

A large wooden barrel with several metal hoops is used as a planter. It is filled with a dense cluster of bright pink flowers with green foliage. The barrel is placed on a bed of small, smooth, light-colored pebbles. The background is a dark, solid color.

# Journal of Medicinal Plant Research

Volume 11 Number 27, 17 July, 2017

ISSN 1996-0875



*Academic  
Journals*

# ABOUT JMPR

**The Journal of Medicinal Plant Research** is published weekly (one volume per year) by Academic Journals.

**The Journal of Medicinal Plants Research (JMPR)** is an open access journal that provides rapid publication (weekly) of articles in all areas of Medicinal Plants research, Ethnopharmacology, Fitoterapia, Phytomedicine etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JMPR are peer reviewed. Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

## Contact Us

Editorial Office: [jmpr@academicjournals.org](mailto:jmpr@academicjournals.org)

Help Desk: [helpdesk@academicjournals.org](mailto:helpdesk@academicjournals.org)

Website: <http://www.academicjournals.org/journal/JMPR>

Submit manuscript online <http://ms.academicjournals.me/>

## Editors

### **Prof. Akah Peter Achunike**

*Editor-in-chief  
Department of Pharmacology & Toxicology  
University of Nigeria, Nsukka  
Nigeria*

### **Associate Editors**

#### **Dr. Ugur Cakilcioglu**

*Elazığ Directorate of National Education  
Turkey.*

#### **Dr. Jianxin Chen**

*Information Center,  
Beijing University of Chinese Medicine,  
Beijing, China  
100029,  
China.*

#### **Dr. Hassan Sher**

*Department of Botany and Microbiology,  
College of Science,  
King Saud University, Riyadh  
Kingdom of Saudi Arabia.*

#### **Dr. Jin Tao**

*Professor and Dong-Wu Scholar,  
Department of Neurobiology,  
Medical College of Soochow University,  
199 Ren-Ai Road, Dushu Lake Campus,  
Suzhou Industrial Park,  
Suzhou 215123,  
P.R.China.*

#### **Dr. Pongsak Rattanachaikunsopon**

*Department of Biological Science,  
Faculty of Science,  
Ubon Ratchathani University,  
Ubon Ratchathani 34190,  
Thailand.*

### **Prof. Parveen Bansal**

*Department of Biochemistry  
Postgraduate Institute of Medical Education and  
Research  
Chandigarh  
India.*

#### **Dr. Ravichandran Veerasamy**

*AIMST University  
Faculty of Pharmacy, AIMST University, Semeling -  
08100,  
Kedah, Malaysia.*

#### **Dr. Sayeed Ahmad**

*Herbal Medicine Laboratory, Department of  
Pharmacognosy and Phytochemistry,  
Faculty of Pharmacy, Jamia Hamdard (Hamdard  
University), Hamdard Nagar, New Delhi, 110062,  
India.*

#### **Dr. Cheng Tan**

*Department of Dermatology, first Affiliated Hospital  
of Nanjing University of  
Traditional Chinese Medicine.  
155 Hanzhong Road, Nanjing, Jiangsu Province,  
China. 210029*

#### **Dr. Naseem Ahmad**

*Young Scientist (DST, FAST TRACK Scheme)  
Plant Biotechnology Laboratory  
Department of Botany  
Aligarh Muslim University  
Aligarh- 202 002,(UP)  
India.*

#### **Dr. Isiaka A. Ogunwande**

*Dept. Of Chemistry,  
Lagos State University, Ojo, Lagos,  
Nigeria.*

## Editorial Board

**Prof Hatil Hashim EL-Kamali**

*Omdurman Islamic University, Botany Department,  
Sudan.*

**Prof. Dr. Muradiye Nacak**

*Department of Pharmacology, Faculty of Medicine,  
Gaziantep University,  
Turkey.*

**Dr. Sadiq Azam**

*Department of Biotechnology,  
Abdul Wali Khan University Mardan,  
Pakistan.*

**Kongyun Wu**

*Department of Biology and Environment Engineering,  
Guiyang College,  
China.*

**Prof Swati Sen Mandi**

*Division of plant Biology,  
Bose Institute  
India.*

**Dr. Ujjwal Kumar De**

*Indian Veterinary Research Institute,  
Izatnagar, Bareilly, UP-243122  
Veterinary Medicine,  
India.*

**Dr. Arash Kheradmand**

*Lorestan University,  
Iran.*

**Prof Dr Cemşit Karakurt**

*Pediatrics and Pediatric Cardiology  
Inonu University Faculty of Medicine,  
Turkey.*

