A Comprehensive Study on the Phytochemistry, Extraction, Biological Evaluation and In Silico Potential of *Onosma bracteatum* by GC/MS

[1]Iqra Farid, [2]Irshad Ahmad, [3]Muhammad Younus, [*4]Muhammad Shahzad Khan, [5]Maryem Safdar, [5]Khalil Ahmad, [6]Jafir Hussain Shirazi, [2]Umair Khurshid, [7]Umer Shaukat, [8]Muhammad Yousuf, [4]Marwah Rehman

[1]Imran Idrees College of Pharmacy, Sialkot, Pakistan

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, The Islamia University of Bahawalpur, 63100, Pakistan

[3]Department of Pharmacognosy, Faculty of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur. 63100, Pakistan

[4]Abbas Institute of Medical Sciences, GC University Faisalabad, Layyah Campus, Layyah, 31200, Pakistan

[5]University College of Conventional Medicine, Faculty of Medicine and Allied Health Sciences, The Islamia University of Bahawalpur, Bahawalpur, 63100, Pakistan

[6]Department of Pharmaceutics, Faculty of Pharmacy, The Islamia University of Bahawalpur, 63100, Pakistan

[7]Johar Institute of professional studies affiliated with Punjab University, Lahore, Pakistan

[8]SHIPS (South Punjab institute of health professionals' studies) Jalal pur Pirwala, Multan

Corresponding author:

Muhammad Shahzad Khan

[4]Abbas Institute of Medical Sciences, GC University Faisalabad, Layyah Campus, Layyah, 31200, Pakistan

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Abstract

Onosma bracteatum is medicinal plant owning bioactive and medicinal properties. This study focuses on phytochemical screening, flavonoids and polyphenolic estimation, and to investigate the antiviral, antioxidant, antibacterial activities and In-silico Docking studies of *Onosma bracteatum.*

The methanol and DCM extracts of leaves, stems, and flowers were prepared, and their percentage yield was calculated. OBLM had the highest TFC (28.39 mg Qu. Eq/gm E) and TPC (191.43 mg GA. Eq/gm E). The antiviral activity assessed against IBV, NDV, and AIV H9N2.

The HA and IC50 values of the extracts and the standard drug acyclovir were measured and reported respectively, OBSM fraction showed moderate antiviral activity against IBV, while OBLM and OBLD fractions had moderate activity against NDV. Acyclovir had the highest antiviral activity. None of the extracts showed activity against AIV H9N2. OBSM fraction showed higher antioxidant activity (219.65 mg AE. g-1 extract), followed by OBLM (74.90 mg AE. g-1 extract) and OBFM (40.81 mg AE. g-1 extract). The antibacterial activity of the methanol and DCM extracts of leaves, stems, and flowers was evaluated against *E. coli, B. bronchiseptica, B. pumilus, and S. epidermidis*. OBLM fraction had moderate activity against *E. coli* and *B. pumilus* (4.43 and 4.34 mm zones of inhibition, respectively). OBSM fraction had moderate activity against *B. bronchiseptica* (3.89 mm zone of inhibition). The standard, ciprofloxacin had strong antibacterial activity against all strains. The GC/MS analysis the extract revealed the presence of alkane, fatty acids, and esters. Methanol extract of flowers (OBFM) revealed the presence of three additional compounds: docosanoic acid, eocosyl ester, trans-2,4-dimethylthiane, S, S-dioxide, and cyclohexene, 1-methyl-3-(1-methylethenyl)-, (. +/-.)-. Finally, In-silico Docking studies were done to observe Binding Affinity and intermolecular interactions of methanolic extract with lipoxygenase, Nitric oxide synthase, xanthine oxidase enzyme.

The study revealed that *Onosma bracteatum* possesses promising antiviral, antioxidant, and antibacterial activities, which may be attributed to its phytochemical constituents. This comprehensive analysis suggested the possibility of novel drug candidates from this medicinal plant.

1. Introduction

From the distant past, plants have been essential to human life. They provided for the practical necessities of early man, including food, clothes, shelter, medicines, decorations, and tools, as well as his spiritual needs. For a very long time, plants have been the primary source of medicinal systems. Nowadays, a number of chemicals extracted from plants have transformed the pharmaceutical and medical fields (1, 2).

Numerous new medications have been discovered as a result of recent developments from natural compounds in conjunction with ethno-botanical research such as Artemisinin, Podophyllotoxin, Vinblastine and Vincristine, Camptothecins and Kaempferol glycoside are used to treat diseases like cancer and malaria (3-6). It is estimated that approximately 20–30% of the world's flora have undergone phytochemical studies in order to produce bioactive compounds with therapeutic significance (7, 8).

Belonging to the Boraginaceae family is *Onosma bracteatum* Wall. In the Unani medical system, it is called Gaozaban, and in the Middle East, it is called Sedge. It is commonly known as rock garden plants and thrives in sunny, dry, or damp conditions (9).

