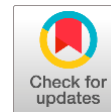


# Antioxidant and antimicrobial activities of *Ocimum basilicum* var. *thyrsoiflora* against some oral microbes



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**Abstract** The accumulation of free radicals is the root cause of many dangerous diseases. Several studies are being conducted to identify plant-based natural antioxidants and antimicrobial elements. *Ocimum basilicum* var. *thyrsoiflora* exhibits anti-inflammatory, antioxidant, antiulcer, antiviral, hypoglycemic, hypolipidemic, antimicrobial, anticancer, wound-healing, etc. This research aimed to determine the *in vitro* antioxidant activity of essential oils extracted from the leaves and seeds of *Ocimum basilicum* var. *thyrsoiflora* by hydroxyl radical, nitric oxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical, and azino-bis (3-ethylbenthiazoline-6-sulphonic acid (ABTS) assays. The antibacterial activity of essential oils extracted from the leaves and seeds of *Ocimum basilicum* var. *thyrsoiflora* was evaluated by the disc diffusion method against *E. coli*, *P. aeruginosa*, *Acinetobacter*, *S. epidermis*, *K. pneumoniae*, *S. aureus*, *E. faecalis*, and *P. mirabilis*. According to the findings, the hydroxyl radical (HO•) scavenging assay method revealed more potent antioxidant activity in *Ocimum basilicum* var. *thyrsoiflora* essential oils than the other methods. The hydroxyl radical scavenging assay was found to have IC<sub>50</sub> values of 115.20 ± 6.45 and 128.35 ± 6.20 for seeds and leaves, respectively. *Ocimum basilicum* var. *thyrsoiflora* essential oils exhibited a strong antibacterial effect against all the tested microorganisms. The highest antibacterial activity was measured in the essential oil extracted from seeds against *P. aeruginosa* (20.06±0.30) at a concentration of 50% essential oil, and the lowest activity was observed in the essential oil extracted from leaves against *P. mirabilis* (7.46±0.45) at a concentration of 12.5% essential oil. As a result, it can be a potent natural source of antioxidants to treat many stress- and anxiety-related diseases and would be a better alternative for the development of new antimicrobial medications to treat a variety of infectious ailments caused by microbes.

**Keywords:** *Ocimum basilicum* var. *thyrsoiflora*, essential oil, antioxidant activity, scavenging activity, free radicals

## 1. Introduction

*Ocimum basilicum* var. *thyrsoiflora* (Lamiaceae) is commonly known as Thai basil. It has narrow, lightly serrated, shiny green leaves that smell sweet and anise-like in aroma and have a hint of licorice and a little spiciness. ([https://en.wikipedia.org/wiki/Thai\\_basil](https://en.wikipedia.org/wiki/Thai_basil)) It is most popular in China, Japan, Turkey, and Iran and is also found in South and Central America, tropical Asia, and Africa (Purushothaman et al 2018). The genus *Ocimum* is particularly well known for its antioxidant properties (Chanwitheesuk et al 2005). It has long been used to treat anxieties, coughs, the common cold, headaches, fevers, diabetes, migraines, neuropathic relief, heart disease, gastrointestinal diseases, insect bites, cramps, sinuses, and several neurological diseases as an anti-inflammatory and antidepressant (Bora et al 2011). Moreover, the plant's flowering tops and leaves are apparent as a galactagogue, carminative, stomachic, and anti-inflammatory in traditional medicine (Sajjadi 2006). Extracts of basil from the roots, stems, flowers, leaves, and seeds have been used in a variety of medical treatments.

Natural antioxidants derived from plants have some advantages over synthetic antioxidants, such as their affordability, ease of use, and minimal to nonexistent negative effects. Herbs and spices are now being targeted as key sources of natural antioxidants as a result of trends toward the preservation of products using natural preservatives (Dessí et al 2001). We recently reported on the chemical composition and radical-scavenging activities of essential oils from various Indian spices and medicinal plants (Kar et al 2017). According to a study performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay for free radical scavenging, bleaching β-carotene in the linoleic acid system, and inhibiting linoleic acid oxidation, basil essential oil (*Ocimum basilicum* L.) showed good antioxidant activity (Hussain et al 2008). The radical-scavenging activities of oils have also



been studied using various assays given the traditional uses of the herbs, which suggest that they may possess antioxidant properties.