**Samuel Adelani Babarinde**

*Department of Crop and Environmental Protection,  
Ladoke Akintola University of Technology,  
Ogbomoso  
Nigeria.*

**Dr.Wafaa Ibrahim Rasheed**

*Professor of Medical Biochemistry National Research Center  
Cairo  
Egypt.*

**ARTICLE**

**Antioxidant and free radical scavenging activities of *Viola odorata* in the search of potential inhibitor of tobacco's free radicals**

**433**

Tania Peshin, Chandra S. Azad and H. K. Kar

## Full Length Research Paper

# Antioxidant and free radical scavenging activities of *Viola odorata* in the search of potential inhibitor of tobacco's free radicals

Tania Peshin<sup>1</sup>, Chandra S. Azad<sup>2</sup> and H. K. Kar<sup>3\*</sup>

<sup>1</sup>University of Medicine and Para-Medical Health Sciences, Guru Gobind Singh Indraprastha University, Sector 16-C, Dwarka, New Delhi, India.

<sup>2</sup>Division of Medicinal and Process Chemistry, CSIR-Central Drug Research Institute, Lucknow, U.P., India.

<sup>3</sup>Department of Dermatology, Paras Hospital, Sector 43, Gurugram, India.

Received 20 May, 2017; Accepted 20 June, 2017

Tobacco use is a leading cause of cancer morbidity and mortality. Out of several possible components of tobacco, the free radical present in tobacco induces oxidative stress which ultimately leads to the damage of DNA. In the present study, an attempt was made to find the natural potential tobacco free radical scavenger, by using different standard assays. *Viola odorata* has been known for diverse therapeutic applications since the ancient time, so in the present study, the tobacco free radical scavenging activity of *V. odorata* was explored. A significant increase in inhibitory concentration (IC<sub>50</sub>) values of *V. odorata* ethanolic extract was observed, when mixed with ethanolic extract of tobacco, which signify that most of the anti-oxidant activity of *V. odorata* has been utilized in the inhibition of tobacco free radicals, leading to increment of IC<sub>50</sub> values in different assays. This positive finding was validated by four different types of assays. The various standard assays like DPPH, nitric oxide, Fe<sup>2+</sup> chelating and hydroxyl radical scavenging were explored to support the study.

**Key words:** *Viola odorata*, *Nicotiana tabacum*, antioxidant property, mixture of extract, tobacco related cancer.

## INTRODUCTION

The two important factors responsible for the pathogenesis of human cancers are environmental factors and genetic disorders, out of which 93% of cancers are caused by the former factor and rest 7% are due to the latter (Seto et al., 2010). Tobacco related cancer (TRC) caused by smoking/chewing tobacco contributes to 30% of the environmental factors, leading to human cancer (Anand et al., 2008). According to the

World Health Organization, one out of two people smoking throughout their lives will end up having TRC (WHO, 2012). In addition to nicotine, tobacco mainly contains polyaromatic hydrocarbons (PAH) and N-nitrosamine carcinogens (Xue et al., 2014). The tobacco specific nitrosamines such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitroso-nornicotine (NNN), are carcinogenic to humans (IARC, 2007). The

\*Corresponding author. E-mail: uimphs.ggsipu@gmail.com. Tel: +91-124-4585555.

NNK and NNN cause cancer by deoxyribonucleic acid (DNA) adductions and mutations by receptor-mediated effect on tumor growth (Xue et al., 2014; Takahashi et al., 2010). Tobacco smokes contains free radicals which induce oxidative damage or stress (Valko et al., 2006). The cause of oxidative stress is the production of oxidant species like reactive oxygen species (ROS) and reactive nitrogen species (RNS). The bulk of ROS are generated by the mitochondrial respiratory chain through incomplete reduction of molecular oxygen to water during oxidative phosphorylation, in addition to during microsomal and peroxisomal oxidations (De Marco, 2003).

The tobacco smoke has large amounts of nitric oxide and other unstable oxidants like hydroquinones, semiquinones and quinones (Xue et al., 2014; Hecht, 2003). These compounds induce redox cycling and are responsible for oxidative damage (Hecht, 2011; Pryor et al., 1998). The NNK leads to increased levels of 8-hydroxy-2'-deoxyguanosine adducts in lung tissues, when orally administered or intraperitoneally injected into mice and rats. The 8-Hydroxy-2'-deoxyguanosine is an important pre-mutagenic lesion produced from ROS that is a marker of DNA oxidative damage (Rosa et al., 1998; Chung and Xu, 1992). The activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) is also responsible for increased level of ROS during tumor progression (Lee and Kim, 2013). So, it has been thought that if any healthy free radical scavenger is consumed along with tobacco, then the concentration of ROS and RNS can be decreased significantly, which subsequently reduce the risk of TRC.