It has historically been used to control urine production and strengthen the body's immunological system. Additionally, it is said to be utilised as a tonic, demulcent, diuretic, asthmatic and bronchitis therapy. Leprosy and syphilis are treated using a decoction. It is said to be helpful in treating excessive thirst, restlessness associated with fever, heart palpitations stomach and bladder discomfort. Wounds and skin conditions are treated with it in folk medicine. It is used as a major constituent in a variety of Ayurvedic and Unani formulations for the treatment of various disorders (10-12). This study aims to confirm chemical constituents of *Onosma bracteatum*, flavonoids and polyphenolic estimation, and to investigate the antiviral, antioxidant, antibacterial activities and In-silico Docking studies of *Onosma bracteatum.*

2. Material and Methods

2.1. Source of Plant Material

Onosma bracteatum wall. Was purchased from the local market of Bahawalpur city, Punjab province in April 2022.

2.2. Extraction and fractionation

Extract obtained by using cold maceration method and concentrated using rotavapor. The dried material was weighed, labeled, and then stored in refrigerator. The percentage yield was calculated by the following formula.

$$Yield\ (\%) = \ (Weights\ of\ solvent\ free\ extract\ (\ g\ ) \times\ 100)/(Dried\ extract\ weight)$$

For this study, the concentrated extract was employed. In addition, the extract was divided into several fractions using the aqua (ENA), n-hexane fraction (ENH), n-butanol fraction (ENB), and chloroform fraction (ENC) in increasing order of polarity, as previously mentioned by Tiwari et al. (2011). Using standard, conventional protocols, ethanol extracts (ENE) and its four fractions (ENH, ENC, and ENB) conducted preliminary qualitative phytochemical screening (13-15).

2.3. Qualitative Phytochemicals analysis

For the analysis of phytochemical tests, first standard drugs were tested and then the plant parts were tested for phytochemical analysis. For Saponin detection standard drug *Glycyrrhiza* was used and OBL (*Onosma bracteatum* leaves) and OBS (*Onosma bracteatum* stem) powder gave positive froth and emulsification test while OBF (*Onosma bracteatum* flower) gave negative froth and emulsification test. For Cardiac glycosides detection, the standard drug *Nerium* leaves were taken and *Keller–Kiliani* test were performed and OBL, OBS, OBF, gave positive results for Keller Killani test. For Anthraquinone glycosides, standard drug Senna and Rhubarb were taken and *borntrager's* test were performed. OBL and OBS gave negative results and OBF gave positive results. For the detection of Alkaloids Tobacco leaves were taken and Mayers, Wagner's and hagners test were performed. OBL gave negative Mayer test and positive results for Wagner's and hagners test. OBS and OBF gave positive magnet ,Wagner and hagners test (16).For detection of Tannins, standard drug Green tea and catechu were taken.$Fecl_3$ test were performed. OBL and OBS gave negative results while OBF gave positive test for tannins. Bromine water test was performed. OBL and OBS gave negative results while OBF gave positive bromine water test. For the detection of flavonoids, standard drug orange peel was used, NaOH test were performed. OBL and OBS gave negative results while OBF gave positive results. Qualitative analysis of phytochemicals was carried out to detect secondary metabolites of plants such as glycosides, alkaloids, tannins, saponins etc., (17).

2.4. (TFC) Total flavonoids content test

A minor alteration to the AlCl3 technique was applied for assessing the TFC in the different test plant extracts. mg of quercetin equivalent per g of dry extract, or mg QE/g, was used to calculate the flavonoids. TFC was estimated using the following formula. (18).

$$\mathrm{TFC} = \mathrm{CV}/(\mathrm{m})$$

Where, the equation derived from the standard curve provided the value of C.

2.5. (TPC) Total phenolic content test

A minor alteration to the technique described in, Folin Ciocalteu's method was used to estimate the TPC of plant extracts. A spectrophotometer was used to measure the absorbance at 725nm (19).

2.6. Determination of Anti-viral Activity

Embryonated eggs of chicken were used for antiviral study of *Onosma bracteatum* as reported in previous studies (20).

2.7.Antioxidant Activity

DPPH method by taking ascorbic acid as standard was used in this study as reported by khan et al (21). Formula used for determining the percentage inhibition given as:

$$Inhibition\ (\%) = [(\mathrm{AB} - \mathrm{AA}) / \mathrm{AB}] \times 100$$

AB = Absorbance of blank sample
AA = Absorbance of test sample:

2.8. Determination of Antibacterial Activity

Table 1. Micro-organisms used for antimicrobial screening.

Sr.no	Test organisms
1	*Escherichia coli*
2	*Bordetella bronchiseptica*
3	*Bacillus pumilus*
4	*Staphylococcus epidermidis*

2.8.1. Preparation of inoculums

By adding nutrient broth that had previously been autoclave-sterilized to the 0.5 McFarland turbidity, all the bacterial cultures were brought to 1×10^5 colony forming units (CFU). Utilizing an analytical weighing balance, each fraction of plant extract was weighed before being separately diluted in its corresponding solvent to create solution. Each disc of 6 mm filter paper received 20 µl of this solution, containing 400 to 800 g of crude plant extract. At room temperature, these discs underwent dehydration. In the same manner, discs of the appropriate solvents were made to serve as the tests' negative controls (22).