The rising prevalence of severe opportunistic fungal and bacterial infections is a major issue. Determining new types of natural compounds that might be useful against bacteria and fungi is therefore extremely important. Plant extracts are part of ongoing research to discover novel compounds with the potential to combat multiple drug-resistant bacteria. Most novel antibiotics coming to market today are derived from natural or semisynthetic sources, with approximately 20% of plants found in the world having undergone pharmacological or biological testing (Mothana et al 2005).

During the last three years, plants with antibacterial activity have received much interest in the search for novel therapeutic agents. Current research has discovered that sweet basil extracts contain antibacterial effects against *Escherichia coli* and *Staphylococcus aureus*, antifungal effects against *Rhizopus solani* and *Aspergillus niger*, and antiviral effects against a few strains (Nguefack et al 2004, Maisuthisakul et al 2008). There is no published information on the antioxidant and antibacterial activities of oils derived from various parts of *Ocimum basilicum* var. *thrysiflora*. Therefore, this research aimed to determine the antioxidant and antimicrobial activities of various plant parts of *Ocimum basilicum* var. *thrysiflora*.

## 2. Materials and Methods

### 2.1. Plant material

The plants *Ocimum basilicum* var. *thrysiflora* (Figure 1) were collected from the Medicinal Plants Knowledge Center (MPKC), Bhubaneswar, Odisha, India, in July 2021. Dr. Pratap Chandra Panda, Senior Scientist, Centre for Biotechnology, Siksha 'O' Anusandhan, Bhubaneswar, India, authenticated the plant. A voucher specimen of the plant (No. 1970/CBT) was deposited in the Centre for Biotechnology, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar.



**Figure 1** (A) Whole plant, (B) Leaf, (C) Seeds of *Ocimum basilicum* var. *thrysiflora*.

### 2.2. Extraction of essential oils

To remove dust, the freshly collected samples were first washed with tap water, followed by distilled water. The seeds and leaves were then air-dried in the shade at room temperature. The air-dried leaves and seeds were crushed and pulverized into a coarse powder in a mortar and pestle. Then, 200 g of coarse powder material was hydrodistilled using a Clevenger apparatus for 6 hours to achieve an oil yield. By letting the oil air-dry on anhydrous  $\text{Na}_2\text{SO}_4$ , moisture traces were eliminated. The oil was then collected in Eppendorf tubes and stored at 4 °C.

### 2.3. Antioxidant Activity

Several *in vitro* experiments, including DPPH, ABTS, and nitric oxide free radical scavenging assays, were used to evaluate the antioxidant activity of oils. All assays were conducted in triplicate, and average values were used.

## 2.4. Chemicals

The following chemicals were purchased from Merck India Ltd.: 2, DPPH, sulphanic acid, sodium nitroprusside, trichloro acetic acid, thiobarbituric acid, methanol, naphthyl ethylene diamine dihydrochloride, dimethyl sulphoxide, ferric chloride, hydrogen peroxide, potassium persulphate, ascorbic acid, and butylated hydroxytoluene (BHT).

## 2.5. Diphenyl-1-picrylhydrazyl (DPPH) assay

Brand-William et al.'s (1995) protocol was used to carry out the DPPH assay. This was done by preparing a 0.1 M DPPH solution in methanol and setting its absorbance at 515 nm to 0.95. Then, 100 L of the sample was mixed with 1 mL of the DPPH solution, and the combined solution was incubated at 37 °C for 30 minutes. Methanol served as the control chemical. At 515 nm, the absorbance was noted after 30 minutes. Ascorbic acid served as the standard. To determine the IC<sub>50</sub> and DPPH scavenging activity, the formula below was employed.

$$\text{DPPH scavenging activity (in \%)} = \frac{[(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \times 100}$$

## 2.6. Nitric oxide (NO) scavenging activity

Alkaline dimethyl sulfoxide (DMSO) was used to measure the nitric oxide scavenging activity (Koleva et al 2002). Dry DMSO was allowed to remain in contact with solid potassium superoxide for at least 24 hours. A 200 mL filtrate was then added to 2.8 mL of an aqueous solution containing 10 mM EDTA, 56 mM nitroblue tetrazolium, and 10 mM potassium phosphate buffer at pH 7.4. At 540 nm, absorbances were measured after adding test solutions at various concentrations (5-100 g/mL) and compared to the control.