*Viola odorata* (family Violaceae) is commonly known as sweet violet and has been used to treat anxiety, insomnia and hypertension (Mousavi et al., 2016). The pharmacological exploration have shown that this plant also has diuretic, laxative (Vishal et al., 2009), antihypertensive and antidyslipidemic (Siddiqi et al., 2012), antibacterial (Pränting et al., 2010), anticancer (Gerlach et al., 2010), hepatoprotective (Qadir et al., 2014), lung-protective (Koochek et al., 2003) and excellent antioxidant activities (Ebrahimzadeh et al., 2010). The plethora of therapeutic applications of *V. odorata*, fortified the authors to evaluate the free radical scavenging activity of the plant extract over the tobacco extract in continuation of their endeavor in the field of natural product chemistry (Khan et al., 2016). To the best of the author's knowledge, this type of cumulative synergistic study is not reported in the literature so far.

## MATERIALS AND METHODS

### Plant material

*V. odorata* was collected from an authorized dealer of Ayurvedic plants, Sadar bazar, New Delhi. The *Nicotiana tabacum* leaves were collected from Munger, Bihar, India. The sample was authenticated by Dr Sunita Garg, CSIR-National Institute of Science Communication and Information Resources, New Delhi, India and

was deposited in the Raw Material Herbarium and Museum, Delhi (RHMD) under voucher number NISCAIR/RHMD/Consult/2017/3081-30. The collected plant material were air dried in shadow and grinded for extraction.

### Preparation of extract

Air dried *N. tabacum* leaves were extracted using 99% ethanol by ultrasonication for 2 h. The combined extract were filtered and concentrated under reduced pressure at 35°C by using rotatory evaporator. The crude extracts were totally dried under high vacuum repeatedly until constant weight was obtained. The dried crude extract was kept over calcium chloride in desiccator for further study.

*V. odorata* (leaves and flower) was firstly extracted with petroleum ether at room temperature for 2 days to remove nonpolar impurities and waxes (fraction CPEEx). The crude extract was then dissolved in diethyl ether and left for 2 days at room temperature (extract CDEEEx). The solution was occasionally stirred with the help of glass rod. After filtration, the crude extract was dissolved in 99% ethanol and left for 2 days at room temperature (extract CEEEx). The combined petroleum ether extract (CPEEx), diethyl ether extract (CDEEEx) and ethanol extract (CEEEx) were concentrated under reduced pressure at 35°C by using rotatory evaporator and desiccated separately for further study (Tang et al., 2010).

### Determination of total flavonoid content

The total flavonoid content was determined by colorimetric aluminum chloride ( $AlCl_3$ ) method with slight modification (Ahn et al., 2007). The 0.5 mL of 2%  $AlCl_3$  in ethanol and 0.1 mL of 1 M potassium acetate were added to 0.5 mL of sample or standard. After 1 h at room temperature, the absorbance of the reaction mixture was measured at 415 nm with a double beam ultraviolet/visible spectrophotometer. The quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) was used as standard.

### Determination of total phenol content

Total phenolic compound contents were determined by the Folin-Ciocalteu reagent and gallic acid (trihydroxybenzoic acid) (Agbor et al., 2014). The method is based on the theory that Folin-Ciocalteu reagent shows blue color by reaction with phenolic compounds. The extract samples (0.5 mL) were mixed with Folin-Ciocalteu reagent (5 mL, 10% v/v, diluted with de-ionized water) and aqueous sodium carbonate (4 mL, 1 M). The mixture was allowed to stand for 30 min at room temperature and the phenols were determined by measuring absorbance at 765 nm. The standard curve was constructed by different concentration of gallic acid (mg/mL) in methanol : water solutions (50:50 v/v).

### Antioxidant activity

#### Assay of free radical scavenging activity (DPPH)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was used for determination of free radical scavenging activity of the extracts based on the method given by Brand-William et al. (1995). This method depends on the decrease in absorbance at 517 nm, of colored solution of DPPH in methanol by reference or extract. Ascorbic acid in methanol (1 mg/mL) was used as reference. Different concentrations of each extract were added to methanolic solution of DPPH (100  $\mu$ M) in equal volumes and left for 20 to

30 min at room temperature. The absorbance was recorded at 517 nm and DPPH radical scavenging activity was calculated as follows:

$$\text{Inhibition (\%)} = \left( \frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} \right) \times 100$$

Where,  $\text{Abs}_{\text{blank}}$  is the absorbance of the control, containing all reagents except extract. The inhibitory concentration ( $\text{IC}_{50}$ ) values represent the concentration of sample, which is required to inhibit 50% of DPPH free radicals.

