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Research Article

Chemical composition, antioxidant, and antimicrobial activities of Bangladesh-origin Jhum-cultivar basil (*Ocimum basilicum* L.) essential oil

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Abstract: Essential oil (EO) from the sweet basil (*Ocimum basilicum* L.) grown in the Jhum cultivations located in Bangladesh was screened for chemical composition, antioxidant, and antimicrobial activities. EO yield from the Jhum-cultivar *O. basilicum* was 1.55% (v/w). Analysis of EO indicated the presence of several bioactive compounds, among which *Geranial* (35.5%) and *cis-citral* (26.2%) are of significant content. The EO showed antioxidant activities inhibiting DPPH radical with a mean value of 45.7% at 2.4 mg mL^{-1} of EO. The EO has susceptibility against Gram-positive and Gram-negative bacteria (*Escherichia coli*, *Salmonella* Typhi, *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus cereus*, and *Micrococcus* spp.), with a notable activity against the Gram-positive bacteria.

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1. INTRODUCTION

Ocimum species (basil), a native herb to Asia and also grows wild in tropical and sub-tropical regions worldwide, is often referred to as the *king of herbs* owing to its popularity (Makri & Kintzios, 2008). Sweet basil (*Ocimum basilicum* L.) is the most commonly grown species of the *Ocimum* genus and also represents a significant fraction of commercial basil cultivars available in the market (Calin-Sanchez *et al*., 2012; Jirovetz *et al*., 2003; Makri & Kintzios, 2008; Shahrajabian *et al*., 2020). As a culinary herb, basil induces distinctive flavor in Iranian, Italian, Chinese, and Indian cuisines (Akbari *et al*., 2018; Makri & Kintzios, 2008; Shahrajabian *et al*., 2020). The medicinal uses and potential health benefits of *O. basilicum* in traditional and

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modern medicine have been reported (Kaefer & Milner, 2008; Twilley *et al*., 2018; Zhan *et al*., 2020). *O. basilicum* is used in ethnomedical practices to treat coughs, stomach-related diseases, involuntary muscle spasms, parasitic worms, and skin infections (Labra *et al*., 2004; Shirazi *et al*., 2014). The essential oil (EO) from *O. basilicum* contains several bioactive chemicals, such as flavonoids, phenol derivatives, phenyl-propanoids, triterpenoids, steroids, and steroidal glycosides (Rezzoug *et al*., 2019; Siddiqui *et al*., 2012; Singh *et al*., 2018; Złotek *et al*., 2017). The constituents in *O. basilicum* EO are known to possess antioxidative, anti-inflammatory, antispasmodic, antibacterial, antifungal, and antioxidant properties (Ahmad *et al*., 2016; Ahmed *et al*., 2019; Burt, 2004; Miguel, 2010; Stanojevic *et al*., 2017; Tankeo *et al*., 2014; Złotek *et al*., 2016). However, *O. basilicum* EO's composition varies depending upon the origin of the plant (Bilal *et al*., 2012; Da-Silva *et al*., 2003), plant genotype, soil and climatic conditions, growing technique, harvest time, irrigation as well as fertilization (Mith *et al*., 2016; Muzika *et al*., 1989).

O. basilicum L. is a popular herb among the indigenous communities living in the Chittagong Hill Tracts (CHT) of Bangladesh. It is consumed both as a food ingredient and in ethnomedicine. The herb is produced via the traditional shifting cultivation farming technique (slash-and-burn agriculture), commonly known as *Jhum* cultivation. The chemical constituents and pharmacological benefits of various *O. basilicum* cultivars have been reviewed (Bakkali *et al*., 2008; Miguel, 2010; Shahrajabian *et al*., 2020; Swamy *et al*., 2016; Zhan *et al*., 2020). However, to our knowledge, EO from any Jhum-cultivar *O. basilicum* variety has not been screened before. Hence, the current study aims to assess the chemical composition, antimicrobial, and antioxidant properties of the EO derived from *O. basilicum* species collected from the Jhum cultivations in CHT, Bangladesh.