## 2.7. Azino-bis (3-ethylbenthiazoline-6-sulphonic acid (ABTS) scavenging activity

The effectiveness of ABTS scavenging activity was analyzed using the Re et al (1999) procedure. To produce a dark ABTS working solution, 2.45 mM potassium oxidopersulphate solution and 7 mM ABTS solution were mixed. The mixture solution was then stored in complete darkness for 12 to 16 hours. After being diluted with 50% methanol, the solution's absorbance at 734 nm was set to 0.7 ( $\pm$  0.02). One milliliter of the ABTS working solution was added to 100 L of the sample, and the absorbance was recorded after 1 and 6 minutes. The following formula was used to calculate the ABTS scavenging activity:

$$\text{ABTS scavenging activity (in \%)} = \frac{[(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \times 100}$$

## 2.8. Hydroxyl radical (HO•) scavenging assay

Using the approach developed by Halliwell and Gutteridge (1981), this activity was determined. First, 100 mL of extract solution, 200 mL of premixed ferric chloride (100 mM) solution (1:1; v/v), 500 mL of 2-deoxyribose (2.8 mM) in phosphate buffer (50 mM, pH 7.4), 100 mL of H<sub>2</sub>O<sub>2</sub> (200 mM), and 200 mL of the reaction mixture (100 mL) were mixed. Then, 100 mL of ascorbate (300 mM) was added to the reaction mixture, which was incubated for 1 hour at 37 °C. Furthermore, 1 mL of TCA solution (2.8% w/v aqueous) and 1 mL of TBA solution (1% w/v in 50 mM NaOH) were added to the reaction mixture. The reaction mixture was then heated in a water bath for 15 minutes and allowed to cool. Using the following formula, the hydroxyl radical scavenging activity at 532 nm was determined.

$$\text{Hydroxyl radical scavenging activity} = (1 - \text{Absorbance of sample} / \text{Absorbance of control}) \times 100$$

## 2.9. Test microorganisms

Some periodontal bacteria were identified, and the test bacterial strains were obtained from the Department of Microbiology, College of Basic Science and Humanities, OUAT, Bhubaneswar. The test bacterial strains were *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Proteus mirabilis*.

## 2.10. Evaluation of antimicrobial activity

The disc diffusion method, developed by Standard Kirby Bauer, was employed to evaluate the antibacterial effect. Standard inoculums were prepared by dipping 1-2 colonies into liquid NB and shaking them for 3 hours. After 3 hours, a sterile spreader was used to spread the liquid bacterial culture onto Mueller-Hinton agar (MHA) plates. Three sterile discs with a 6 mm diameter were placed on each agar plate with bacteria. The discs were impregnated with DMSO-dissolved oils at test concentrations of 12.5%, 25%, and 50%. After 24 hours of incubation at 37 °C, the zones of inhibition around the discs were measured and reported in mm. Each bacterium was screened in triplicate, with amoxicillin serving as the positive control and DMSO solvent serving as the negative control.

## 2.11. Statistical analysis

The analyses were carried out in triplicate, and the mean and standard error of the mean (SEM) of the observations were calculated. Tukey's test ( $p < 0.05$ ) was used to assess the variations in the means of the IC<sub>50</sub> for different samples following the completion of an analysis of variance (ANOVA).