#### Assay of nitric oxide-scavenging activity

The theory of the assay depends on the estimation of nitrite ion by Griess reagent (Jagetia and Baliga, 2004). The nitrite ion is formed by the reaction of nitric oxide with oxygen, in which nitric oxide is generated by the sodium nitroprusside at biological pH. The Griess reagent usually contains 0.2% naphthylethylenediamine dihydrochloride (NED), and 2% sulphanilamide in 5% phosphoric acid. The competitive scavenging activity of nitrite ion with oxygen leads to lesser production of nitrite ions. To carry out the experiment, sodium nitroprusside (10 mM), in phosphate buffer at pH 7, was mixed with different concentrations of each extracts dissolved in methanol and left for 2 to 2.5 h at room temperature under dark and anhydrous condition. The absorbance was recorded at 546 nm after adding 0.5 mL of Griess reagent in the experimental mixture. Quercetin was used as positive control or reference.

#### Assay of metal chelating activity

Ferric ions ( $\text{Fe}^{2+}$ )-chelating activity of extracts were determined by following the method of Dinis et al. (1994) and Gülçin et al. (2004) with minor modification. The  $\text{Fe}^{2+}$  capacity was recorded spectrophotometrically at 562 nm. Briefly, the plant extract at different concentrations in methanol was added into 0.1 mL of  $\text{FeCl}_2$  (1 mM) followed by addition of 0.1 mL of ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate) solution (5 mM). The experiment solution was incubated at room temperature for about 10 to 15 min. Finally, the absorbance value of the mixture was quantified spectrophotometrically at 562 nm using same formula used for DPPH assay. Where  $\text{Abs}_{\text{blank}}$  was the absorbance of the control, and  $\text{Abs}_{\text{sample}}$  was the absorbance of the extract or positive control.

#### Assay of hydroxyl radical scavenging activity

The assay for hydroxyl radical scavenging activity was developed according to Kim et al. with minor modifications (Kim and Minamikawa, 1997; Topal et al., 2016). The extract at different concentrations in methanol was mixed with 0.4 mL of  $\text{FeSO}_4$  (10 mM), 0.4 mL ethylenediaminetetraacetic acid (EDTA, 10 mM), 0.4 mL of 2-deoxyribose, 0.2 mL of phosphate buffer and 250  $\mu\text{L}$  of hydrogen peroxide. The experimental mixture was incubated at room temperature for about 4 to 5 h. In the next step 1 mL of trichloroacetic acid (2.5%) and 1 mL thiobarbituric acid (1%) were added to the experimental mixture and heated for 5 minutes at 80°C. After cooling, its absorbance was measured at 520 nm. The hydroxyl radical scavenging activity (%) was calculated using the same formula which was used for DPPH activity. The  $\text{IC}_{50}$  was calculated by plotting the inhibition percentage against extract concentrations.

#### Determination of antioxidant properties of mixture of extracts

The combined activity of both extracts was determined by mixing

the different w/w combination. The scavenging activity of the combined extract was determined by the described assays. For this, the mixture of extract was incubated at room temperature with occasionally shaking for about 3 to 3.5 h and then heated at 50 to 60°C for 10 min. This experimental results obtained by interval of times showed that 3 to 3.5 h are sufficient time for this kind of study. The different possible combinations of w/w ratio of mixture were examined with 50 to 10% of tobacco extract with *V. odorata* extract. The most important results are shown in the current manuscript.

#### Statistical analysis

The data of various analyses were expressed as mean  $\pm$  standard deviation. All tests were carried out in triplicate to improve the accuracy. The data reported in the present manuscript were analysed using one way analysis of variance (ANOVA) followed by Dunnet's test. In the experiments, P values of <0.05 were taken to be significant.

## RESULTS

### Total phenolic and total flavonoid contents

The total phenolic and total flavonoid contents in the different extracts expressed as gallic acid equivalent and quercetin equivalent are shown in Figure 1. The total phenolic and total flavonoid contents were greater in the ethanolic extract [CEEx] than in the diethyl ether extract [CDEEEx]. The petroleum ether extract [CPEEx] showed no positive results in the phytochemical screening. The results were calculated from the standard gallic and quercetin calibration curves ( $R^2 = 0.99$ ).