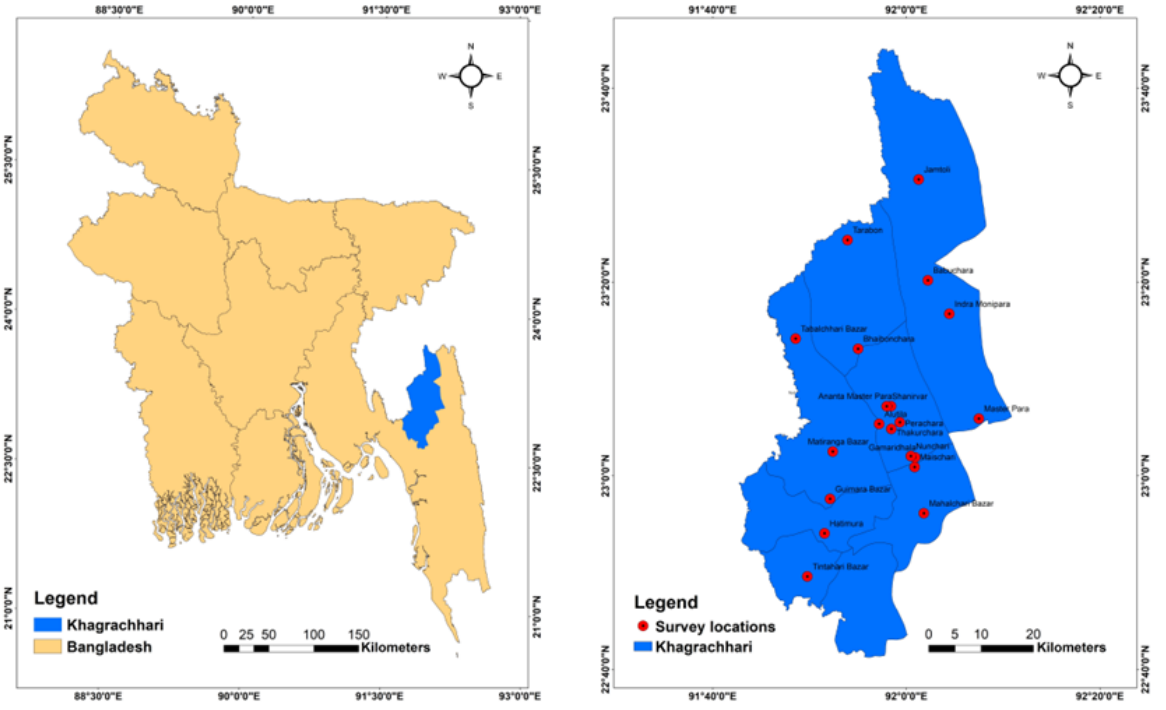
2. EXPERIMENTAL

2.1. Materials

2.1.1. *Sample*

The *O. basilicum* species were collected from the *Khagrachhari* hill district in Bangladesh. The study location and sampling points are shown in Figure 1.

Figure 1. Location map showing the sampling points.



2.1.2. *Chemicals and reagents*

2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Shanghai Shenmin Industrial Co., Shanghai, China), tetrazolium chloride (Sigma-Aldrich, St. Louis, MO), and dimethyl sulfoxide (DMSO) (Merck, Darmstadt, Germany) were used without further purification.

2.1.3. *Microorganisms*

The food-borne pathogens *Staphylococcus aureus* (ATCC6538), *Bacillus cereus* (ATCC14574), *Micrococcus* spp. (ATCC4698), *Salmonella* Typhi (ATCC14028), *Vibrio cholerae* (ATCC14035), and *Escherichia coli* (ATCC25922) were used for antimicrobial activity monitoring.

2.2. Methods

2.2.1. *Separation of EO*

Fresh leaves of *O. basilicum* were subjected to hydrodistillation for 2 h, assisted by the Clevenger apparatus. Next, the distilled EO was dried on anhydrous sodium sulfate. Afterward, the filtrate was transferred into a sealed vial and preserved at 4 °C for further analyses.