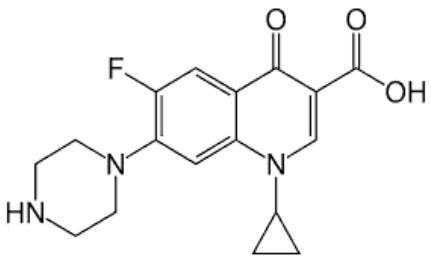

Figure **Error! No text of specified style in document.**.1: Ciprofloxacin

Nutrient broth was utilized to culture bacterial strains, while Muller Hinton agar was employed for antibacterial tests .All growth and assay media's composition is listed above.20 g of nutritional agar and 38 g of Muller Hinton agar were each suspended in 1 liter of deionized and distilled water before being autoclaved for 15 minutes at 121 °C. 20 to 25 ml of it were then added to sterile petri plates, which were then given time to solidify at room temperature before being kept at 4 °C until they were required for antibacterial activity. It was then allowed to cool to 60 °C. For the antibacterial test, the Muller Hinton agar was inoculated for each crude plant extract with a standard bacterial suspension in sterile nutritive broth and Saboruad's dextrose agar, respectively. To remove extra liquid, a sterile brush was moved up against the test tube wall after being soaked in the bacterial culture. After incubation, a uniform lawn of bacterial growth was obtained by spreading these inoculums of the appropriate bacteria on the Muller Hinton agar. To do this, the swab was gently pushed along the x-axis across the full surface of the agar. The plate was then spun 90 degrees, and the swab was moved along the remaining two

axes, namely the y and z axes. This guarantees that the bacteria will develop uniformly following the incubation time. Then, using flamed forceps, filter paper discs of each extract, a negative control, and a disc of reference antibiotics were positioned at the right distances (23).

2.9. Gas Chromatography Mass Spectrometry

GC-MS apparatus Agilent's 6890 series GC-MS and HP's 5973 mass selective detector were used in this (24, 25).

2.10. In-silico Molecular Docking Studies

Molecular Docking studies are particularly helpful in molecular biology and computer-aided drug development. A well-focused search database that includes several methods for ligand preparation and the helpful PDB (Protein Data Bank) format, which is required for molecular retrieval. *Onosma brateatum* enzyme preparation was done using discovery studies, and standards were obtained from the PubChem database in SDF (Structural Data Format) format. The Babel used as a tool for ligand molecule preparation. Vina, a component of PyRx, was loaded with ready-to-use receptors and ligands.

The receptor's target was prepared by eliminating several undesirable materials, including cofactors, water molecules, and ligand molecules that were already attached. To prepare the ligand for the docking procedure, the PDB format of the three-dimensional structure is translated to the Autodock format. The receptor target that was developed was loaded into the PyRx programme and stored as a macromolecule. The interacting ligand's binding energy values for undesired compounds such cofactors, water molecules, and ligand molecules that are already connected. PDB format of a three-dimensional structure is transformed to Autodock format for the docking process in order to prepare the ligand. After being prepared, the receptor target was loaded into the PyRx virtual screening programme and saved as a macromolecule. Using the Auodock –Vina programme from PyRx, molecular docking was done for a subset of molecules. The interacting ligand target complexes' binding energies were noted, and the complexes were visually analysed using the Discovery Studio software (26, 27).

3. Results and Discussion

3.1. Extraction Yield

Extraction of *Onosma bracteatum* plant parts was done in methanol and DCM solvents and then the results were taken that 800 grams of leaves were taken, they gave methanol extraction as 6.54 grams and DCM fraction as 9.24 grams .When stems were taken 800 grams, it gave 5.94 grams of methanol extract and 8.32 grams of DCM extract and flowers were taken 400 grams ,it's methanolic extract were 6.27 grams and DCM fraction was 7.99grams.

Table 2. Results of extraction of flowers, leaves and stem of *O. bracteatum.*

Plant Parts	Solvents used	Extract weight	Abbreviations	Weight of plant powder
Leaves	DCM	9.24gm	OBLD	800 gm
	Methanol	6.54gm	OBLM	800 gm
Stems	DCM	8.32gm	OBSD	800 gm
	Methanol	5.94gm	OBSM	800 gm
Flowers	DCM	7.99gm	OBFD	400 gm
	Methanol	6.21gm	OBFM	400 gm

DCM=Dichloromethane, OBL = *Onosma bracteatum* leave, OBS= *Onosma bracteatum* stem, OBF=*Onosma bracteatum* flowerS.

3.2. Qualitative Phytochemical analysis

The results of phytochemical analysis of *Onosma bracteatum* showed that it contains Alkaloids, Glycosides, Phenols, Tannins, Saponins and Flavonoids. Secondary metabolites contribute to different therapeutic activities.

Table 3. Results of qualitative analysis of flower, stem, and leaves of O. bracteatum

Phytochemicals	Standard used	Test name	Standard	Obl	Obs	Obf
Saponins	*Glycyrrhiza*	Froth	+	+	+	-
	glabra	Emulsification	+	+	+	-
Cardiac glycosides	Nerium leaves	Keller killani	+	+	+	+
Anthraquinone	*Senna*	Borntrager's test	+	-	-	+
glycosides	*Rhubarb*					
Alkaloids	Tobacco	Mayers	+	-	+	+
		Wagners	+	+	+	+
		Hagners	+	+	+	+
Tannins	Green tea	$Fecl_3$ test	+	-	-	+
	Catechu	Bromine water test	+	-	-	+
Flavonoids	Orange peel	NaOH	+	-	-	+

