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Antioxidant, Antibacterial and Antifungal Properties of Black Pepper Essential Oil (*Piper nigrum* Linn.) and Molecular Docking and Pharmacokinetic Studies of Its' Major Component

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ABSTRACT

The present study aimed to investigate chemical composition of essential oil (EO) from black pepper extract through steam distillation and evaluate by GCMS with *In vitro* antimicrobial and antioxidant activities and *In silico* studies. In total, thirteen volatile compounds identified by GCMS analysis. Among them, main components are d-norandrostane (14.874%), Valencene (13.297%), 1H-3a-7-Methanoazulene octahydro-1,9,9-trimethyl-4-methylene-(11.591%), (-)-spathulenol (8.193%), aromandendrene (8.398%), and naphthalenedecahydro-4a-methyl-1-methylene-7-(1-methylethylidene) (7.794%). The EO extracted from black pepper, displayed moderate antibacterial activity against ten bacterial strains (two and eight numbers of *Gram-positive* and *Gram-negative*, respectively) compared with Ceftriaxone as standard. In case of antifungal study, the EO exhibited a greater zone of inhibition with 13.7±1.5 mm against *Trichoderma harzianum*, compare to Amphotericin B as standard (11.7±1.5 mm). The results of antioxidant efficacy of extracted EO revealed good activity with IC₅₀ value 35.83±2.92 µg/mL as compared to standard ascorbic acid (27.34±1.86 µg/mL). *In silico* studies satisfy the experimental values.

Keywords: Black pepper, Essential oil, GCMS, Antimicrobial activities, Antioxidant activities, Molecular docking.

INTRODUCTION

Many plant extracts have been use to treat infectious diseases of human for centuries due to their therapeutic properties. Generally, most

of the plants bear important chemical compounds called phytochemicals, which can generate specific physiological action against microorganisms on human body¹. The treatment of infectious diseases caused by various pathogens has become an



alarming threat due to the extensive use of antibiotics and the rapid development of multidrug resistance against microorganisms². Essential oil and other extracts of plants draw a greater interest due to their medicinal significance³. Essential oil extracted from different plant showed antiviral, antibacterial, antifungal, antioxidant and insecticidal properties⁴⁻⁵. Some essential oils used in the treatment of cancer, aromatherapy, food preservation, and fragrance industries⁶. Therefore, it has great importance to extract essential oils from different parts of plant i.e. fruits, leaves, roots, flowers, seeds, and barks⁷. Steam distillation, hydro distillation, and fermentation techniques usually employed to extract these oils from various plants⁸. These oils are complex mixtures of many volatile components, which are chemically nonpolar in nature. Black pepper (*Piper nigrum* Linn.) is a popular spice known as 'King of Spices' or 'Black Gold', and in Bangladesh known as 'Golmorich'. It belongs to the Piperaceae family^{9,10} and is native to Bangladesh, India, Thailand, Brazil, China, Indonesia, Malaysia, Mexico, and Vietnam¹¹. This widely used due to its aroma properties with nutrition and medicinal values¹⁰. In Ayurvedic, black pepper is use in the treatment of rheumatism, diarrhea, headache, dysentery, cholera, to remove excessive gas from gastrointestinal tract, and to increase the flow rate of urine. It also use in treatment of digestive problems, stomach disorders, neuralgia, and scabies^{12,13}. Considering the above-mentioned biological importance of EOs, the present study aimed to extract essential oil from black pepper and evaluate its *In vitro* anti-microbiological and antioxidant activities using disc diffusion test and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, respectively. In this study, 2, 8 and 6 number of *Gram-positive* bacteria, *Gram-negative* bacteria and fungal strains, respectively, were used for antimicrobial assay, and *In silico* molecular docking and pharmacokinetic analysis performed in support of experimental results. The results present in this paper will useful to identify the biologically potent volatile compounds.