### 3. Results

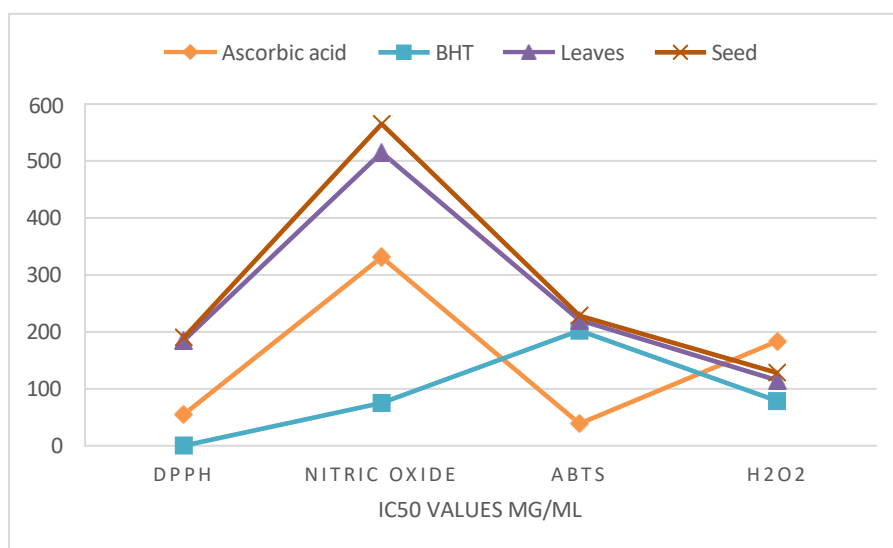
#### 3.1. Antioxidant activity

Various analytical assays were used to evaluate the plant's *in vitro* antioxidant activities. In comparison to standard ascorbic acid and BHT, all antioxidant assays significantly support the antioxidant potential of plants. Table 1 and Figure 2 provide the IC<sub>50</sub> values for the hydrogen peroxide radical, ABTS radical, nitric oxide radical, and DPPH radical scavenging activities of essential oils extracted from leaves and seeds.

**Table 1** Free radical scavenging activity of *Ocimum basilicum var. thyriflora* essential oil.

Essential Oil/Standards	IC <sub>50</sub> value ± SEM (µg/mL) * by methods			
	DPPH	Nitric oxide	ABTS	H <sub>2</sub> O <sub>2</sub>
Ascorbic acid	55.4 ± 20.12 <sup>a</sup>	331.34 ± 0.08 <sup>a</sup>	39.00 ± 0.15 <sup>a</sup>	183.66 ± 12.89 <sup>d</sup>
BHT	0.18 ± 0.00 <sup>b</sup>	75.00 ± 0.00 <sup>b</sup>	202.35 ± 0.10 <sup>a</sup>	78.33 ± 7.07 <sup>b</sup>
Seeds	185.33 ± 2.08 <sup>c</sup>	515.05 ± 8.25 <sup>d</sup>	220.58 ± 0.81 <sup>b</sup>	115.20 ± 6.45 <sup>c</sup>
Leaves	190.25 ± 2.00 <sup>b</sup>	565.25 ± 8.10 <sup>d</sup>	228.67 ± 0.49 <sup>b</sup>	128.35 ± 6.20 <sup>b</sup>

\*Average of determinations made in triplicate. The letters (a-d) in the mean ± SEM indicate significance ( $p < 0.05$ ).



**Figure 2** Free radical scavenging activity of *Ocimum basilicum var. thyriflora* essential oil.

#### 3.2. *In vitro* antioxidant activity of essential oil extracted from leaves

The essential oil extracted from leaves exhibited a strong hydroxyl radical scavenging effect with an IC<sub>50</sub> value of 128.35 ± 6.20 µg/mL, which is significantly lower than the standard value of ascorbic acid. At 190.25 ± 2.00 µg/mL for DPPH, 565.25 ± 8.10 µg/mL for nitric oxide radical, and 228.67 ± 0.49 µg/mL for ABTS. The IC<sub>50</sub> value of the essential oil extract was higher than that of the ascorbic acid and BHT standards. The results show that the essential oil extracted from the leaves has a moderate antioxidant effect.