OBL = *Onosma bracteatum* leave, OBS= *Onosma bracteatum* stem., OBF=*Onosma bracteatum* flower. + = Present - = Absent

3.3. Total Flavonoid Contents

Table 4. Absorbance measured at different Quercetin Concentrations

Sr no	Conc. of Quercetin µg/ml	Average Absorbance ± S. D
1	20	0.524 ± 0.005
2	30	0.6569 ± 0.002
3	40	0.7154 ± 0.001
4	80	0.9969 ± 0.001
5	100	1.136 ± 0.009

Table 5. Flavonoid Content (TFC) of *Onosma bracteatum*

Sr no	Extracts	mgQu.Eq/gm E±S.D
1	OBLM	28.39189
2	OBSM	27.33784
3	OBFM	17.75676

Measured Absorbance at Different Gallic Acid Concentrations

Table 6. Absorbance measured at different Gallic Acid Concentrations

Sr No.	Conc. of Gallic Acid µg/ml	Average Absorbance ± S. D
1	0	0.2042 ± 0.001
2	10	0.6227 ± 0.002
3	20	0.6955 ± 0.005
4	30	0.7858 ± 0.001
5	40	0.8751 ± 0.003
6	50	0.9011 ± 0.001
7	60	0.9823 ± 0.004
8	80	1.1171 ± 0.005
9	100	1.2414 ± 0.001

3.4. The total Phenolic content (TPC)

Table 7. Total Phenolic Content (TPC) of *Onosma bracteatum*

Sr no	Extracts	Mg GA.Eq/gm E±S.D
1	OBLM	191.4265
2	OBSM	56.38235
3	OBFM	75.05882

3.5. Antiviral Activity

IC50 values of *Onosma bracteatum* against IBV, which is a type of coronavirus that infects chickens. Virus control is the dilution factor of the virus that causes 50% hemagglutination. IC50 is the concentration of the sample that inhibits 50% of the viral replication as in Table 8.

Table 8. HA titer and IC_{50} values of *Onosma bracteatum* against IBV

Samples	HA titer	Virus control	IC_{50}(mg/o.1mL) H_2O
OBLM	8	1024	-
OBLD	32	1024	-
OBSM	00	1024	6.25
ACYCLOVIR	00	1024	0.781

The result shows the HA titer and IC50 values of *Onosma bracteatum* against NDV, which is a type of avian paramyxovirus that causes Newcastle disease in poultry. The results show that OBLM and OBLD fractions had no hemagglutination activity but had moderate antiviral activity compared to the standard drug acyclovir.

Table 9. HA titer and IC_{50} values of *Onosma bracteatum* against NDV

Samples	HA titer	Virus control	IC_{50}(mg/o.1mL) H_2O
OBLM	00	2048	12.5
OBLD	00	2048	6.25
OBSM	16	2048	-
ACYCLOVIR	00	2048	0.781

Acyclovir had no hemagglutination activity, but had the lowest IC50 value, indicating the highest antiviral activity. This suggests that *O. bracteatum* fractions have different mechanisms of action against AIV H9N2, and that acyclovir is more effective than *O. bracteatum* in inhibiting AIV H9N2 replication.

Table 10. HA titer and IC_{50} values of *Onosma bracteatum* against AIV H9N2

Samples	HA titer	Virus control	IC_{50}(mg/o.1mL) H_2O
OBLM	02	2048	-
OBLD	00	2048	6.25
OBSM	00	2048	12.5
ACYCLOVIR	00	2048	0.390

IBV=avian infectious bronchitis virus, NDV=Newcastle disease virus, H9=influenza virus, HA, titer 0-8=exceptionally strong, HAtitre16-32=strong, HAtitre64-128=moderate, HA titer 256-2048=not active