EXPERIMENTAL

Material and GCMS analysis

The black pepper collected from the local market of Chattogram, Bangladesh, wash with distilled water and dry under subdue sunlight for several days. The dry samples transform to powder

using blender machine. The powder sample (370.0 g) subjected to extraction with *n*-hexane using Clevenger apparatus (Germany) for 72 hours. *n*-Hexane extract dried by using anhydrous Na₂SO₄ and followed by filter and solvent removed by using vacuum evaporator to obtain black pepper essential oil. The EO stored in an airtight container and preserved in refrigerator at 8°C earlier used by separation and analysis of essential oil was performed by GCMS (gas chromatography mass spectrometer). Simadzu GC-17A gas chromatograph fitted with RTS-5MS capillary column (30cm×0.25mm diameter) coupled with a mass spectrometer (MS 2010 plus). Column temperature maintained at 260°C and packed by diethylene glycol succinate (10%). Ultra-high pure helium gas (99.99%) used as carrier with flow rate 1.0 mL/min maintaining a constant pressure (90 kPa).

Antimicrobial activity assay

The 'disc diffusion test' employed to determination of *In vitro* antimicrobial activity and Mueller Hinton agar and potato dextrose agar were use as base media¹⁴. These incubated 24 h and monitored continually to check any contamination. The standardized test organism inoculation on incubated media by using sterile cotton bar and the filter paper discs (6 mm dia.) contain test sample at desire concentration located softly on pre-inoculated agar media surface and aerobic incubation done for 24 h at 37°C and 48 h at 26°C for bacterial and fungal pathogen, respectively. Each paper disc contained 25 µL of sample in DMSO that contained 300 µg of extracted EO, here dimethyl sulfoxide (DMSO) use as control. In addition, 10 µL of Ceftriaxone or Amphotericin B in DMSO was use on paper disc as standard. The Petri dishes incubated for 24 h and the diameters of inhibition zones (mm) measured by a measuring scale. All the tests done repeated for three times. In this study, two numbers of *Gram-positive* bacteria named *Staphylococcus aureus* and *Bacillus magneterium*; moreover, eight numbers of *Gram-negative* bacteria, named *Salminella paratyphi*, *Enterotoxigenic Escherica coli*, *Salminella typhi*, *Shigella flexneri*, *Shigella sonnei*, *Shigella boydii*, *Escherica coli*, *Shigella dysentery* were used to determine antimicrobial activities. Beside this, six numbers of fungal strains namely *Aspergillus niger*, *Panysalium notatum*, *Candida albican*, *Aspergillus flavus*, *Neurospora crassa*, *Trichoderma harzianum* were used to check antifungal activity.

Antioxidant activity assay

DPPH radical scavenging method employed to find out antioxidant efficacy of EO according to a method described by Shah and co-workers¹⁵. In this method, solutions were prepared separately in ethanol with 10 mL of DPPH radical (0.1 mM), essential oil at desire concentration (31.25, 62.5, 125, 250, 500 µg/mL) and ascorbic acid as standard. 4 mL DPPH radical solution with 100 µL sample solutions were vortex and incubated at 26°C for 15 min and maximum absorbance observed at 517 nm with a blank sample (2 mL DPPH with 2 mL ethanol) by an UV spectrophotometer. The test performed three times. Inhibition (%) by the radicals calculated with equation:

$$\text{Inhibition (\% of DPPH free radical)} = \left[\left(1 - \frac{Ab_{\text{sample}}}{Ab_{\text{control}}} \right) \times 100 \right]$$

Here, Ab_{control} is absorbance of DPPH radical and Ab_{sample} is absorbance of DPPH with essential oil. The concentration-inhibition curves utilized to determine IC_{50} of essential oil and ascorbic acid.