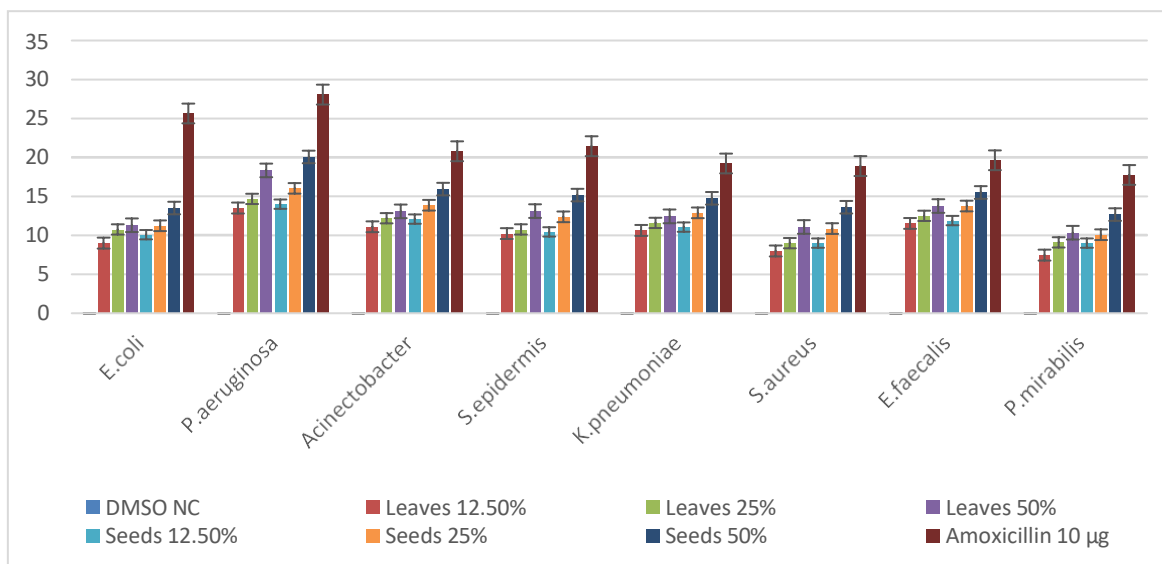
#### 3.3. *In vitro* antioxidant activity of essential oil extracted from seeds

The essential oil from seeds has an IC<sub>50</sub> value of 115.20 ± 6.45 µg/mL, which is significantly lower than the standard value for ascorbic acid and indicates a strong hydroxyl radical scavenging effect. The IC<sub>50</sub> value of seed oil was higher than the ascorbic acid and BHT standards, at 185.33 ± 2.08 µg/mL for DPPH, 515.05 ± 8.25 µg/mL for nitric oxide radical, and 220.58 ± 0.81 µg/mL for ABTS scavenging effect. The outcomes revealed moderate antioxidant activity for the essential oil derived from seeds.

#### 3.4. Antimicrobial activity

The antimicrobial effect of the essential oils of *Ocimum basilicum var. thyriflora* was tested against eight pathogenic bacteria, as shown in Table 2 and Figure 3. *Ocimum basilicum var. thyriflora* essential oils exhibited a strong antibacterial effect against all the tested microorganisms. The antimicrobial activity was evaluated by measuring the zone of inhibition.





**Figure 3** Antibacterial activity of different concentrations of essential oils extracted from leaves and seeds of *Ocimum basilicum var. thrysiflora*.

**Table 2** Antibacterial activity of different concentrations of essential oils extracted from leaves and seeds of *Ocimum basilicum var. thrysiflora*.

Oil concentration of samples	Diameter of zone of inhibition (mm ±SD)							
	Name of the microorganisms							
	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>Acinetobacter</i>	<i>S.epidermis</i>	<i>K.pneumoniae</i>	<i>S.aureus</i>	<i>E.faecalis</i>	<i>P.mirabilis</i>
DMSO (NC)	-	-	-	-	-	-	-	-
Leaves (12.5%)	9.01±0.5	13.5±0.5	11.10±0.65	10.23±0.91	10.63±0.55	8±0.5	11.53±0.50	7.46±0.45
Seeds (12.5%)	10.08±0.6	14.00±0.8	12.09±0.23	10.45±0.86	11.06±0.43	9±0.4	11.89±0.60	9.01±0.23
Leaves (25%)	10.76±0.36	14.68±0.2	12.20±0.36	10.76±0.25	11.6±0.52	9±0.75	12.5±0.5	9.1±0.36
Seeds (25%)	11.23±0.42	16.03±0.23	13.87±0.67	12.38±0.75	12.89±0.25	10.87±0.35	13.76±0.2	10.08±0.24
Leaves (50%)	11.3±0.6	18.33±0.32	13.08±0.21	13.11±0.56	12.43±0.42	11.08±0.8	13.76±0.3	10.34±0.85
Seeds (50%)	13.5±0.5	20.06±0.30	15.93±0.40	15.16±0.47	14.76±0.92	13.6±0.6	15.5±0.5	12.66±0.56
Amoxicillin (10 µg) (PC)	25.63±0.09	28.07±0.56	20.87±1.02	21.45±1.22	19.23±0.89	18.90±0.66	19.65±0.8	17.76±1.05

\*Values are displayed as the mean zone of inhibition (mm) ± standard deviation of three replicates.

