content of *V. tessellata* extract

Extracts	Total polyphenol content (GAE mg/gm)
Chloroform extract	98.25±0.11
Ethanol extract	202.00±0.21

GAE: Gallic acid equivalent

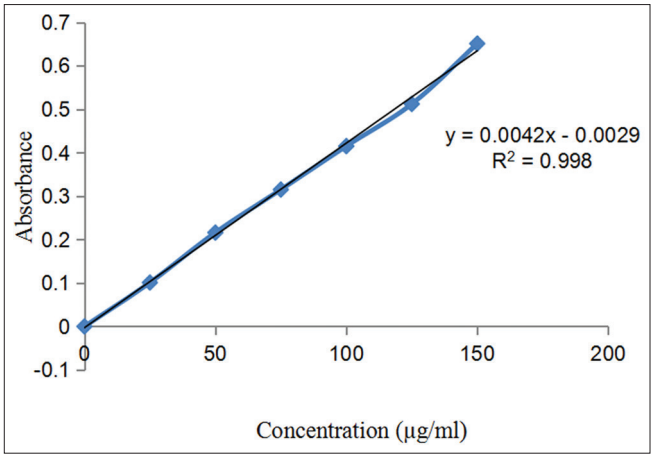

Figure 1: Calibration curve of gallic acid in distilled water

plotted in distilled water for determining absorption data [Table 3]. The linear equation of quercetin was found to be y = 0.0115x–0.0264 [Figure 2]. The content of flavonoids in chloroform and ethanol extracts of *V. tessellata* is shown in Table 4. The concentrations of flavonoids in chloroform and ethanol extracts of *V. tessellate* were 23.57 ± 0.47 and 114.48 ± 0.12 QE mg/gm, respectively. The ethanol extracts displayed the highest amount of flavonoids content compared to chloroform extracts. According to Min and Chun-Zhao (2005), the concentration of flavonoid in plant extract depends on the polarity of solvents. Hence, the maximum concentration of flavonoids was observed in ethanol extract.[17]

The polyphenol and flavonoids impart chief role in the eradication of free radicals from the body due to its antioxidant property. The antioxidant property of extract can be used for the prevention and cure of various diseases allied with free radicals. It has been reported that compounds such as the flavonoids, which contain hydroxyl, are responsible for the radical scavenging effects of most plants. The mechanism of action of the flavonoids is through scavenging or chelating processes.[15]

The primary findings of TPC and TFC of *V. tessellata* leaves extract demonstrated that extract possesses antioxidant activity and are able to produce antidepressant activity. The higher concentration of flavonoids and polyphenol was observed in ethanol extract compared to chloroform extracts. Hence, the ethanol extract of *V. tessellata* was elected for the investigation of antidepressant activity.

Antidepressant Activity

FST and TST are the most commonly used preliminary screening tests for illustrating potential antidepressant drugs.

Table 3: Absorbance by quercetin in different concentration

Concentration	Absorbance
0	0
25	0.225
50	0.558
75	0.812
100	1.139
125	1.438
150	1.681

Table 4: Estimation of total flavonoid content of *V. tessellata* extract

Extracts	Total flavonol content (QE mg/gm)
Chloroform extract	23.57±0.47
Ethanol extract	114.48±0.12

FST

Table 5 exhibited the animals treated with the ethanol extract of *V. tessellata* at doses of 200 mg/kg and 400 mg/kg significantly decreased the immobility time of animal compared to the normal group in FST. The animals treated with imipramine (10 mg/kg) significantly decreased the immobility time of animals. The increase in the motor activity of animals which elevate depressed mood by decreasing immobility time of animals. The decreased in immobility time of animals in FST expressed the antidepressant-like activity.

TST

Table 6 demonstrated the animals treated with the ethanol extract of *V. tessellata* at doses of 200 mg/kg and 400 mg/kg significantly decreased the immobility time of animal compared to normal group in TST. The animals treated with imipramine (10 mg/kg) significantly decreased the immobility time of animals. The increase in the motor activity of animals which elevate depressed mood by decreasing immobility time of animals. The decreased in immobility time of animals in TST expressed the antidepressant-like activity.