Molecular docking study

Three-dimensional crystal structure of target protein 1JJ¹⁶, 1KZN¹⁷ and 5JBO¹⁸ of *S. aureus*, *E. coli* and *T. harzianum*, respectively, retrieved in PDB format from protein data bank archive (<https://www.rcsb.org/>) and human antioxidant enzyme 1HD2¹⁹ was used for investigate the antioxidant activity of EO from black pepper by docking method. The water molecules, heteroatoms, and inhibitors removed and hydrogen atoms added to the amino acid residues through PyMol software (ver. 2.4). Swiss-PdbViewer employed for minimize energy of the target proteins. Structure optimization done by Gaussian 09 software and the optimized structure subjected for docking study against selected proteins²⁰.

ADME analysis

Pharmacokinetics properties of major component of essential oil from black pepper determined by online web tool SwissADME²¹.

RESULT AND DISCUSSION

Characterization of essential oil by GCMS

Yield of obtained essential oil founded

24.67% (w/w). The presence of volatile compounds in the EO identified by GCMS analysis comparing their retention time and molecular weight with the reference compounds in NIST mass spectra library (<https://www.nist.gov/nist-research-library>) and GCMS chromatogram shown in Fig. 1. Thirteen compounds identified by GCMS analysis and presented in Table 1. The major compounds identified as d-Norandrostane(5 α ,14 α) (14.874%), Valencene (13.297%), 1-H-3a-7-Methanoazulene octahydro-1,9,9-trimethyl-4-methylene-(11.591%), (-)-spathulenol (8.193%), Aromandendrene (8.398%), and Napthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethylidene) (7.794%) and shown in Fig. 2. Other minor compounds identified as Bicyclo[7.2.0]undec-4-ene-4,11,11-trimethyl-8-methylene- (6.594%), Bicyclo[3.1.1]heptan-3-ol,6,6-dimethyl-2-methylene-(6.594%), 8-methylene-D-Limonene (5.159%), Linalool isobutyrate (3.728%), Alpha-Terpinol (3.692%), Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-(2.890%), Caryophyllene oxide (2.578%), Ledene oxide-(II) (1.094%) and Disipro[2.1.2.4]undecane (1.458%).

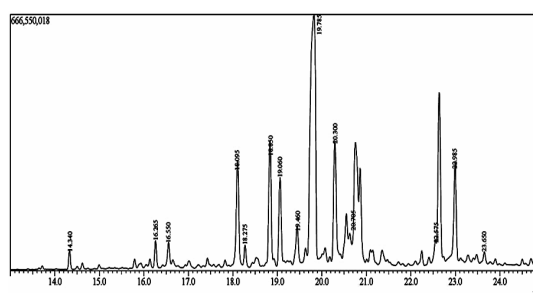


Fig. 1. GCMS chromatogram of EO from black pepper

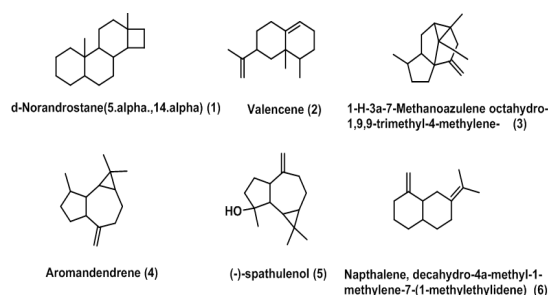


Fig. 2. Chemical structure of major constituents in EO extract from black pepper

Table 1: Chemical constituents of EO extract of black pepper

Compound	R _t	m/z	(%) Area	Similarity
Disipro[2.1.2.4]undecane	12.244	93.0	1.458	83
8-methylene-D-Limonene	12.643	68.0	5.159	98
Linalool isobutyrate	14.311	93.0	3.728	98
Alpha-Terpinol	16.265	132.0	3.692	98
Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-	16.557	91.0	5.168	95
Valencene	18.075	136.0	13.297	84
Napthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	18.273	161.0	3.312	94
1-H-3a-7-Methanoazulene octahydro-1,9,9-trimethyl-4-methylene-	18.821	161.0	11.591	98
Napthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)	19.062	147.0	7.794	98
Bicyclo[7.2.0]undec-4-ene-4,11,11-trimethyl-8-methylene-	19.448	69.0	6.594	97
d-Norandrostane(5.alpha.,14.alpha)	19.787	175.0	14.874	81
Aromandendrene	20.299	107.0	8.398	97
Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-	20.862	131.0	2.890	60
Caryophyllene oxide	22.552	69.0	2.758	92
(-)-spathulenol	23.004	131.0	8.193	96
Ledene oxide-(II)	23.654	91.0	1.094	95