2.2.2. *Antimicrobial activity analysis*

The disc diffusion method (Kim *et al*., 1995) was used to study the activity of EO against the above-mentioned food-borne bacteria. The fresh culture of organisms was adjusted at 1×10^8 CFU mL^{-1} by comparing with the 0.5 Mc Farland standard (optical density, 0.80–0.12). The cultured organism was swabbed into the Mueller-Hinton Agar (MHA) plates. The sterile paper disc (dia, 6 m) was soaked with different EO concentrations (10, 15, 20 µL of 1:1 (v/v) dilution with 0.5% DMSO). The air-dried cultures were incubated at 37 °C for 24 h in MHA plates. One filter paper disc was placed per Petri dish to avoid a possible additive activity. DMSO (0.5%, 10 µL) was used as the negative control, and standard antibiotic chloramphenicol (10 µg) was used as the positive control. Obstruction zones were measured using a transparent ruler on an mm scale. If the zone of inhibition is measured >15 mm, it will be termed as *strongly inhibitory*, 10 to 15 mm as *moderately inhibitory*, and <10 mm as *not inhibitory*.

2.2.3. *Bactericidal concentration analysis*

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were tested according to the broth dilution method (Mith *et al*., 2016; Sakkas *et al*., 2018; Veiga *et al*., 2019). The stock solution of EO was prepared by diluting EO with 2 to 5% DMSO in a ratio of 1:1. 10^{-1} dilution of Mueller-Hinton Broth (MHB) was prepared by mixing 4.5 mL of sterilized MHB with 0.5 mL of freshly prepared stock solution. The micro-dilution tests were performed in sterile round-bottomed 96-well microplates. First, 100 µL of 10^{-1} MHB was transferred into 1st wells of the microtiter plate. Next, 50 µL of previously prepared sterile MHB was moved into the subsequent 2–12 wells of a microtiter plate. Two-fold dilution was performed by transferring 50 µL from the 1st to the 11th well. (12th well: drug-free well/ growth control). Next, 10^{-2} dilutions of prepared 0.5 Mc Farland standard inoculation (1.5×10^8 CFU mL^{-1}) of six human pathogenic bacteria were prepared. From this dilution, 50 µL of inoculum was transferred from the 1st to 12th wells (except the 11th one used for a negative control). Then, the microtiter plate was incubated at 37 °C for 18 to 24 h. MIC was recorded according to the inhibition of visible growth. To determine the MBC, we picked the area where no increase was observed.

2.2.4. *Antioxidant activity analysis*

The antioxidant activity of *O. basilicum* EO was examined by the DPPH radical scavenging method (Mensor *et al*., 2001). The antioxidant activities of EO were assessed by measuring the ability to scavenge DPPH stable radicals (Saha *et al*., 2017). EO sample (1.5 mL) was prepared

in different concentrations using methanol. A reaction mixture of 0.5 mL consisting of a 0.5 M acetic acid buffer solution of pH 5.5 and 1 mL of 0.2 mM DPPH in methanol was prepared. The sample solution and reaction mixture were combined and kept in the dark for 30 min after incubation at room temperature. A chemical reaction occurred between DPPH and EO samples. The unused DPPH concentration was analyzed at 517 nm using a UV-1601 spectrometer (Shimadzu, Kyoto, Japan).

The reference standard was ascorbic acid. DPPH radical scavenging concentration was computed using the following equation.

$$\text{DPPH radical scavenging concentration (\%)} = [1 - \frac{A_{\text{sam}}}{A_{\text{cont}}} \times 100]$$

Here, A_{cont} = absorbance of the control, A_{sam} = absorbance of sample solution. Then, % inhibition was plotted against the used respective concentrations.

2.2.5. *Chemical composition analysis*

The EO's gas chromatographic-mass spectrometric (GC-MS) analysis was performed using GC system 7899A with MS detector 5975C (Agilent Technologies, Santa Clara, CA). A fused silica capillary column of (5% phenyl)-methylpolysilloxane (HP-5MS) (length, 30 m; diameter, 0.250 mm; film, 0.25 μm) was used. The GC parameter was set as follows: inlet temperature, 250 °C; oven temperature, 60 °C for 0 min, 10 °C per min to 200 °C for 2 min, and 12 °C per min to 300 °C for 5 min. The total analysis time was 29.33 min with a column flow rate of 2.43 mL min^{-1} helium gas.

The mass spectrometer (MS) was an electron ionization type. MS quad temperature was set at 150 °C, and the source temperature was set at 230 °C. The detector voltage was 0.2 kV, and the mass range was 50–550 m/z. The Total Ionic Chromatogram (TIC) was used to determine each compound's peak area and percentage concentration.