The male animals were used to screen the potential antidepressant-like activity of the *V. tessellata*. The constraints observed in both model are immobility time of animals. The ethanol extract of *V. tessellata* reduced the immobility period during the forced swimming and TST in comparison with normal group and displayed a dose-dependent antidepressant activity. The characteristic behavior evaluated in FST and TST, termed immobility, has been considered to reflect behavioral despair similar to that seen in the human depression, and hence any reduction in immobility time of animals reflects antidepressant activity.[6] Hence, the findings of ethanol extract of *V. tessellata* correlated with the above statements. Consequently, the findings showed that the ethanol extract of *V. tessellata* showed a significant dose-dependent activity and offers good percentage protection as compared to normal group.

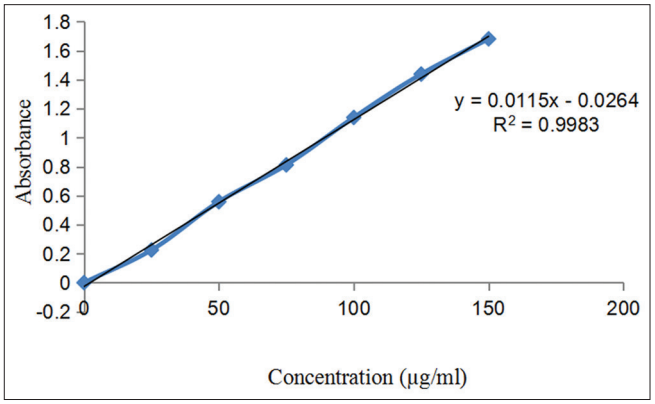

Figure 2: Calibration curve of quercetin in distilled water

Table 5: Effect of ethanol extract of *V. tessellata* on immobility time in FST rats

Groups	FST duration of immobility (Sec)	Percent reduction in time of immobility
Normal	192.27±2.63	-
Imipramine (10 mg/kg)	69.15±4.71*	64.03
Ethanol extracts of *V. tessellata* 200 mg/kg	92.34±6.85*	51.97
Ethanol extracts of *V. tessellate* 400 mg/kg	78.54±5.43*	59.15

Valuesexpressed as Mean±SEM, *n*=6 animals; **P*<0.05 compared with normal groups, FST: Forced swimming test, *V. tessellate: Vanda tessellata*

Table 6: Effect of ethanol extract of *V. tessellata* on immobility time in TST rats

Groups	TST duration of immobility (Sec)	Percent reduction in time of immobility
Normal	208.63±5.82	-
Imipramine (10 mg/kg)	79.18±2.43*	62.04
Ethanol extracts of *V. tessellata* 200 mg/kg	115.41±4.17*	44.68
Ethanol extracts of *V. tessellate* 400 mg/kg	85.57±3.92*	58.98

Valuesexpressed as Mean±SEM, *n*=6 animals; **P*<0.05 compared with normal groups, TST: Tail suspension test, SEM: Structural equation modeling, *V. tessellata: Vanda tessellata*

The oxidative stress characterizes imbalance between oxidation-reduction reactions. This happens due to the reduced ability of the antioxidant defense system to efficiently eliminate the excess of the oxygen-derived species production, prompting the toxicity of oxygen and its detrimental effects. Further, it has been observed enhanced oxidative stress in patients suffering from depression.[18] The polyphenol and flavonoids component scavenge the oxygen-derived species and play an important role in controlling the depressive disorders. The findings indicate that the antioxidant property of leaves of *V. tessellata* validates the antidepressant activity of the plant.

CONCLUSION

The result of the present study showed that TPC and TFC are found the maximum in ethanol extract of *V. tessellata* leaves. The highest antioxidant activity of *V. tessellata* leaves extract might be useful in preventing the progress of various oxidative conditions. The ethanol extracts of *V. tessellata* leaves demonstrated significant antidepressant activity. Further research will be carrying out in future to explore the potential and isolation of bioactive compounds responsible for the antidepressant activity.

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