### 3.5. Antibacterial activity of essential oils of seeds of *Ocimum basilicum var. thrysiflora*

The antibacterial activity of different concentrations of essential oils of *Ocimum basilicum var. thrysiflora* seeds was determined. The maximum antibacterial activity was observed in the essential oil extracted from seeds against *P. aeruginosa* (20.06±0.30) at a concentration of 50% essential oil, and the minimum activity was noted against *S. aureus* (9.00±0.4) at a concentration of 12.5% essential oil. Essential oils extracted from seeds showed antibacterial activity against all the tested bacteria. The 50% essential oil extracted from seeds exhibited lower antibacterial activity against *P. mirabilis* (12.66±0.56). Twenty-five percent essential oils exhibited the maximum antibacterial effect against *P. aeruginosa* (13.87±0.67) and the minimum against *P. mirabilis* (10.08±0.24), while 12.5% showed the strongest antibacterial effect against *P. aeruginosa* (14.00±0.8).

### 3.6. Antibacterial activity of essential oils of leaves of *Ocimum basilicum var. thrysiflora*

The antibacterial activity of different concentrations of essential oils of *Ocimum basilicum var. thrysiflora* leaves was studied. The strongest antibacterial effect was evaluated in the essential oil extracted from leaves against *P. aeruginosa* (18.33±0.32) at a concentration of 50% of the essential oil and the lowest effect was evaluated against *P. mirabilis* (7.46±0.45) at a concentration of 12.5% of the essential oil. Essential oils extracted from leaves showed antibacterial activity against all tested bacteria. The 50% essential oil extracted from seeds exhibited lower antibacterial activity against *P. mirabilis* (10.34±0.85). Twenty-five percent essential oils exhibited the maximum antibacterial effect against *P. aeruginosa* (14.68±0.2) and minimum antibacterial effect against *S. aureus* (9.00±0.75), while 12.5% showed the maximum antibacterial activity against *P. aeruginosa* (13.5±0.5).

## 4. Discussion

Free radicals have a significant effect on pathogenic manifestations. Phytoconstituents derived from plants play a major role in shielding the antioxidant defense system (Umamaheswari et al 2008). Several methods have been used to



investigate the plant's antioxidant properties (Kil et al 2009), and it has been found that phytoconstituents are the best synthetic alternatives (Vongtau et al 2005, Oluyemi et al 2007).

*Ocimum basilicum* var. *thyrsoflora* is well recognized for its essential oil and the presence of active phytoconstituents such as alkaloids, carbohydrates, steroids, proteins, glycosides, tannins, flavonoids, terpenoids, and phenolic compounds (Lyczko et al 2020). All of these compounds have potent antioxidant properties that actively scavenge the radicals responsible for lipid peroxidation (Chanwitheesuk et al 2005). According to pharmacological studies, these constituents exhibit antimicrobial, antioxidant (Bora et al 2011), antiviral (Chiang et al 2005), anti-inflammatory, hypolipidemic (Amrani et al 2009), anti-platelet aggregation, anti-carcinogenic, antiulcerogenic, and antithrombotic activities. Phenolic compounds' antioxidant properties are mostly related to their (Beric et al 2008) redox activities, and the antioxidant properties of most of these phytochemicals have been linked to lower cancer mortality rates in a diversity of human populations (Oyas 2013).

DPPH, a purple bleaching solution high in free radicals, is commonly used to assess a plant's electron-donating ability (Nunes et al 2012, Kar et al 2017).

The plant has a significant ability to quench ABTS radicals because of its significant ABTS radical quenching ability. Therefore, it may be used to alleviate radical-related stress (Sahreen et al 2010).

The harmful reactive oxygen radical hydrogen peroxide ( $H_2O_2$ ) damages cells when it is converted into the hydroxyl radical, which can lead to DNA mutations and lipid peroxidation (Gülçin et al 2003). According to current research, essential oil extracts can quench this radical. This is possible because of the phenolic compounds that help to convert  $H_2O_2$  to  $H_2O$ .