Antimicrobial activity test

In vitro antimicrobial activity test of EO determined two numbers of *Gram-positive* bacteria, eight numbers of *Gram-negative* bacteria and six numbers of fungal strains using disc diffusion method. The results tabulate in Table 2 and Table 3 that obtained from this study. The extracted black pepper essential oil displayed moderate activity against used bacteria, compared to Ceftriaxone

as standard (Table 2). The EO showed the highest activity with a zone of inhibition (20.7 ± 0.6 mm) against *Shigella boydii* compared with standard Ceftriaxone (24.7 ± 0.6 mm). The EO also showed good antifungal activity against all of the fungal strains with one exception *Neurospora crassa* (Table 3). The EO exhibited greater zone of inhibition with 13.7 ± 1.5 mm against *Trichoderma harzianum* with standard Amphotericin B (10.7 ± 1.5 mm).

Table 2: Antibacterial activity of EO extract of black pepper and Ceftriaxone

Bacteria	Strains	Inhibition Zone (mm)	
		EO	Ceftriaxone
Gram (+)ve bacteria	<i>Staphylococcus aureus</i>	16.0 \pm 1.0	40.3 \pm 0.6
	<i>Bacillus magneterium</i>	16.7 \pm 1.5	50.0 \pm 1.0
Gram (-)ve bacteria	<i>Salminella paratyphi</i>	19.0 \pm 1.0	49.0 \pm 1.0
	<i>Enterotoxigenic Escherica coli</i>	15.0 \pm 2.0	43.3 \pm 1.5
	<i>Salminella typhi</i>	18.0 \pm 1.0	42.7 \pm 1.5
	<i>Shigella flexneri</i>	14.3 \pm 2.5	37.7 \pm 1.5
	<i>Shigella sonnei</i>	16.3 \pm 0.6	39.7 \pm 0.6
	<i>Shigella boydii</i>	20.7 \pm 0.6	24.7 \pm 0.6
	<i>Escherica coli</i>	19.3 \pm 0.6	37.3 \pm 1.2
	<i>Shigella dysentary</i>	22.7 \pm 0.6	45.7 \pm 1.5

Table 3. Antifungal activity of EO extracts of black pepper and Amphotericin B.

Strains	Inhibition Zone (mm)	
	EO	Amphotericin B
<i>Aspergillus niger</i>	11.0 \pm 1.0	15.0 \pm 1.0
<i>Panysalium notatum</i>	7.3 \pm 1.2	15.3 \pm 0.6
<i>Candida albican</i>	15.0 \pm 1.0	8.3 \pm 0.6
<i>Aspergillus flavus</i>	10.3 \pm 2.5	16.0 \pm 1.0
<i>Neurospora crassa</i>	-	17.0 \pm 1.0
<i>Trichoderma harzianum</i>	13.7 \pm 1.5	10.7 \pm 1.5

Antioxidant activity by DPPH assay

Antioxidant efficacy of EO determine by DPPH free radical scavenging method using a standard (ascorbic acid). IC₅₀ values denote the desire concentration of sample, which inhibits 50% DPPH radicals. The concentrations and inhibitions of EO and ascorbic acid present in Fig. 3. The IC₅₀ values calculated from concentration against inhibition curves and EO shown good activity (IC₅₀ 35.83 \pm 2.92 μ g/mL) compare with ascorbic acid (IC₅₀ 27.34 \pm 1.86 μ g/mL) as standard (Table 4).