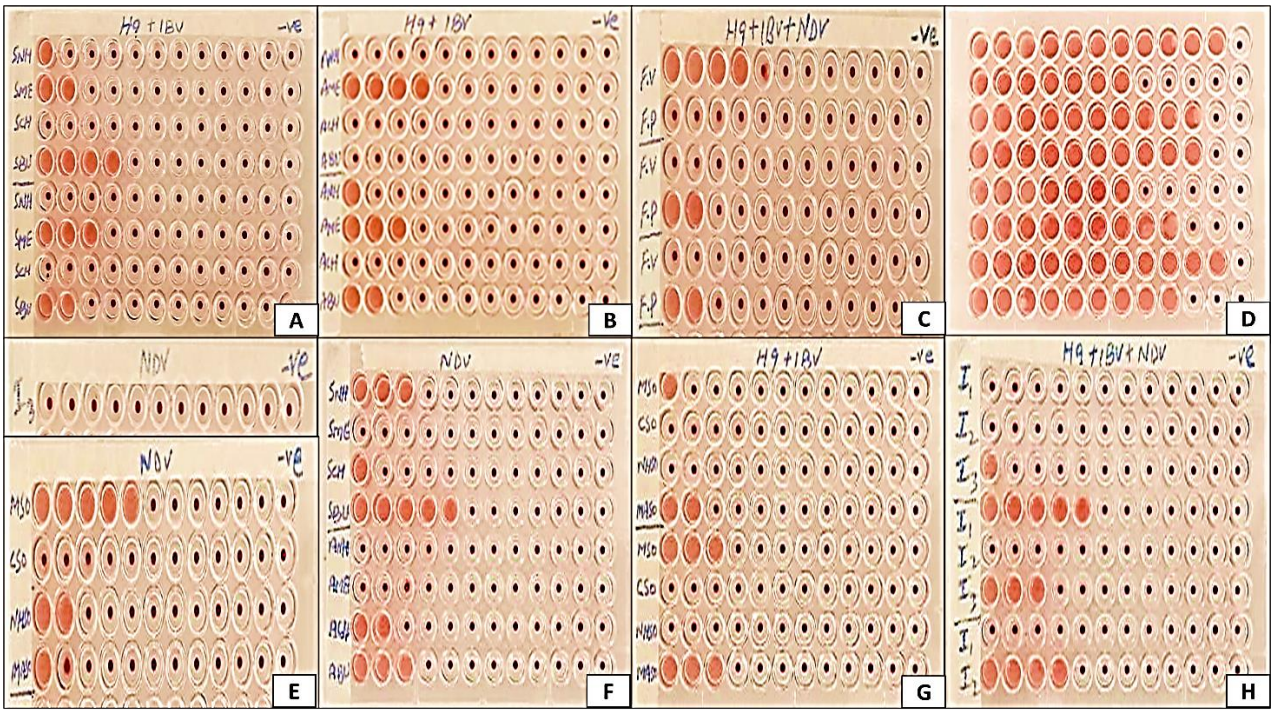

Figure 1. Anti-Viral Activity

(A, B) Influenza, (C, D) Influenza and Newcastle Disease Virus, (E, F) Newcastle disease virus, (G) Influenza Infectious Bronchitis Virus, (H) Influenza, Infectious Bronchitis Virus, and Newcastle disease Virus.

3.6. Antioxidant Activity

The absorbance of ascorbic acid was measured at 300.69 nm. The average absorbance of ascorbic acid solutions decreases as the concentration increases, from 1.412 ± 0.001 at 3.93017 µg/ml to 1.2777 ± 0.001 at 63.5 µg/ml.

Table 11. Absorbance measured at different Ascorbic acid concentrations

SR no	conc. of ascorbic acid µg/ml	Average Absorbance ±S. D
1	3.93017	1.412 ± 0.001
2	7.9825	1.399 ± 0.002
3	15.535	1.378 ± 0.005
4	31.35	1.345 ± 0.001
5	63.5	1.2777 ± 0.001

The results of antioxidant activity using DPPH for *Onosma bracteatum* are described in table below in table 12.

Table 12. Antioxidant activity of *Onosma bracteatum* by DPPH methods

Sr.no	Extract /Fractions	mg AE. g-1 extract ±SD
1	Ascorbic acid	7.66±2.38µg/ml
2	OBLM	74.90±4.72µg/ml
3	OBFM	40.81±0.82µg/ml
4	OBSM	219.65±9.75µg/ml

3.7. Antibacterial activity

Escherichia coli (ATCC10536), Bordetella bronchiseptica (ATCC4617), Bacillus pumilus (ATCC14884), Staphylococcus epidermidis (ATCC12228) were used to assess the activity. Antibacterial potential of extracts of *Onosma bracteatum* OBLM, OBSM, OBFM was tested against these strains.

Table 13. Antibacterial activity of extract of *Onosma bracteatum* against 4 bacterial strains

Bacterial strains	Zone of Inhibition (mean±SD)		
Conc.	OBLM (50mg/ml)	OBSM (100mg/ml)	STD (25mg/ml)
E.Coli	4.43±0.05	3.25±0.04	24.33±0.15
B. Bronchiseptic	2.75±0.04	3.89±0.03	32.3±0.26
B. Pumilus	4.34±0.04	2.92±0.02	18.3±0.15
S. Epidermidis	2.33±0.03	1.93±0.02	21.4±0.25

3.8. Gas Chromatography with mass spectrometry GC/MS

The GCMS chromatogram of *Onosma bracteatum* methanolic fraction revealed a total of 3 peaks of compounds which identified by comparing their peaks, retention time, height (percent), and patterns of mass spectra fragmentation to those of known compounds that are present in the National Institute of Standards and Technology (NIST).

Table 14. Tentative identification and biological activity of compounds

SR	Compound Name	Retention time	Molecular weight	Molecular Formula	Class	Biological Activity	Reference
1	Docosanoic Acid, Eocosyl Ester	3.11	648	C44H88O2	Ester	Anti-Inflammatory activity	(28)
2	Trans-2,4-Dimethylthiane, S, S-Dioxide	4.34	162	C7H14O2S	Alkane	Anti-inflammatory Antioxidant	(29)
3	Cyclohexene, 1-methyl-3-(1-methylethenyl)-, (. +/-.)-	15.76	136	C10H16	Alkane	Anti-microbial activity	(30)

3.9. In Silico Activity

Binding Affinity and intermolecular interactions of compounds with lipoxygenase, Nitric oxide synthase, xanthine oxidase enzyme.