3. RESULTS AND DISCUSSION

3.1. Composition of EO

It has been reported that basil EO yields can vary significantly from 0.07 to 1.92% in different basil assays (Zheljazkov *et al*., 2008). The yield of light yellow EO obtained with a characteristic pleasant aroma was 1.55% v/w. Considered EO content was expressed as mL per 100 g raw sample.

The Jhum-cultivar *O. basilicum* EO has 19 bioactive compounds representing 99.95% of the total mass (Figure 2 and Table 1), with a significant presence of geranial (35.5%), followed by cis-citral (26%), cyclopentanol (5%), and linalool (4.94%). Hence, the biophysical and biological properties of EOs depend on the identified 19 compounds.

A previous study on the Bangladesh-origin *O. basilicum* EO (sample collection location: Dhaka) showed methyl chavicol and gitoxegenin as the major constituents (Hossain *et al*., 2010), while geranial has been reported as the significant EO constituent in several *O. basilicum* varieties (Shahrajabian *et al*., 2020; Zhan *et al*., 2020). Nerol and geraniol are oxidative forms of neral and geranial (Patora *et al*., 2003). Geraniol induces antibacterial and antifungal properties (Lira *et al*., 2020). It has been assumed that geranial mixed with other constituents to form geraniol and thus accelerated the antibacterial properties of Jhum-cultivar *O. basilicum* EO. Turpentine alcohols, such as linalool, exhibit potent antibacterial activity against bacterial strains (Uysal *et al*., 2011). In India, under different climatic conditions, it has been reported that *O. basilicum* EO is also rich in linalool, geraniol, geranial, caryophyllene, and limenone (Maurya *et al*., 2022).

Figure 2. GC-MS spectrum of Bangladesh-origin Jhum-cultivar *O. basilicum* EO.

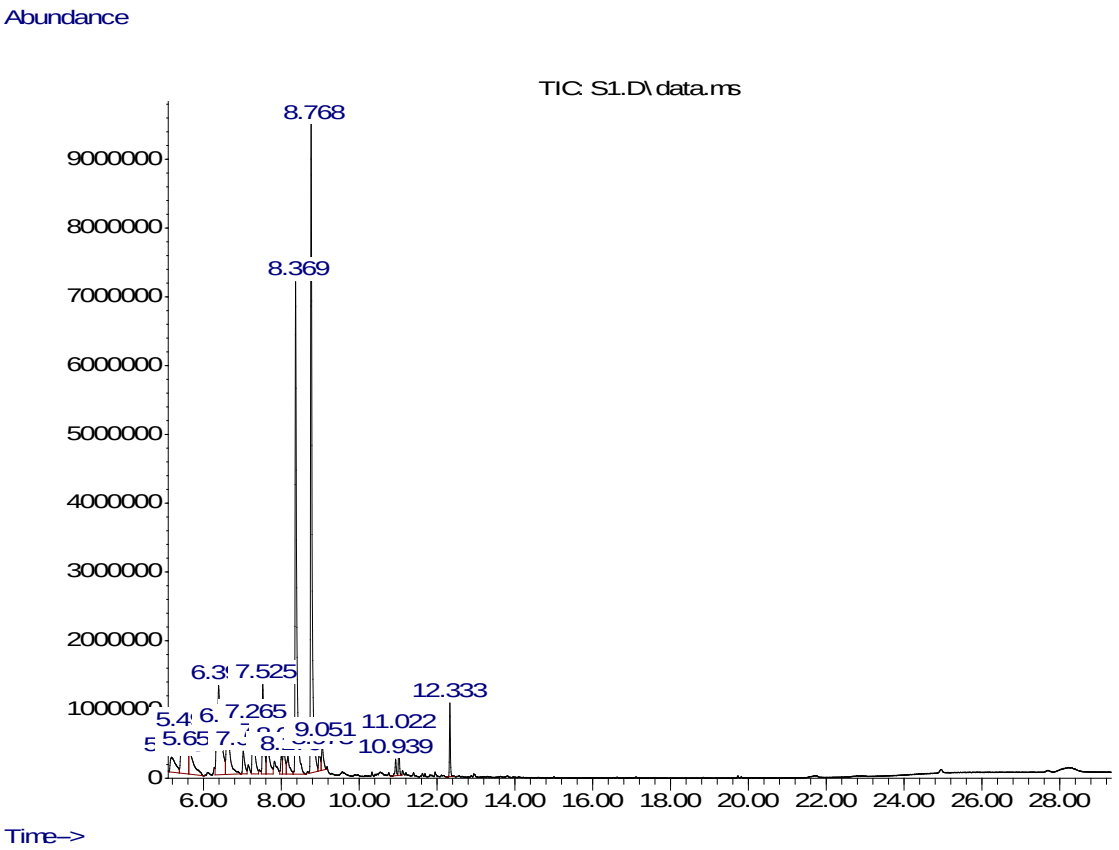


Table 1. Major constituents in Bangladesh-origin Jhum-cultivar *O. basilicum* EO.

