



Ethyl Oleate, Hexadecenoic Acid, Phytol Profiling of *Onosma bracteatum*: Antibacterial, Anticancer and Molecular Docking Studies

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Research studies on phytochemicals and antibiotics utilizing extracts from medicinal plants have proliferated in the past few years. The phytochemicals profiling, antioxidant capacity, antibacterial properties, antiproliferative potential and molecular docking studies of ethanolic extract of *Onosma bracteatum* was examined in this study. The extract prepared into ethanol (80%) and identify the presence of phytochemical compounds using GC-MS profiling. The antibacterial potential was investigated in relation to *Staphylococcus aureus* and *Escherichia coli*. Further to evaluate the anticancer potential of ethanolic extract of *O. bracteatum*, the antiproliferative assay (MTT cytotoxicity assay) was performed against Ln-18 (human malignant glioma cell line) and A-549 (Human lung cancer cell line) cells. Ethyl oleate, hexadecenoic acid and phytol were highest in GC-MS and docked against DNA gyrase B (*E. coli*), β -lactamase (*S. aureus*) and BCL-2 (apoptosis inhibitor). In antioxidant assay, 78% maximum inhibition of free radical scavenging activity was observed. The zone of inhibition was in the ranges from 9 mm (*E. coli*) to 20 mm (*S. aureus*). Furthermore, the ethanolic extract of *O. bracteatum* demonstrated antiproliferative activity against the cancer cell lines LN-18 and A-549 with growth inhibitions of 84% and 81%. The ethyl oleate was found to be best compound with -8.4 (kcal/mol) binding score in the molecular docking studies. The results showed that *O. bracteatum* is a good source of phytochemicals and antibacterial compounds with promising anticancerous potential.

Keywords: *Onosma bracteatum*, Phytochemical compounds, Antibacterial activity, Anticancer activity, Docking studies.

INTRODUCTION

Plants have long been a vital source of natural materials for preserving human health, but in the past 10 years, studies into natural medicines has increased significantly. Apart from providing essential human necessities like food, clothes and shelter, plants are a significant source of medicinal materials. Since the beginning of time, human groups have utilized medicinal plants to cure and prevent a wide range of illnesses and these uses have been passed down to succeeding generations [1]. In last 30 years, the pharmaceutical industry has developed several new antibiotics; yet, microbes have become more resistant to these medications [2]. The antimicrobial medicine utilization is still questionable in the future due to the growing problem of microorganism resistance. Therefore, necessary steps must be taken to overcome this issue, such as limiting the

use of antibiotics and carrying out ongoing studies to produce new medications, either natural or synthetic [3].

Cancer is the deadliest illness in the world and a serious health concern among other life-threatening diseases. It is typified by the fast spreading of aberrant cells as well as dysregulated cell division, growth and death [4]. The development of the human health care system has been greatly influenced by traditional medicinal plants, since plant based compounds make up around 70% of all currently prescription pharmaceutical medications [5,6]. The majority of the biomedical action of plants comes from their secondary metabolites [7]. By analyzing chemical structures prior to synthesis, *in silico* methods have proven particularly useful in predicting new medicines and prospective targets [8].

Onosma bracteatum is one of member belongs to family Boraginaceae generally referred as 'Gaozaban' and 'Gojihva'.

Prior research has indicated that the genus *Onosma* possesses a range of therapeutic attributes including anticancer, antiaging, antimicrobial, antiasthmatic, antioxidant, antibacterial, anti-diarrheal, psychoimmunomodulatory and wound healing activity [9,10]. Evidence demonstrating the abundance of vital bioactive phytochemicals found in the *Onosma* genus [11]. The study aims to evaluate the biochemical properties of *O. bracteatum* and highlight its biological activity, with a focus on its phytochemical, antioxidant, antibacterial properties, anticancer and molecular docking analysis.

EXPERIMENTAL

Collection of plant material and preparation of plant extract:

The aerial parts of *Onosma bracteatum* including stems and leaves, collected from registered company named Drug analysis laboratory (CIN-U24233PB2008PTC032243), Herbal Health Research Consortium Pvt. Ltd., Amritsar, India. The material was crushed to powder form and solvent extraction of 25 g powdered plant material was done by soaking in organic solvents 80% ethanol in 500 mL (200 + 150 + 150 sequentially in repeated maceration). The mixture was left in solvent for maceration period of 3 days. Then the mixture was filtered by using Whatman filter paper. After filtration, the supernatant was collected and dried fully using hot air oven at 50-55 °C.