Table 15. In-silico Docking studies of methanolic extract against lipoxygenase

Sr no	Ligands	Binding affinity	Amino acids	TYPE of Interaction
1	Cyclohexane	-6.1	ALA: 453	Alkyl
			LEU : 448	Alkyl
			PPHE : 544	Alkyl
2	Trans-2,4-Dimethylthiane, S, S-Dioxide	-5.5	TYR : 383	Hydrogen bonding
			PHE : 402	Alkyl
			TYR : 81	Alkyl
3	Docosanoic acid docosyl ester	-5.8	PHE : 169, 102	Alkyl
			VAL : 175	
			LEU : 615	Alkyl
			PRO : 621	Alkyl
			LYS : 83	

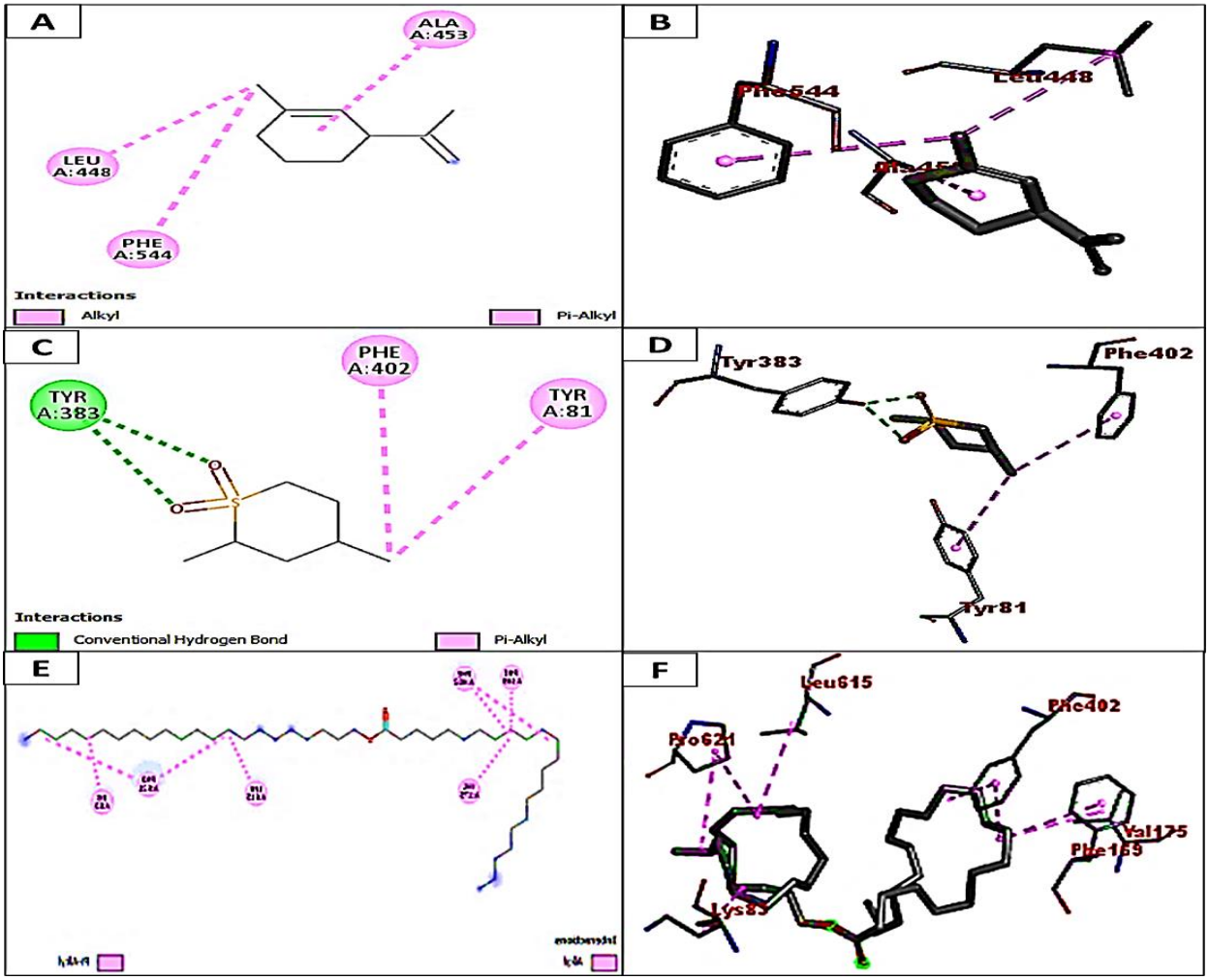

Figure 2. Docking Of Cyclohexene, Trans-2,4-Dimethylthiane, S, S-Dioxide, Docosanoic Acid Docosyl Ester performing with lipoxygenase (A) Cyclohexene 2d, (B) 3d, (C) Trans-2,4-Dimethylthiane, S, S-DIOXIDE 2d, (D), 3d (E) Docosanoic acid docosyl ester 2d, (F) 3d

Table 16. In-silico Docking studies of methanolic extract against nitric oxide

Sr No.	Ligands	Binding affinity	Amino acids	Type of Interaction
1	Dimethylthiane	-6.5	PHE 488	Alkyl
			TYR 491	Alkyl
			LEU 125	Alkyl
2	Cyclohexene, 1-methyl-3-(1-methylethenyl)-	-6.1	PHE 369	Alkyl
			TRP 194	Alkyl
			LEU 209	Alkyl
			CYS 200	Alkyl
			TYR 489	Alkyl
3	Docosyl	-5.8	ALA 262	Alkyl
			MET 355	Alkyl
			VAL 352	Alkyl
			TRP 463	Alkyl
			ARG 381	Alkyl
			TYR 489	Alkyl
			PHE 369	Alkyl
			ALA 197	Alkyl
			TRP 194	Alkyl
			PRO 350	Alkyl
			CYS 200	Alkyl