### Free radical scavenging activities

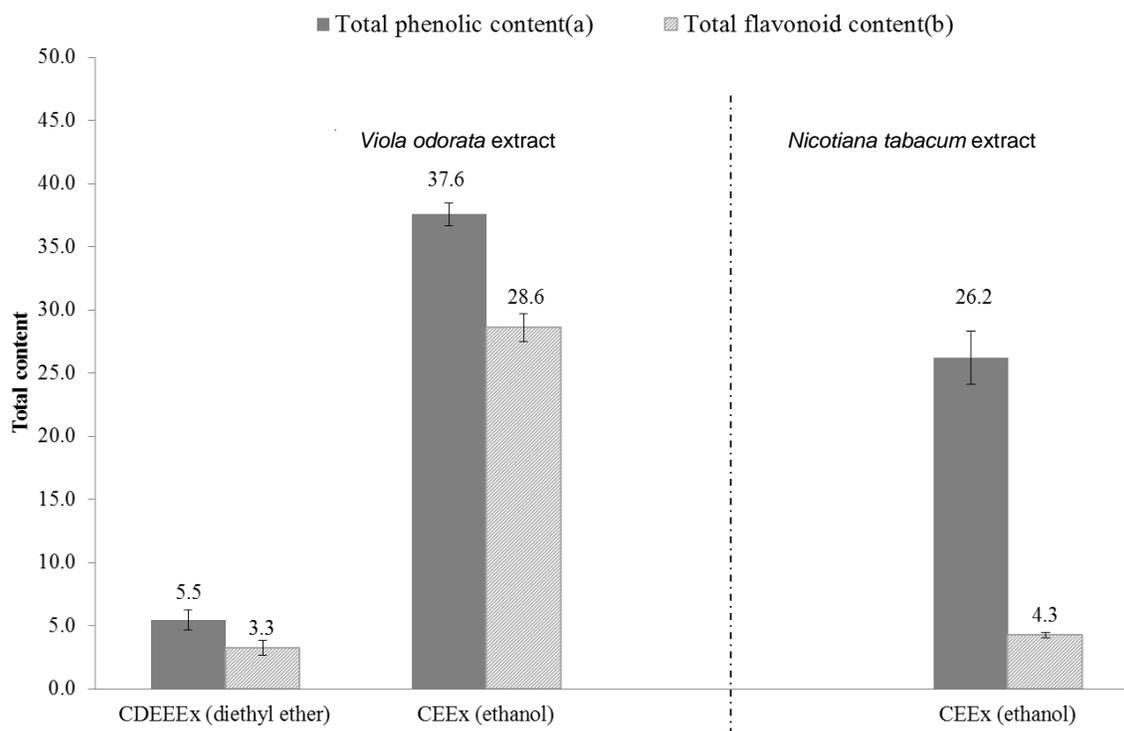
The DPPH free radical scavenging activity of ethanolic extracts of *V. odorata*, *N. tabacum* and mixture of both are reported in Table 1. It was found that the radical scavenging activities of the extract were in linear relation with concentration, that is, increased with the concentration. The  $\text{IC}_{50}$  value of CEEx (*V. odorata*) and CEEx (*N. tabacum*) were  $112.36 \pm 0.8 \mu\text{g/mL}$  and  $312.68 \pm 0.6 \mu\text{g/mL}$ , respectively, thus signifying that CEEx (*V. odorata*) has a very good antioxidant property. The  $\text{IC}_{50}$  of ascorbic acid (vitamin C) was found to be  $4.98 \pm 0.3 \mu\text{g/mL}$  for DPPH assay. The  $\text{IC}_{50}$  of nitric oxide radical scavenging activity (NORSA) of CEEx (*V.odorata*) was found to be  $1.26 \mu\text{g/mL}$ , which may not be considered good as compared to the  $\text{IC}_{50}$  of Quercetin ( $19.86 \pm 0.6 \mu\text{g/mL}$ ). The CEEx extracts of *V. odorata* and *N. tabacum* also showed good metal chelating activity, which was  $168.43 \pm 0.2 \mu\text{g/mL}$  and  $168.43 \pm 0.2 \mu\text{g/mL}$ , respectively. The hydroxy radical scavenging activity of CEEx extracts of *V. odorata* and *N. tabacum* were found to be  $228.65 \pm 0.9 \mu\text{g/mL}$  and  $356.36 \pm 0.8 \mu\text{g/mL}$ , respectively.