Quantification of total phenolic content (TPC) and total flavonoid content (TFC): The TPC content in the ethanolic extract of *O. bracteatum* was determined using Folin-Ciocalteu reagent with a slight modification as reported by Phuyal *et al.* [12] and gallic acid was served as standard reference. The absorbance of mixture was measured at 750 nm using UV-Vis spectrophotometer and TPC was expressed as mg GAE/g dry powder extract. Similarly, TFC was also calculated by measuring the absorbance at 510 nm using the AlCl₃ colorimetric method and quercetin served as standard reference. The quercetin equivalents (mg QE g⁻¹) per gram of dry extract were used to calculate the amount of TFC.

Antioxidant activity: The 2-diphenyl-1-picrylhydrazyl (DPPH) was used to determine free radical scavenging activity with methodology previously established by Nithianantham *et al.* [13]. The stock solution was prepared by dissolving DPPH (24 mg) in ethanol (100 mL). The ethanolic extract samples (100 µL) was mixed with 3 mL DPPH working solution in varying concentration ranges from 100 µg/mL to 1000 µg/mL. A standard measurement was typically 3 mL of DPPH containing solution in 100 µL of ethanol and the samples left for incubation of 30 min in dark. The ascorbic acid was used as standard reference, with same range of concentrations (100 µg/mL to 1000 µg/mL). The absorbance was therefore determined at 517 nm. The % inhibition was established using following formula:

$$\text{Inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c is absorbance of control and A_s is absorbance of sample.

Qualitative analysis by GC-MS: Analysis of ethanolic extract of *O. bracteatum* was carried out at Central University

of Punjab, Bhatinda, India using the GC-MS system. Injector temperature was maintained at 230 °C. Flow of carrier gas was maintained at a rate of 1.0 mL/min and an injection volume of 0.20 µL was employed. The MS scan parameters was included in terms of electron impact ionization voltage and molecular mass range. The identification of components was based on comparison of their mass spectra with those of NIST05 library.

Antibacterial activity: Agar well diffusion method was applied to evaluated the antibacterial potential of the ethanolic extract of *O. bracteatum* against tested micro-organisms *i.e.*, *E. coli* (MTCC 42) and *S. aureus* (MTCC 87) over the entire agar surface of the plates. The extract solution at different concentrations (100-1000 µg/mL) was introduced into the wells of agar plates, spread with tested microorganisms. The plates are then incubated under suitable conditions depending upon the tested microorganisms. The standard antibiotic chloramphenicol (30 mcg/disc) was compared to the plant extract in terms of its ability to inhibit or suppress the growth of microorganisms, namely its zone of inhibition's diameter.

Antiproliferative assay: Using the MTT test, the ethanolic extract was assessed for its potential to inhibit cell proliferation in Ln-18 (human malignant glioma cell line) and A-549 (human lung cancer cell line) cells. The selected cell lines were seeded with 96-well plate (8 × 10³ cells/well) cultured to confluency and then treated for 24 h with varying dosages of the samples (15.625-500 µg/mL). Subsequently, MTT (20 µL) were introduced into every well and the plates were kept in a CO₂ (5%) incubator for 4 h. After discarding the supernatant, 0.1 mL of DMSO was added to dissolve the formazan and the readings were then measured at 570 nm [14].

$$\text{Inhibition of growth (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A₀ is the absorbance of control (untreated cells); A₁ is the absorbance of extract treated cells.

Molecular docking: The natural compounds ethyl oleate, hexadecenoic acid and phytol has been retrieved from the GC-MS analysis of *O. bracteatum* extract, selected as ligand molecules. The 3D structure of ligand molecules was downloaded from the PubChem database. Open Babel was used for converting .sdf file of ligand into PDB file. Ligands were prepared by Auto dock vina [15]. In order to examine the antibacterial activity of compounds of *O. bracteatum* extract, three target proteins were chosen which include DNA gyrase B (PDB ID: 1EI1), β-lactamase (PDB ID: 1ALQ) and BCL 2 (PDB ID: 2O2F) were chosen as target proteins. The 3D structure of these targeted proteins were downloaded from RCSB PDB database in PDB format. The target proteins were prepared for docking *via* the Autodock tools software. The .pdb files was converted to pdbqt files. The molecular docking analysis of all the selected phytocompounds and targeted proteins were subjected to AutoDock tools (MGLTools 1.5.6.) AutoDock Vina 1.1.2, using the script standard method. The interaction of docked protein-ligand structures were visualized by Discovery Studio Visualizer tool [16].