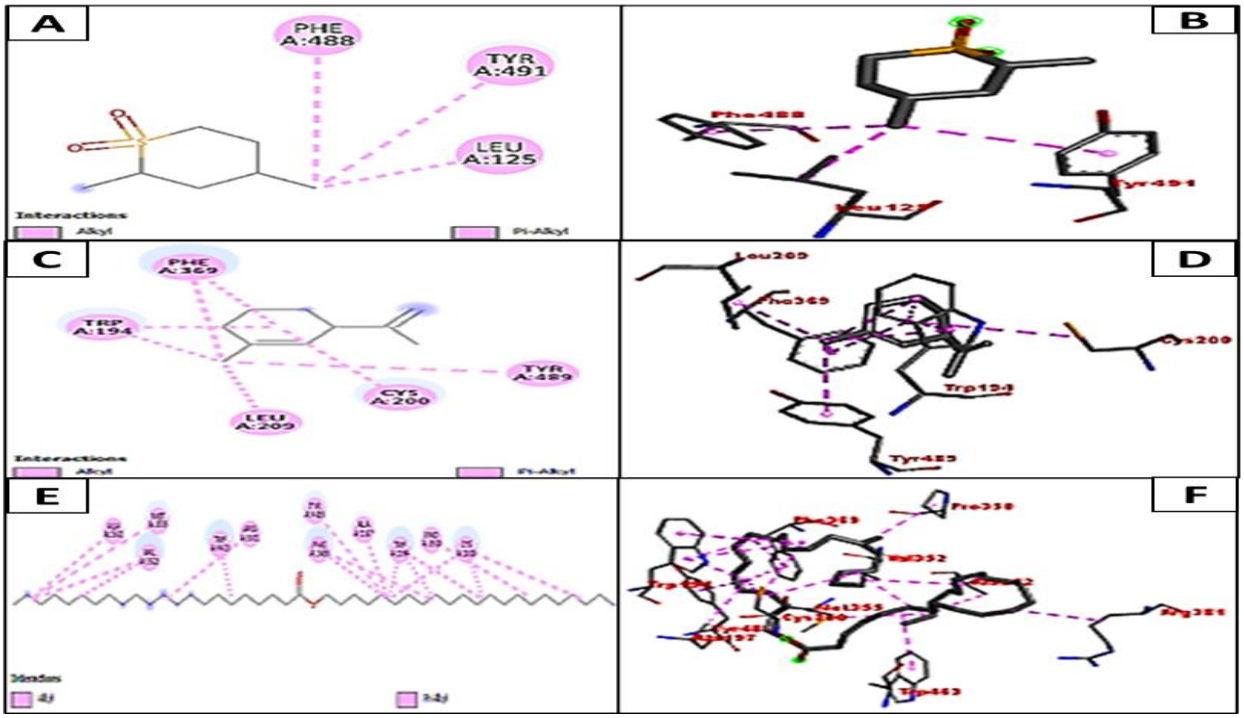

Figure 3. Docking Of Cyclohexene, Trans-2,4-Dimethylthiane, S, S-Dioxide, Docosanoic Acid

Docosyl Ester Performing with Nitric Oxide Synthase (A) Cyclohexene 2d, (B) 3d, (C) Trans-2,4-Dimethylthiane, S, S-Dioxide 2d, (D), 3d (E) Docosanoic Acid Docosyl Ester 2d, (F) 3d

Table 17. In-silico Docking studies of methanolic extract against Xanthine Oxidase

Sr No.	Ligands	Binding Affinity	Amino acids	Type Of Interaction
1	2,4-dimethyl	-6.3	ARG 161	Hydrogen bonding
2	Cyclohexene,1-methyl-3-(1-methylethenyl)	-4.2	LEU 157	Alkyl
			TYR 153	Alkyl
3	Docosanoic acid, docosyl ester	-4.5	ALA 106	Alkyl
			PRO 118	Alkyl
			VAL 121	Alkyl
			VAL 88	Alkyl
			ARG 37	Alkyl
			THR 94	Hydrogen bond
			SER 93	Hydrogen bond