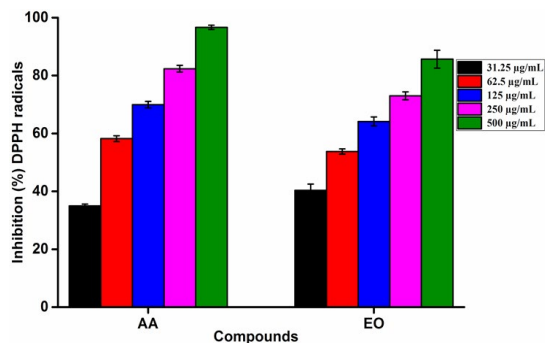


Fig. 3. Inhibition (%) DPPH radicals of EO extract of black pepper and ascorbic acid

Table 4: Antioxidant efficacy of EO extract of black pepper and Ascorbic acid

Compounds	IC ₅₀ (µg/mL)
Essential Oil	35.83±2.92
Ascorbic acid	27.34±1.86

Molecular docking

Molecular docking performed with software Gaussian 09, PyRx 0.8, and Pymol for explore the interaction of target compound of EO with selected proteins. Protein structure optimization were done by DFT technique on B3LYP/6-31+G (d,p) basis set up. Compound 1 occupy highest percentage area (14.874%) in GCMS chromatogram (Table 1). Molecular docking study of compound 1 perform against selected PDB protein 1JJJ, 1KZN and 5JBO that are crystals of *S. aureus*, *E. coli* and *T. harzianum*, respectively. Binding affinity of highest negative value chose for final appearance. The predicted interaction profiles of compound and target proteins illustrated in Fig. 4. Compound 1 showed binding affinity -7.7 Kcal/mol, when docked against 1JJJ protein. It displayed Pi-sigma interactions with PHE306 at a distant of 3.50Å. In addition, several alkyl and Pi-alkyl interactions were involved with LYS305, PHE273, PHE306, and PHE306 at a distance of 4.42 Å, 5.24 Å, 4.81 Å, and 4.98 Å, respectively. The binding affinity -6.2 Kcal/mol obtained when the compound 1 docked against 1KZN protein. It exhibited alkyl interactions with VAL120, LEU132, ILE78, LEU132, and LEU154 at a distance of 5.23 Å, 4.69 Å, 3.81 Å, 4.10 Å, and 4.73 Å, respectively. It also exhibited Pi-alkyl interaction with TYR145 at a distant of 5.13 Å. The highest binding affinity -9.0 Kcal/mol obtained when compound 1 docked against 5JBO protein. It showed several Pi-alkyl interactions with TRP357 at

various distances. On the other hand, compound 1 showed binding affinity -6.9 Kcal/mol when docked with human peroxiredoxin 5 (antioxidant enzyme) 1HD2 protein (Fig. 5) shown alkyl interactions with ARG86, ALA90, LEU96 at a distance of 4.73 Å, 4.12 Å, and 4.19 Å, respectively.

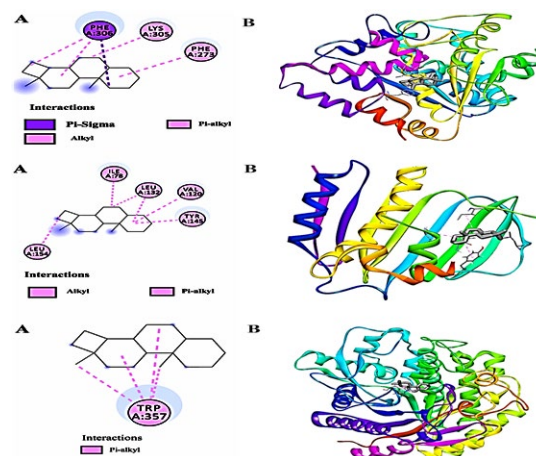


Fig. 4. Molecular docking studies of compound 1 against 1JJJ, 1KZN, and 5JBO protein receptor. (A) 2D Interaction sketches. (B) 3D Docking prediction