Statistical analysis: The three distinct values' means and standard deviations were displayed as the results.

RESULTS AND DISCUSSION

Quantification of total phenolic content (TPC) and total flavonoid content (TFC): After maceration, the dried ethanolic extract of *Onosma bracteatum* was obtained with 26% extract yield and used for further quantification of phyto-constituents. Using gallic acid as a standard, the Folin-Ciocalteu method was used to evaluate the total phenolic content of the ethanolic extract of *O. bracteatum*. The calibration curve was constructed using the absorbance values measured at various gallic acid concentrations. The regression equation of the calibration curve ($Y = 0.0017x$; $R^2 = 0.9926$) was used to compute the total phenolic content of the extract. The TPC was observed in range of 26.2 ± 0.9 mg GAE/g. Similarly, the TFC of extract, was determined using the calibration curve's regression equation ($Y = 0.0005x$; $R^2 = 0.9969$). Similar trends were also seen by the TFC values and the TPC values. The TFC was observed in the range of 16.85 ± 0.8 mg QE/g. Previously, the TPC and TFC was also reported in *O. bracteatum* in 2021 by Rajapara *et al.* [17]. The result of the study concluded the TPC and TFC as 14.5 ± 0.00047 mg GAE/g, 11.2 ± 0.0014 mg GAE/g respectively.

Antioxidant activity: Plants contain active polyphenols that protect DNA, scavenge reactive oxygen species (ROS) and help in the creation of genes and proteins that catalyze the breakdown of toxins and maintain cellular redox balance [18]. The *Onosma* genus has been shown to possess antioxidant properties and to be useful in preventing a variety of pharmacological activities [19-22]. In present investigation, several ethanolic extract concentrations ranging from 100 μ g/mL to 1000 μ g/mL were used to measure radical scavenging activity of *O. bracteatum* by DPPH method. The maximum percentage inhibition by DPPH method was found about 78 ± 0.5 % at highest concentration (1000 μ g/mL), in ethanolic extract. Similarly, ascorbic acid exhibited 82 ± 0.8 % inhibition at 1000 μ g/mL concentration (Fig. 1). The IC_{50} value was calculated for ethanolic extract and ascorbic acid; 524.76 μ g/mL and 414.53 μ g/mL respectively. Rahman *et al.* [23] reported significant antioxidant activity by similar method, in extract of *Tabebuia pallida* grown in Bangladesh.

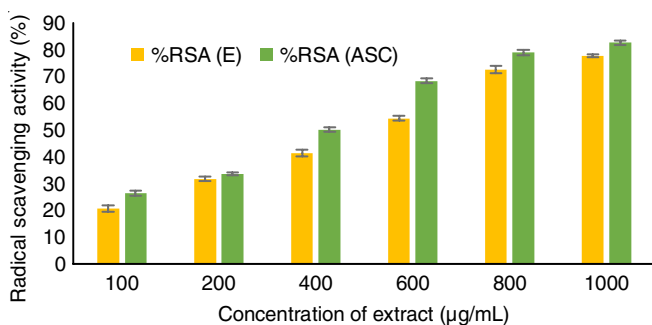


Fig. 1. DPPH free radical scavenging activity (%) of ethanolic extract of *Onosma bracteatum*