Compound name	RT*	Absolute height	%Concentration
Mentha-1,4,8-triene	5.178	304652	1.12
Thymene	5.493	671859	2.46
l-Phellandrene	5.648	388687	1.42
Linalool	6.392	1349647	4.94
Santolina triene	6.603	716933	2.62
Geraniol acetate	7.027	390986	1.43
Limonene oxide	7.267	785715	2.88
Cyclopentanol	7.524	1369252	5.01
Fumaric acid	7.645	507564	1.85
Ethanone	8.005	321851	1.18
Chrysanthenone	8.062	448254	1.64
Camphene	8.171	315577	1.16
cis-Citral	8.371	7152777	26.19
Geranial	8.766	9693337	35.50
Piperidine	8.972	406767	1.48
Pentanamide	9.052	520585	1.90
Caryophyllene	10.941	272579	0.99
Bergamotene	11.021	621629	2.28
Bisabolene	12.331	1065430	3.90
			Total, 99.95%

*RT, Retention time

The low contents of bergemotene, bisabolene, caryophyllene, llimonene oxide, and santolena triene have contributed to the antibacterial activity. However, it is also possible that the rest secondary elements may be involved in some form of synthesis with other active compounds (Marino *et al*., 2001). The composition of Jhum-cultivar *O. basilicum* EO shows significant differences from the same species cultivated in Pakistan, Kenya, Iran, and Serbia, Egypt (Table 3). Such differences in composition can be attributed to the geographical diversity and variations in the drying process or EO extraction approach.

3.2. Antimicrobial Activity

The effect of *O. basilicum* EO on Gram-positive and Gram-negative bacteria growth at three different volumes (1:1 v/v dilution with DMSO) was studied. The antibacterial behavior of Jhum-cultivar *O. basilicum* EO is shown in Table 2. *O. basilicum* EO has shown a more substantial impact on Gram-positive bacteria, except for *V. cholerae*, than on Gram-negative bacteria, as evident from the MIC and MBC values (Tables 3 and 4). *Micrococcus* spp. showed the highest zone of inhibition (Figure S1. See Appendix A. Supplementary information). The MIC and MBC values of *B. cereus* (0.69 and 1.39 mg mL^{-1}) were minimum, while *S.* Typhi and *E. coli* showed maximum MIC and MBC (5.59 and 11.18 mg mL^{-1}). The cell wall of Gram-negative bacteria can prevent the penetration of antimicrobial compounds of EO (Burt, 2004; Nazzaro *et al*., 2013), which might be the reason for higher resistance to EO by Gram-negative bacteria than Gram-positive bacteria. The results of this study are similar to other investigations that have already been published (Anand *et al*., 2011; Hossain *et al*., 2010; Hussain *et al*., 2008; Sakkas *et al*., 2018; Silva *et al*., 2015; Stanojevic *et al*., 2017).

The impact of origin diversity on *O. basilicum* EO's antimicrobial behavior has also been observed (Table 4). For instance, *O. basilicum* EO collected from India exhibits MIC 12.5 and 6.25 mg mL^{-1} for *S. aureus* and *E. coli*, respectively, which were higher than the findings from the current work (Anand *et al*., 2011; Sakkas *et al*., 2018).

Table 2. Antibacterial activity of Bangladesh-origin Jhum-cultivar *O. basilicum* EO.