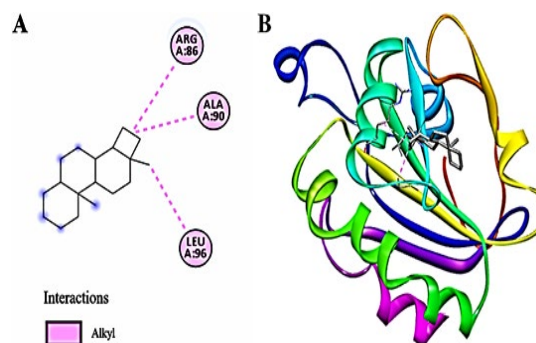


Fig. 5. Molecular docking study of compound 1 against 1HD2 protein receptor. (A) 2D Interaction sketches. (B) 3D Docking prediction

ADME analysis

To develop an effective drug in early preclinical trial is significant to know ADME (absorption, distribution, metabolism and excretion) including their pharmacokinetic drug like properties. Selected major six compounds (1–6) showed good ADME properties. The bioavailability score all of the compounds (>0.50) confirmed more drug-likeness properties. Most of the compounds showed low GI adsorption and BBB permeation except compound 4 and 5. Noticeably, there is no P-glycoprotein (P-gp) substrate to justify high GI adsorption. Compounds 4 and 5 can easily pass blood brain barrier (BBB),

and can bind to specific receptors (Table 5). Any component showed better effectiveness with insignificant toxicity when they exhibited interactions with at least two isoenzymes of the cytochrome P (CYP) family. The compounds (1–6) with high affinities (low Kp) [(-5.44) to (-2.78)] describes higher strength of drug binding with receptor. The outcome of this study revealed that all selected compounds

be strongly support for oral bioavailability. The radar chart of bioavailability placed inside the color zone with a small polygon closer to the centre represents pharmacokinetics, physicochemical and drug-likeness properties²². The radar plot chart of compounds (1–6) (Fig. 6) denoted that all of the components are entirely inside the pink area, which indicates their good drug-likeness properties²³.

Table 5: *In silico* ADME profile for major constituents of EO of black pepper

Entry	Compounds					
	1	2	3	4	5	6
Lipinski	Yes	Yes	Yes	Yes	Yes	Yes
TPSA (Å ²)	0.00	0.00	0.00	0.00	20.23	0.00
Log(Po/w)	5.45	4.41	4.61	4.34	3.26	4.06
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55
GI adsorption	Low	Low	Low	Low	High	Low
BBB permeate	No	No	No	Yes	Yes	No
P-gp substrate	No	No	No	No	No	No
CYP1A2 inhibitor	Yes	No	Yes	Yes	No	No
CYP2C9 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes
CYP2C9	Yes	Yes	Yes	Yes	No	Yes
CYP2D6	No	No	No	No	No	No
CYP134	No	No	No	No	No	No
log Kp (cm/s)	-2.78	-3.83	-4.15	-4.20	-5.44	-4.42

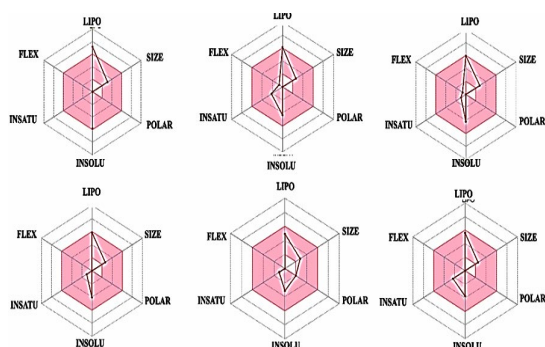


Fig. 6. Radar plot chart of major constituents of EO of black pepper

CONCLUSION

n-Hexane extract of black pepper

essential oil showed moderate antibacterial activities and good antifungal and antioxidant activities that compare to their standard. The experimental results supported by *In silico* prediction and molecular docking studies. This *n*-hexane extract black pepper essential oil could be further screen as a potential source of biologically important drug candidates.

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