GC-MS profiling: Plants in the *Onosma* genus have an abundance of bioactive substances, such as flavonoids, alkannin and shikonin, which have antibacterial, analgesic and wound-healing properties [24]. The bioactive compounds present in

whole plant ethanolic extract of *O. bracteatum* can contribute to the medicinal quality of the plant. According to their peak area, the most common phytochemical components in the ethanolic extract of *O. bracteatum* are ethyl oleate (14.99%), hexadecenoic acid (11.97%), phytol (8.96%), stigma-5-en-3-ol, oleate (7.95%), 13-docosenamide, (Z)-(5.14%) and stigmasterol (3.36%). In extract, the most prevalent component was shown to be ethyl oleate. Previously, the antibacterial potential of ethyl oleate was checked, that was extracted from *Phyllanthus amarus* [25]. Published reports suggested that hexadecenoic acid exhibits the following properties: it is a flavouring agent, hemolytic, antimicrobial, cytotoxic, hypocholesterolemic, nematocide, antiandrogenic and it inhibits 5α -reductase [26]. An effective antidiabetic drug was found in the ethanolic extract of *O. bracteatum* containing a plant phytosterol called strig-5-en-3-ol, oleate, which was extracted from the ethyl acetate extract of *Adathoda vasica* [27]. Similarly, octadecanoic acid and phytol show anti-inflammatory activities [26,28]. The GC-MS spectrum of ethanolic extract determined 74 compounds with 8 significant peaks of various compounds according to their retention time (RT) as shown in Fig. 2. Based on the results, the main compounds identified were ethyl oleate, hexadecenoic acid and phytol (Fig. 3). The highest peak with maximum hit (27) was identified that ethyl oleate was primary compound. Some of phytochemicals are listed in Table-1.

Antibacterial activity: The results revealed that Gram-positive bacteria *i.e.* *S. aureus* showed more inhibition to extract than the Gram-negative bacteria *E. coli*. Correspondingly, Moghaddam *et al.* [29] showed that extract of *O. dichroanthum* Boiss. inhibited Gram-positive bacteria more effectively than Gram-negative bacteria. The antibacterial activity of ethanolic extract derived from *O. bracteatum* was compared with standard antibiotic chloramphenicol. Variable antibacterial effects of plant extract at different concentration (100-1000 μ g/mL) were observed against bacterial strains (*E. coli* and *S. aureus*). In ethanolic extract of *O. bracteatum*, the zone of inhibition ranging from 9 mm (*E. coli*) to 21 mm (*S. aureus*) as shown in Fig. 4. According to Jeyaseelan & Jashothan [30], it was found that the hot and cold extracts of ethanol of *Ricinus communis* L. showed significantly higher inhibition on *S. aureus* than both cold and hot extracts of methanol.

Antiproliferative activity: To check the cytotoxicity of ethanolic extract of *O. bracteatum*, two cell lines Ln-18 and A549 were used at different concentration (15.625 to 500 μ g/mL) of extract. Treatment-response curves were obtained by plotting the percentage of growth inhibition against the amounts of extracts treatment. After 24 h of treatment with concentrations of ethanolic extract on both cell lines, % growth inhibition was observed maximum 84% in Ln-18 and 81% in A-549 with IC_{50} value 75.48, 87.57 μ g/mL, respectively (Fig. 5). Kamaly *et al.* [31] investigated the antiproliferative activity of hexane fraction of *Heliotropium bacciferum* effectively inhibit the growth of A549 and MCF-7 cancer cells with IC_{50} values of 104.14 and 83.84 μ g/mL, respectively.

Molecular docking: The crystal structure of three proteins DNA gyrase B (PDB ID: 1EI1) and β -lactamase (PDB ID: 1ALQ) and BCL 2(PDB ID: 2O2F) were successfully docked