The main focus of the current manuscript is the scavenging properties of combined extract of *V. odorata* and *N. tabacum*. The combined DPPH assay revealed

**Table 1.** Free radical scavenging activities of ethanolic extract of *V. odorata*, *N. tabacum* and mixture of both.

Extract	DPPH radical scavenging IC <sub>50</sub> (µg/mL) <sup>a</sup>	Nitric oxide radical scavenging IC <sub>50</sub> (mg/mL) <sup>b</sup>	Metal chelating activity IC <sub>50</sub> (µg/mL)	Hydroxyl radical scavenging IC <sub>50</sub> (µg/mL)
CEEx ( <i>V.odorata</i> )	112.36 ± 0.8	1.26 ± 0.9	168.43 ± 0.2	228.65 ± 0.9
CEEx ( <i>N.tabacum</i> )	312.68 ± 0.6	Not determined	242.43 ± 0.2	356.36 ± 0.8
CEEx ( <i>V. odorata</i> + <i>N. tabacum</i> ) (80% w/w)	235.16 ± 0.2	1.41 ± 0.3	223.62 ± 0.2	315.12 ± 0.3

<sup>a</sup>IC<sub>50</sub> of ascorbic acid (Vitamin C) was 4.98 ± 0.3 µg/mL for DPPH assay; <sup>b</sup>IC<sub>50</sub> of quercetin was 19.86 ± 0.6 mg/mL for nitric oxide scavenging assay. All values are mean ± standard deviation. IC<sub>50</sub>, concentration required to inhibit 50% of free radicals.



**Figure 1.** Total phenol and flavonoid contents of different extract of *V. odorata* and *N. tabacum*. (a) Expressed as mg gallic acid equivalent per gram of dry extract; (b) expressed as mg quercetin equivalent per gram of dry extract. All values are mean ± standard deviation.

the scavenging activity of mixture of extract with IC<sub>50</sub> 235.16 ± 0.2 µg/mL. The other assays, that is, NORSA, metal chelating activity and hydroxyl radical scavenging gave the IC<sub>50</sub> values of 1.41 ± 0.3, 223.62 ± 0.2 and 315.12 ± 0.3 µg/mL, respectively. For this combined extract analysis, the best found combination, that is, 80% w/w of *V. odorata* and *N. tabacum*, was used.

## DISCUSSION

The DPPH assay is a widely accepted and used model for exploration of radical scavenging ability of various

extracts and samples. The DPPH is a stable nitrogen-centered free radical, in which the color changes from violet to yellow upon reduction. The potential compound or substance capable of inhibiting this free radical is treated as antioxidants and consequently a radical scavenger. It has been accepted that higher total phenol and flavonoids contents lead to good DPPH-scavenging activity (Xu and Chang, 2007). From Figure 1, it is shown that the ethanolic extract of *V. odorata* has good antioxidant activity as expressed in terms of DPPH scavenging activity. The activity of combined ethanolic extract significantly decreased (CEEx (*V. odorata*)) which can be rationalized on the basis of competitive inhibition

of tobacco extract free radicals. The free radicals responsible for TRC significantly was inhibited by the *V. odorata*, which is shown by increase in IC<sub>50</sub> values (235.16 ± 0.2 from 112.36 ± 0.8 µg/mL). As NORSA is found to be in the order of mg/mL, nevertheless significant increase in IC<sub>50</sub> was observed in the same case from 1.26 ± 0.9 to 1.41 ± 0.3 mg/mL. These results also favor the hypothesis that *V. odorata* has the capability to inhibit the ROS present in tobacco which leads to TRC.

The Fe<sup>2+</sup> catalyzed the oxidative processes, leading to the formation of hydroxyl radicals and hydro peroxide decomposition reactions. Minimizing the concentration of Fe<sup>2+</sup> is directly correlated with the decrease in oxidative damage, because it causes the production of oxyradicals (Ak and Gülçin, 2008). The ferrozine formed red colored complex and in the presence of other chelating agent, the red color fades. The absorbance of Fe<sup>2+</sup>-ferrozine complex varied linearly, which means that the activity was increased with increase in concentration from 12.5 to 1000 µg/mL. The IC<sub>50</sub> for Fe<sup>2+</sup> chelating ability of CEEEx (*V. odorata*) was 168.43 ± 0.2 (µg/mL) and it significantly increased to 223.62 ± 0.2 (µg/mL) in the presence 20% of tobacco extract w/w (Table 1). This also gave the support that *V. odorata* has a significant tobacco free radical scavenging activity. The principal for hydroxyl radical scavenging activity, is the conversion of 2-deoxyribose to malondialdehyde, which react with thiobarbituric acid giving rise to a pink pigment. The increase in IC<sub>50</sub> value from 228.65 ± 0.9 to 315.12 ± 0.3 µg/mL signify the importance of *V. odorata* as a free radical scavenger of tobacco.