Pripdeevech et al (2010) reported that the essential oil of *Ocimum basilicum* var. *thyrsoflora* exhibits a potent scavenging ability for DPPH radicals with an  $IC_{50}$  value of  $98.33 \pm 2.08 \mu\text{g/mL}$ . According to Oonsivilai et al (2013), *Ocimum basilicum* var. *thyrsoflora* ethanol extract had the highest antioxidant activity by FRAP assay at a value of  $0.0186 \pm 0.00 \text{ mmol Fe}^{2+}/\text{g}$ . Naidu et al (2016) reported that the scavenging activity of DPPH radicals increased with increasing concentration, i.e., 73.75%, with an  $IC_{50}$  value of  $22 \mu\text{g/mL}$ . *Ocimum basilicum* var. *thyrsoflora* essential oil had 68% linalool and exhibited the highest antioxidant activity. As a result, it is suggested that plant leaves contain significant antioxidant activity and therefore could be utilized as a significant source of antioxidants.

According to Adam and Omer (2015), *Ocimum basilicum* leaf extract had the highest antibacterial activity against *E. coli* and *P. aeruginosa* (7.8 mm inhibition zone) at the lowest concentration of 6.25 mg/ml and the lowest antimicrobial activity against *S. aureus* (4.4 mm inhibition zone). Astuti (2016) reported that basil essential oil exhibited antibacterial activity against *S. mutants* with an  $IC_{90}$  value of 0.23%. In an experiment, Kaya et al (2008) found that the methanol extracts of *Ocimum basilicum* exhibited an antimicrobial effect against *S. aureus* (15 mm), *Shigella specie* (13 mm), *P. aeruginosa* (13 mm), *E. coli* RSHI (14 mm), and *E. coli* ATCC 25922 (13 mm). There was no difference between the acetone and chloroform extracts. Reports revealed that *Ocimum basilicum* var. *thyrsoflora* exhibited antibacterial activity against ampicillin-resistant *E. coli* and *S. aureus* with MICs of  $12.5 \mu\text{L/mL}$  and  $6.25 \mu\text{L/mL}$ , respectively (Avetisyan et al 2017). The report revealed that ethanol, methanol, and chloroform were used for the extraction of antifungal and antibacterial compounds from different parts of the medicinal plants (Kar et al 2018a, b, c).

## 5. Conclusions

In this study, we found that the essential oils of *Ocimum basilicum* var. *thyrsoflora* showed potent antioxidant and antibacterial activities. It showed a stronger antioxidant effect when tested using the hydroxyl radical ( $HO\bullet$ ) scavenging assay technique than other methods. It could be utilized as a widely available source of naturally occurring antioxidants and an excellent source of food supplements. Antioxidants found in natural products play a key role in preventing the harmful effects of free radicals. In addition, antioxidants derived from plants are less expensive and safer than synthetic alternatives. This suggests that the *Ocimum basilicum* var. *thyrsoflora* essential oils obtained from the plant's seeds and leaves have antibacterial properties. Therefore, it would be preferable to isolate antimicrobial compounds from the leaves and seeds of *Ocimum basilicum* var. *thyrsoflora* to develop novel antimicrobial therapeutics to treat various infectious ailments caused by microbes. From this study, we concluded that due to the high antioxidant and antimicrobial activity of *Ocimum basilicum* var. *thyrsoflora*, it might be beneficial to incorporate natural into therapeutic medicines for better implications on human health.

## 6. List of abbreviations

- DPPH - 2-Diphenyl-1-picrylhydrazyl.
- ABTS - Azino-bis (3-ethylbenthiazoline-6-sulphonic acid).
- MPKC - Medicinal Plants Knowledge Center.
- BHT - Butylated hydroxytoluene.
- DMSO - Dimethyl sulfoxide.
- ANOVA - Analysis of variance.
- SEM - Standard error of mean.
- SD - Standard Deviation.

NC - Negative Control.  
PC - Positive Control.  
MIC - Minimum inhibitory concentration.

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### Ethical considerations

Not applicable.

### Conflict of Interest

The authors declare that they have no conflict of interest. The authors Akankshya Sahu and Gayatree Nayak contributed equally to this work.

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