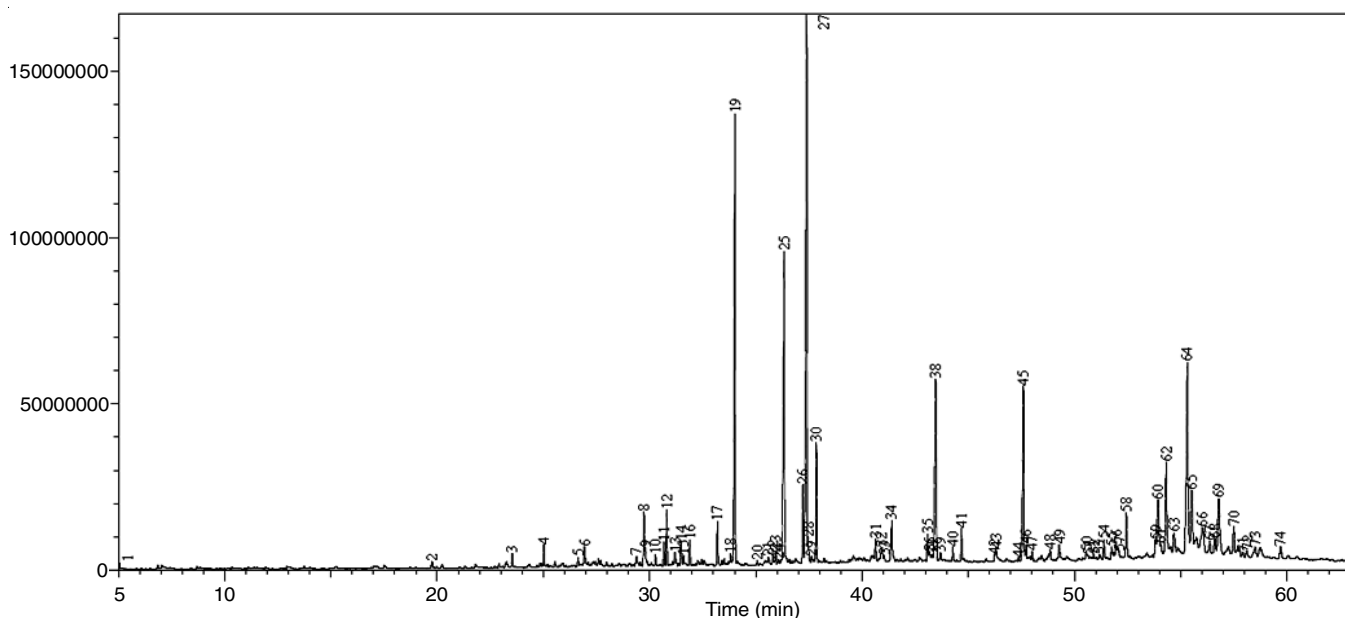


Fig. 2. A typical GC-MS profile of ethanolic extract of *Onosma bracteatum*

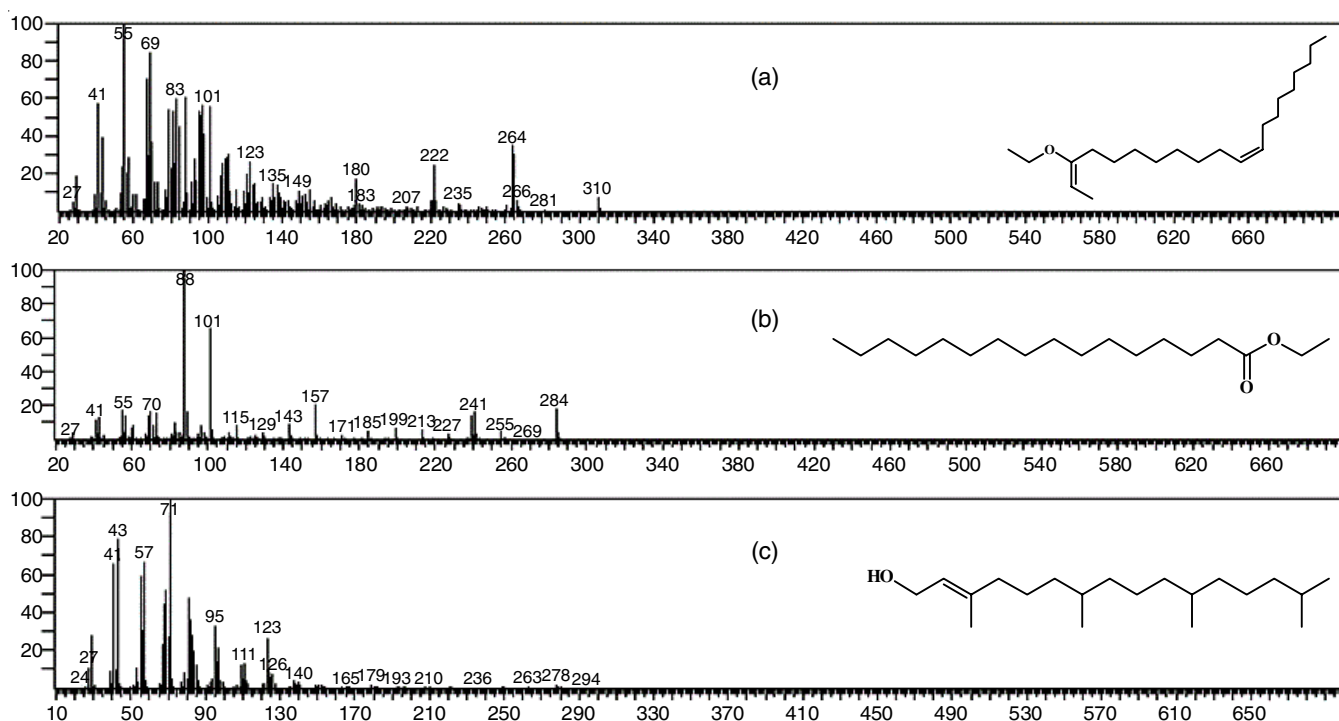


Fig. 3. Chromatogram of ethyl oleate, hexadecenoic acid and phytol (a,b,c) from GCMS analysis of ethanolic extract of *Onosma bracteatum*

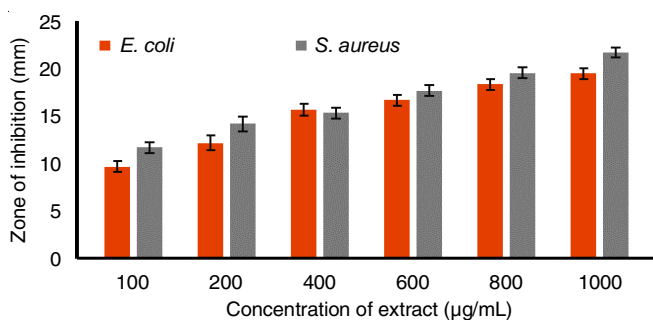


Fig. 4. Antibacterial activity of ethanolic extract of *Onosma bracteatum*

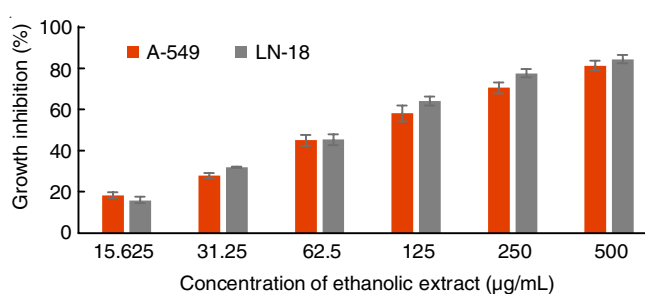


Fig. 5. Antiproliferative activity of *Onosma bracteatum* ethanolic extract against Ln-18 and A-549 cell lines

TABLE-1
LIST OF SOME PHYTOCONSTITUENTS PRESENT IN THE ETHANOLIC EXTRACT OF *Onosma bracteatum* BY GC-MS

S. No.	Retention time (min)	Compound name	m.f.	Area (%)
1	37.38	Ethyl oleate	C ₂₀ H ₃₈ O ₂	14.99
2	34.01	Hexadecenoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	11.97
3	36.32	Phytol	C ₂₀ H ₄₀ O	8.96
4	55.30	Stigma-5-en-3-ol, oleate	C ₄₇ H ₈₂ O ₂	7.95
5	43.47	1(2 <i>H</i>)-Naphthalenone, 3,4-dihydro-6-m	C ₁₉ H ₁₈ O ₃	6.84