## Conclusion

In conclusion, it has been found that *V. odorata* has a good antioxidant property, as proved by the ethanolic extract of the same. The various standard assays like DPPH, nitric oxide, Fe<sup>2+</sup> chelating and hydroxyl radical scavenging was explored to sum up the good antioxidant property of *V. odorata*. In addition to this, *V. odorata* ethanolic extract was found to be a good scavenger of tobacco free radicals, responsible for tobacco related cancer as significant increment of IC<sub>50</sub> value was observed in 20% w/w of tobacco and *V. odorata extract*. The detailed study to find out a more proper tobacco free radical scavenger is under investigation in our laboratory. This finding results in a possible solution to mix the highly antioxidant indexed therapeutically important plant to tobacco so that the emission of cancer causing free radicals can be inhibited and this may reduce the chance of occurrence of tobacco related cancer.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

## ACKNOWLEDGEMENT

One of the authors (TP) thank AIRF, JNU, India and R&D Indian Oil Limited, Faridabad, India for providing instrumental facilities. The authors thank Gautam Budhha University, Greater Noida for providing some laboratory facilities.

## REFERENCES

- Agbor GA, Vinson JA, Donnelly PE (2014). Folin-Ciocalteu reagent for polyphenolic assay. *Int. J. Food Sci. Nutr. Diet* 3(8):147-156.
- Ahn MR, Kumazawa S, Usui Y, Nakamura J, Matsuka M, Zhu F, Nakayama T (2007). Antioxidant activity and constituents of propolis collected in various areas of China. *Food Chem.* 101(4):1383-1392.
- Ak T, Gülçin İ (2008). Antioxidant and radical scavenging properties of curcumin. *Chem. Biol. Interact.* 174(1):27-37.
- Anand P, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharm. Res.* 25(9):2097-2116.
- Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* 28(1):25-30.
- Chung FL, Xu Y (1992). Increased 8-oxodeoxyguanosine levels in lung DNA of A/J mice and F344 rats treated with the tobacco-specific nitrosamine 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone. *Carcinogenesis* 13(7):1269-1272.
- De Marco F (2013). Oxidative stress and HPV carcinogenesis. *Viruses.* 5(2):708-731.
- Dinis TC, Madeira VM, Almeida LM (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Arch. Biochem. Biophys.* 315(1):161-169.
- Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, Bekhradnia AR (2010). Antioxidant and free radical scavenging activity of *H. officinalis* L. var. *angustifolius*, *V. odorata*, *B. hircana* and *C. speciosum*. *Pak. J. Pharm. Sci.* 23(1):29-34.
- Gerlach SL, Rathinakumar R, Chakravarty G, Göransson U, Wimley WC, Darwin SP, Mondal D (2010). Anticancer and chemosensitizing abilities of cycloviolacin O2 from *Viola odorata* and psyle cyclotides from *Psychotria leptothyrsa*. *Biopolymers* 94(5):617-625.
- Gülçin İ, Şat İG, Beydemir Ş, Elmastaş M, Küfrevioğlu Öİ (2004). Comparison of antioxidant activity of clove (*Eugenia caryophyllata* Thunb) buds and lavender (*Lavandula stoechas* L.). *Food Chem.* 87(3):393-400.
- International Agency for Research on Cancer Working Group on the Evaluation of the Carcinogenic Risks to Humans (IARC) (2007). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans VOLUME 89. Smokeless tobacco and some tobacco-specific N-Nitrosamines. World Health Organization International agency for research on cancer. Available at: <https://monographs.iarc.fr/ENG/recentpub/mono89.pdf>
- Jagetia GC, Baliga MS (2004). The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants in vitro: a preliminary study. *J. Med. Food* 7(3):343-348.
- Khan MF, Azad CS, Kumar A, Saini M, Narula AK, Jain S (2016). Novel Imbricatic acid derivatives as protein tyrosine phosphatase-1B inhibitors: Design, synthesis, biological evaluation and molecular docking. *Bioorg. Med. Chem. Lett.* 26(8):1988-1992.
- Kim JW, Minamikawa T (1997). Hydroxyl radical-scavenging effects of spices and scavengers from brown mustard (*Brassica nigra*). *Biosci. Biotechnol. Biochem.* 61(1):118-23.
- Koochek MH, Pipelzadeh MH, Mardani H (2003). The effectiveness of *Viola odorata* in the prevention and treatment of formalin-induced lung damage in the rat. *J. Herbs Spices Med. Plants* 10(2):95-103.
- Lee JW, Kim JH (2013). Activation of the leukotriene B4 receptor 2-reactive oxygen species (BLT2-ROS) cascade following detachment confers anoikis resistance in prostate cancer cells. *J. Biol. Chem.*