Pathogen		Zone of inhibition (mm)[a]			DMSO[b]	Chloramphenicol[c]
		10 µL	15 µL	20 µL		
Gram-positive Bacteria	*S. aureus*	22.7 ± 0.6	24.8 ± 1.0	29.2 ± 0.8	0	25
	B. cereus	33.4 ± 0.8	40.8 ± 1.1	46 ± 1.3	0	28
	Micrococcus spp.	41.8 ± 1.1	46.6 ± 0.5	50.1 ± 1.0	0	25
Gram-negative bacteria	*S.* Typhi	8.7 ± 0.6	9.5 ± 0.5	10 ± 1.0	0	20
	V. cholerae	33.3 ± 0.8	41.3 ± 1.2	45.8 ± 0.8	0	35
	E. coli	8.7 ± 0.6	10 ± 0.0	10 ± 0.0	0	20

[a] Diameter of inhibition zone (mm) around the disc (6 mm); [b] 10 µL 0.5% DMSO was used as negative control; [c] standard antibiotic 10 µg chloramphenicol was used as a positive control.

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Bangladesh-origin Jhum-cultivar *O. basilicum* EO.

Pathogen		MIC, mg mL^{-1}	MBC, mg mL^{-1}
Gram-positive Bacteria	*S. aureus*	2.89	5.59
	B. cereus	0.69	1.39
	Micrococcus spp.	2.89	5.59
Gram-negative bacteria	*S.* Typhi	5.59	11.18
	V. cholerae	2.89	5.59
	E. coli	5.59	11.18

Table 4. Comparison of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and major constituents of *O. basilicum* EO from different regions of the world.

Major constituents (%)	Antimicrobial activity		Country		Reference
	MIC	MBC	Pathogen		
Linalool (32.83), Elemol (7.44), Geranyl acetate (6.18)	–	–	–	Algeria	Hadj Khelifa *et al*., 2012
Methyl Chavicol (36.7), Trimethoquinol (10.3), Gitoxigenin (9.3	125, 62.5, 500, 250 (µg mL^{-1})	–	*S. aureus*, *B. cereus*, *E. coli*, *S.* Typhi	Bangladesh (northern Dhaka)	Lira *et al*., 2020
Linalool (48.4), 1,8-Cineole (12.2), Eugenol (6.6)	–	–	–	Egypt	Abou El-Soud *et al*., 2015; Farouk *et al*., 2016
Methyl chavicol (70), Linalyl acetate (22.54)	12.5, 6.25 (mg mL^{-1})	–	*S. aureus*, *E. coli*	India	Sharafati Chaleshtori *et al*., 2018; Srivastava *et al*., 2014
Methyl Chavicol (40.5), Geranial (27.6), Neral (18.5)	18, 18, 9 (µg mL^{-1})	–	*S. aureus*, *B. cereus*, *E. coli*	Iran	Moghaddam *et al*., 2011; Sajjadi, 2006
Linalool (31.6), Methyl chavicol (23.8), *β*-Elemene (6.9)	4.5 µg mL^{-1}	5.5, 6.6 (µg mL^{-1})	*S. aureus*, *E. coli*	Serbia	Sokovic *et al*., 2008; Stanojevic *et al*., 2017
Linalool (61.7), 1,8-Cineole (17.2), Borneol (8.5)	Resistant, 5, 2.5 (mg mL^{-1})	–	*S. aureus*, *B. cereus*, *E. coli*	Nigeria	Usman *et al*., 2013
Linalool (69.87), Geraniol (9.75), *p*-Allylanisole (6)	Sensitive	–	*S. aureus*, *E. coli*, *B. cereus*	Oman	Al Abbasy *et al*., 2015; Hanif *et al*., 2011
Linalool (60), Cadinol, Epi-*α* (12), *α*-Bergamotene (9.2)	Sensitive	–	*S. aureus*, *E. coli*	Pakistan	Hussain *et al*., 2008
Methyl chavicol (93.4), (E)-*β*-ocimene (2.24)	100, 50, 25,50 (µg mL^{-1})	>200, 100, 50, 50 (µg mL^{-1})	*S. aureus*, *B. cereus*, *E. coli*, *S.* Typhi	Thailand	Bunrathep *et al*., 2007; Phanthong *et al*., 2013
Copaene (25.5), *p*-Menth-2-en-1-ol (7.7), Eugenyl acetate (4.8)	Sensitive	–	*S. aureus*, *E. coli*	Ethiopia	Unnithan *et al*., 2013
Linalool (65.38), Eugenol (5.26)	Sensitive	–	*S. aureus*, *E. coli*	Romania	Stefan *et al*., 2013
Linalool (57.08), , *α*-Bergamotene (2.27–	1≤ mg mL^{-1}	–	*S. aureus*	Turkey	Daneshian *et al*., 2009