6	47.58	13-Docosenamide, (Z)-	C ₂₂ H ₄₃ NO	5.14
7	54.32	Stigmasterol	C ₂₉ H ₄₈ O	3.36
8	56.79	Lanosterol	C ₃₀ H ₅₀ O	3.09
9	37.85	Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	2.83
10	53.93	Lathosterol	C ₂₇ H ₄₆ O	2.42
11	55.51	Cholest-5-en-3-ol, 24-propylidene-,(3.1	C ₃₀ H ₅₀ O	2.10
12	37.22	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	2.02
13	56.03	9,19-Cyclolanost-24-en-3-ol, (3β)	C ₃₃ H ₅₀ O	1.54
14	41.39	Heptadecanoic acid, ethyl ester	C ₁₉ H ₃₈ O ₂	1.43
15	29.73	Docosanoic acid, ethyl ester	C ₂₄ H ₄₈ O ₂	1.36
16	52.43	Vitamin E	C ₂₉ H ₅₀ O ₂	1.33
17	30.80	2-Pentadecanone,6,10,14-trimethyl-	C ₁₈ H ₃₆ O	1.26
18	57.50	Cholest-4-en-3-one	C ₂₇ H ₄₄ O	1.04
19	33.19	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	1.03
20	51.95	Cholesterol	C ₂₇ H ₄₈ O	0.66
21	31.88	Lidocaine	C ₁₄ H ₂₂ N ₂ O	0.64
22	31.48	8-Pentadecanone	C ₁₅ H ₃₀ O	0.59
23	40.93	Ethyl 9-hexadecenoate	C ₁₈ H ₃₄ O ₂	0.57
24	46.29	Triacetyl heptafluorobutyrate	C ₃₄ H ₆₁ O ₂ F ₇	0.57
25	51.38	γ-Tocopherol	C ₂₈ H ₄₈ O ₂	0.57