- 288(42):30054-30063.
- Mousavi SH, Naghizade B, Pourgonabadi S, Ghorbani A (2016). Protective effect of *Viola tricolor* and *Viola odorata* extracts on serum/glucose deprivation-induced neurotoxicity: role of reactive oxygen species. *Avicenna J. Phytomed.* 6(4):434-441.
- Pränting M, Lööv C, Burman R, Göransson U, Andersson DI (2010). The cyclotide cycloviolacin O2 from *Viola odorata* has potent bactericidal activity against Gram-negative bacteria. *J. Antimicrob. Chemother.* 65(9):1964-1971.
- Pryor WA, Stone K, Zang LY, Bermúdez E (1998). Fractionation of aqueous cigarette tar extracts: fractions that contain the tar radical cause DNA damage. *Chem. Res. Toxicol.* 11(5):441-448.
- Qadir MI, Ali M, Saleem M, Hanif M (2014). Hepatoprotective activity of aqueous methanolic extract of *Viola odorata* against paracetamol-induced liver injury in mice. *Bangladesh J. Pharmacol.* 9(2):198-202.
- Rosa JG, Prokopczyk B, Desai DH, Amin SG, El-Bayoumy K (1998). Elevated 8-hydroxy-2'-deoxyguanosine levels in lung DNA of A/J mice and F344 rats treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and inhibition by dietary 1, 4-phenylenebis (methylene) selenocyanate. *Carcinogenesis* 19(10):1783-1988.
- Seto M, Honma K, Nakagawa M (2010). Diversity of genome profiles in malignant lymphoma. *Cancer Sci.* 101(3):573-578.
- Siddiqi HS, Mehmood MH, Rehman NU, Gilani AH (2012). Studies on the antihypertensive and antidyslipidemic activities of *Viola odorata* leaves extract. *Lipids Health Dis.* 11(1):6.
- Takahashi H, Ogata H, Nishigaki R, Broide DH, Karin M (2010). Tobacco smoke promotes lung tumorigenesis by triggering IKK $\beta$ -and JNK1-dependent inflammation. *Cancer cell.* 17(1):89-97.
- Tang J, Wang CK, Pan X, Yan H, Zeng G, Xu W, He W, Daly NL, Craik DJ, Tan N (2010). Isolation and characterization of cytotoxic cyclotides from *Viola tricolor*. *Peptides* 31(8):1434-1440.
- Topal F, Nar M, Gocer H, Kalin P, Kocyigit UM, Gülçin İ, Alwaseel SH (2016). Antioxidant activity of taxifolin: an activity–structure relationship. *J. Enzyme Inhib. Med. Chem.* 31(4):674-683.
- Valko M, Rhodes CJ, Moncol J, Izakovic MM, Mazur M (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.* 160(1):1-40.
- Vishal A, Parveen K, Pooja S, Kannappan N, Kumar S (2009). Diuretic, laxative and toxicity Studies of *Viola odorata* aerial parts. *Pharmacol. online* 1:739-48.
- WHO (World Health Organization) (2012). WHO global report on mortality attributable to tobacco. In WHO global report on mortality attributable to tobacco. Available at: [http://www.who.int/tobacco/publications/surveillance/rep\\_mortality\\_attributable/en/](http://www.who.int/tobacco/publications/surveillance/rep_mortality_attributable/en/)
- Xu BJ, Chang SK (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *J. Food Sci.* 72(2):S159-166.
- Xue J, Yang S, Seng S (2014). Mechanisms of cancer induction by tobacco-specific NNK and NNN. *Cancers* 6(2):1138-1156.



# Journal of Medicinal Plant Research

## Related Journals Published by Academic Journals

- *African Journal of Pharmacy and Pharmacology*
- *Journal of Dentistry and Oral Hygiene*
- *International Journal of Nursing and Midwifery*
- *Journal of Parasitology and Vector Biology*
- *Journal of Pharmacognosy and Phytotherapy*
- *Journal of Toxicology and Environmental Health Sciences*

**academicJournals**