Major constituents (%)	Antimicrobial activity MIC	MBC	Country Pathogen		Reference
3.70), Naphthalene (11–14.6)					
Methyl eugenol (52.60), Caryophyllene (18.75), Germacrene-D (9.19)	–	–	–	Indonesia	Ni Putu Ermi Hikmawanti, 2019
α-Terpineol (59.78), *β*-caryophyllene (10.54), *α*-humulene (3.90)	–	–	–	Burkina Faso	Bayala *et al*., 2014
Linalool (37), Eugenol(6.3), Geraniol (8.9)	Sensitive	–	*S. aureus*, *E. coli*	Nepal	Yonzon *et al*., 2005
Geranial (35.5), Cis-citral (26.19), Cyclopentanol (5.01)	2.89, 0.69, 2.89, 5.59, 2.89, 5.59 (mg mL^{-1})	5.59, 1.39, 5.59, 11.18, 5.59, 11.18 (mg mL^{-1})	*S. aureus*, *B. cereus*, *Micrococcus* spp., *E. coli*, *V. cholerae*, *S.* Typhi	Bangladesh (Jhum-cultivar)	Present work

'–'= No information

3.3. Antioxidant Activity

The antioxidant activity of Jhum-cultivar *O. basilicum* EO is shown in Table 5. The EO removed 45.70 ± 0.041% free radical at a concentration of 2.39 mg mL^{-1} attributable to the EO constituents' antioxidant properties. Similar levels of inhibition (45.0±0.008) were shown by reference antioxidant ascorbic acid at a concentration of 0.002 mg mL^{-1}. Both the EO and the ascorbic acid showed antiradical activity in a concentration dependent manner. Geranial and cis-citral were the major constituents in the Jhum-cultivar *O. basilicum* EO. Citral isomers, such as trans-citral, cis-citral, and geraniol, have potential antioxidant activity compared to the Trollox standard (Jaradat *et al*., 2016). Besides, citral is a combination of neral and geranial, and both constituents were present in the Jhum-cultivar *O. basilicum* EO. The *O. basilicum* EO has shown antioxidant activity closer to the Serbian variety (2.38±0.01 mg mL^{-1}, 90 min of incubation) (Stanojevic *et al*., 2017). Egyptian *O. basilicum* EO also exhibited a significantly higher scavenging ability for DPPH (Farouk *et al*., 2016).

Table 5. Scavenging effect (%) of positive control and *O. basilicum* EO of Bangladesh-origin Jhum-cultivar on DPPH at different concentrations.

Samples	EO concentration (mg mL^{-1})	Antiradical activities (%)
Essential oil	1.19	29.65±0.011
	2.39	45.70±0.041
Ascorbic acid	0.0013	29.68±0.009
	0.002	45.0±0.008

4. CONCLUSION

The Bangladesh-origin Jhum-cultivar *O. basilicum* EO was studied to explore the bioactive contents plus antibacterial and antioxidant activities. The Jhum-cultivar *O. basilicum* EO confirmed the impacts of origin diversity on EO's composition and the antibacterial and antioxidant response pattern. Therefore, additional studies are required to explore the potential

of Jhum-cultivar *O. basilicum* EO as a source of natural antioxidants for use in the food or pharmaceutical sectors of Bangladesh.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s).

Authorship contribution statement

Suman Barua: Conception, Design, Supervision, Fundings, Analysis and/or Interpretation, Writing. **Kajalika Dewan**: Data Collection and/or Processing, Analysis and/or Interpretation, Literature Review. **Saiful Islam**: Data Collection and/or Processing, Writing. **Suman Mojumder**: Data Collection and/or Processing, Writing. **Ovi Sikder**: Data Collection and/or Processing, Writing. **Rajib Sarkar**: Data Collection and/or Processing, Writing. **Hiroshi Hasegawa:** Analysis and/or Interpretation, Critical Review. **Ismail M.M. Rahman:** Conception, Supervision, Fundings, Analysis and/or Interpretation, Critical Review.

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Appendix A. Supplementary information

Figure S1. Antibacterial activity (maximum zone of inhibition) of Bangladesh-origin Jhum-cultivar *O. basilicum* EO against *Micrococcus* spp.