against the phytochemicals ethyl oleate, hexadecenoic acid and phytol (Figs. 6 and 7). The best compound *i.e.* ethyl oleate scored -8.4 kcal/mol (Table-2) with DNA gyrase B protein forming alkyl interactions Ile-94, Val-120, Val-167, Val-43 with residues present in the target protein. Etmnani *et al.* [32] studied the binding affinity of β-lactamase inhibitor of *Staphylococcus aureus* against phytochemicals from *Rosmarinus officinalis*, *Ocimum basilicum*, *Eucalyptus globulus* and *Thymus vulgaris*.

Conclusion

Onosma bracteatum has remarkable antibacterial and antioxidant capabilities, which are thought to be due to the phytochemicals it possesses. A large number of phytoconstituents, including alkaloids, terpenoids, flavonoids and phenols, were identified in the ethanolic extract. The ethanolic extract was observed with significant antioxidant activity. The ethanolic extract of *O. bracteatum* showed maximum inhibitory effect against Gram-positive and Gram-negative bacteria, *E. coli* and *S. aureus*. The antioxidant and antibacterial properties of ethanolic extract are due to the presence of several phytochemicals. Further *in vitro* and *in vivo* studies will give a clear path in

drug development. Also, computer aided drug designing or molecular simulations, further revealed appropriate active binding sites of ligand and receptor involved in various diseases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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TABLE-2
BINDING AFFINITIES OF ETHYL OLEATE, HEXADECENOIC ACID AND PHYTOL AGAINST DNA GYRASE B AND β-LACTAMASE

Compounds	Binding affinity (kcal/mol)			Interacting amino acids
	DNA gyrase B	β-Lactamase	BCL 2	
Ethyl oleate	-8.4	-5.3	-8.4	Ile, Val (DNA gyrase B) Lys, Asn, Leu (β-lactamase), Val, Phe, Tyr, Leu (BCL 2)
Hexadecenoic acid	-3.5	-4.3	-4.4	Leu, Ala (DNA gyrase B) Asn, Glu, Lys, Ser, Tyr, Ile (β-lactamase), Gly (BCL 2)
Phytol	-3.8	-4.1	-5.4	Pro, Arg, Phe, Ile, His (DNA gyrase B) Leu, Lys (β-lactamase), Val, Tyr, Ala, Phe, Gly (BCL2)

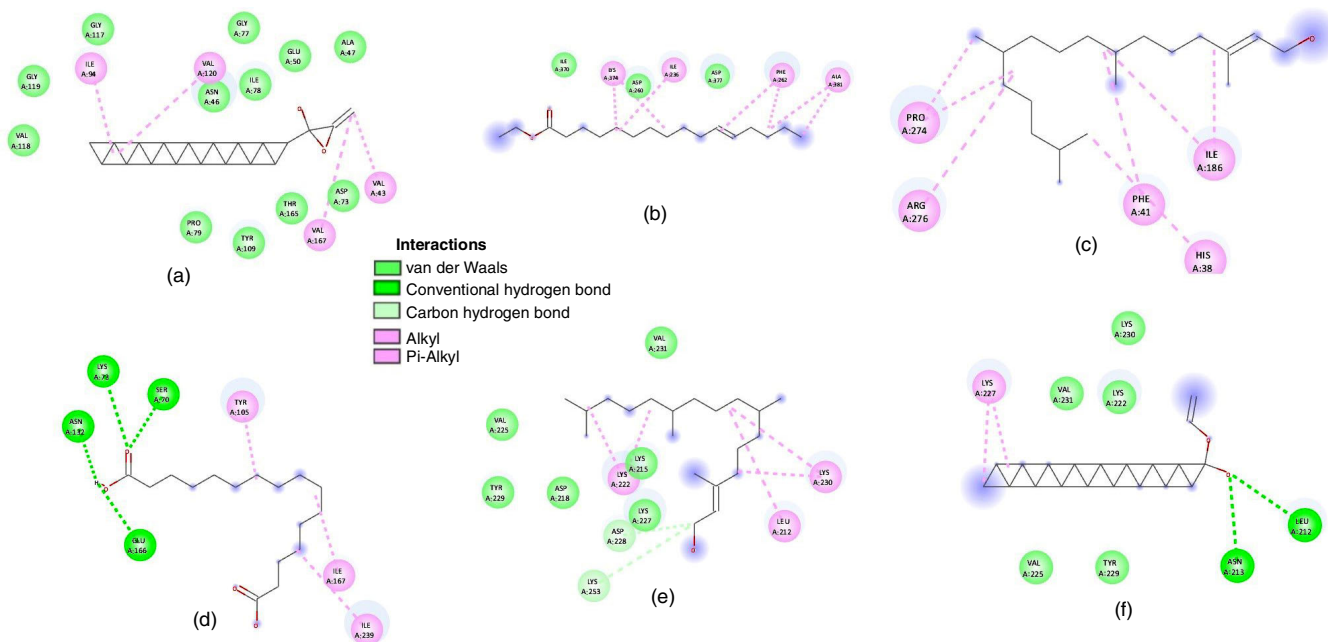


Fig. 6. 2D interactions of DNA gyrase B (a) ethyl oleate-DNA gyrase B, (b) hexadecenoic acid-DNA gyrase B, (c) phytol-DNA gyrase B, (d) hexadecenoic acid-β-lactamase, (e) phytol-β-lactamase, (f) ethyl oleate-β-lactamase

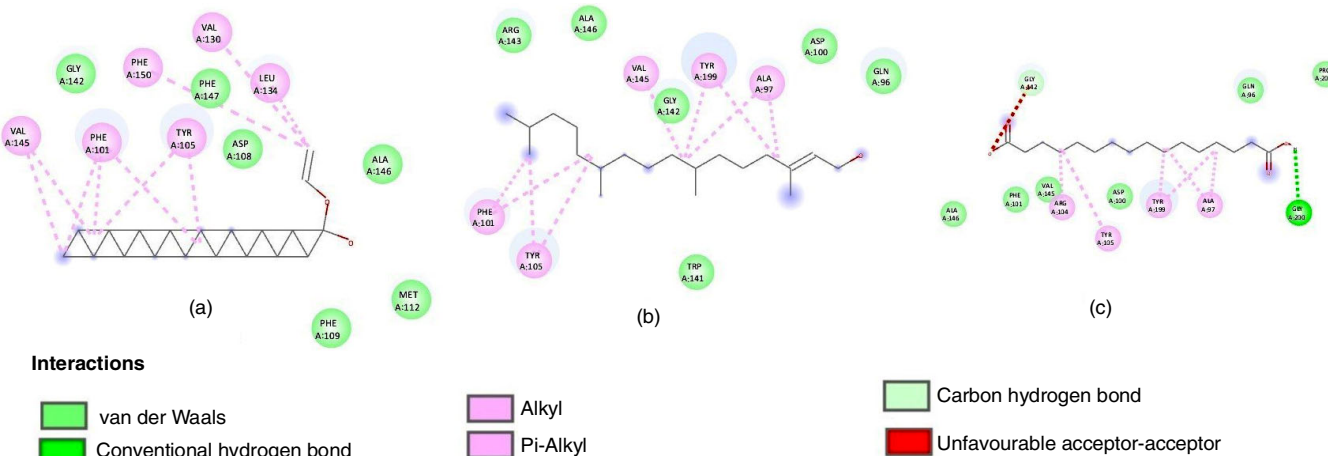


Fig. 7. 2D interactions of ethyl oleate, phytol, hexadecenoic acid (a,b,c) against BCL 2

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