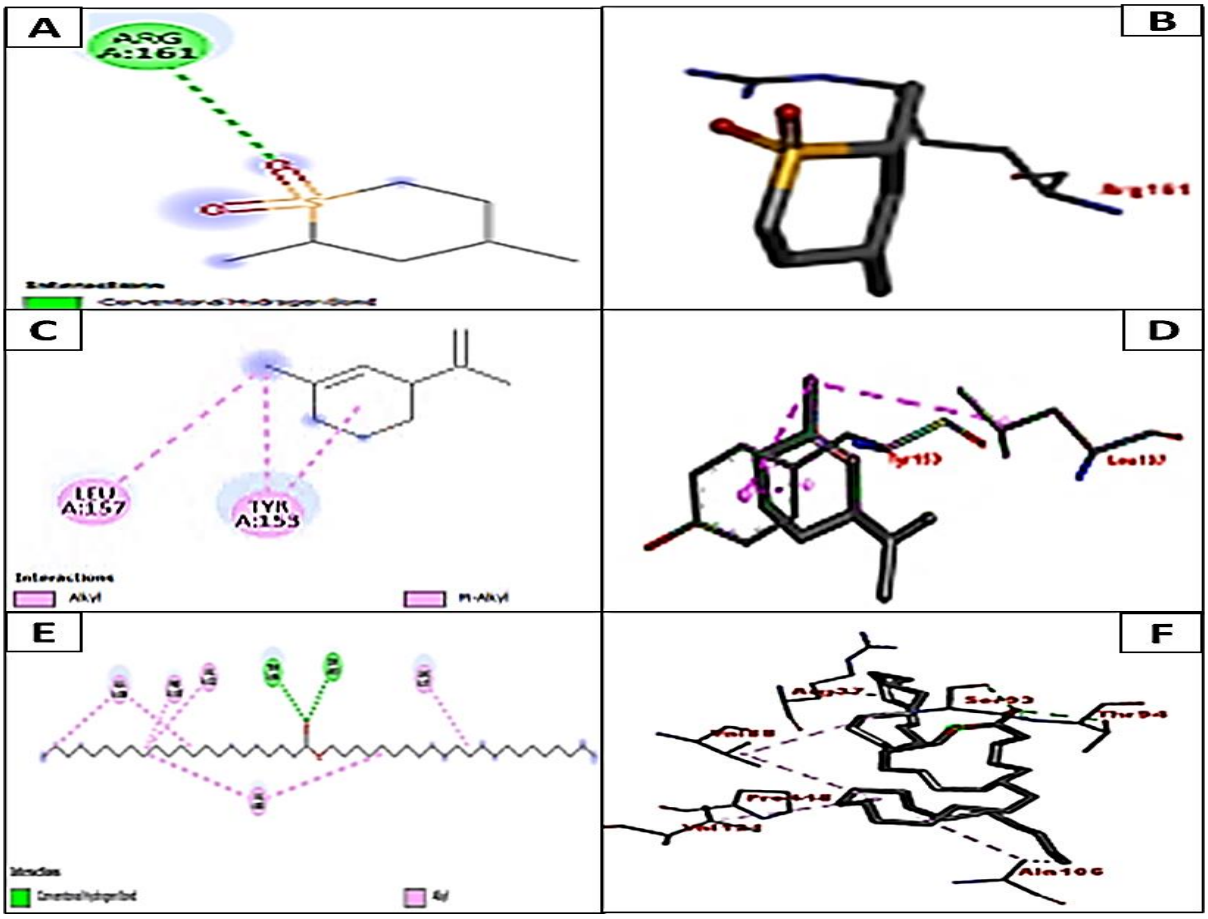

Figure 4. Docking Of Cyclohexene, Trans-2,4-Dimethylthiane, S, S-Dioxide, Docosanoic Acid

Docosyl Ester Performing with Xanthine Oxidase A) Cyclohexene 2d, (B)3d, (C) Trans-2,4-Dimethylthiane, S,S-Dioxide 2d, (D) ,3d (E) Docosanoic Acid Docosyl Ester 2d, (F) 3d

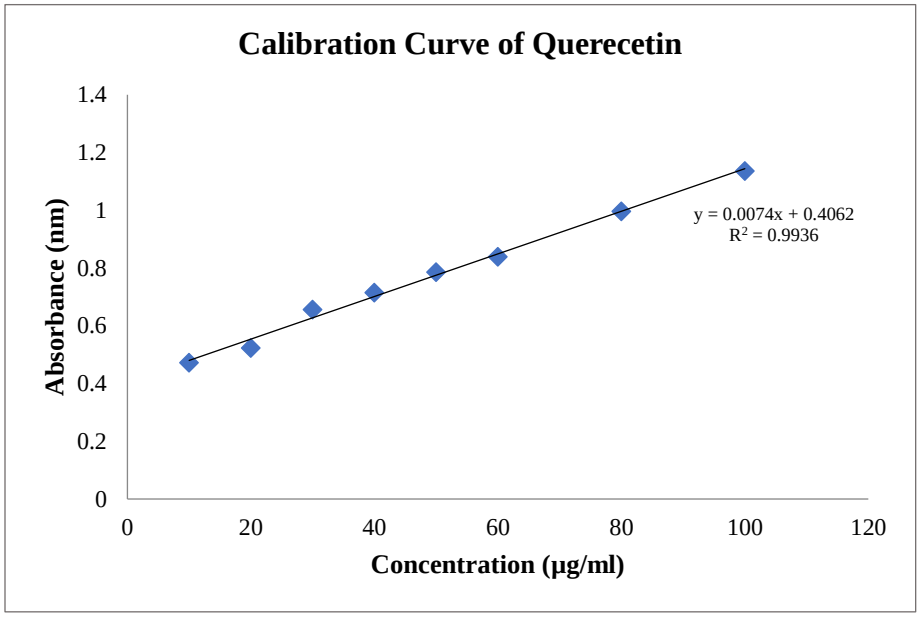

Figure 5. Calibration curve of quercetin for TFC

A graphical representation of the TFC of *Onosma bracteatum* is shown in fig below.

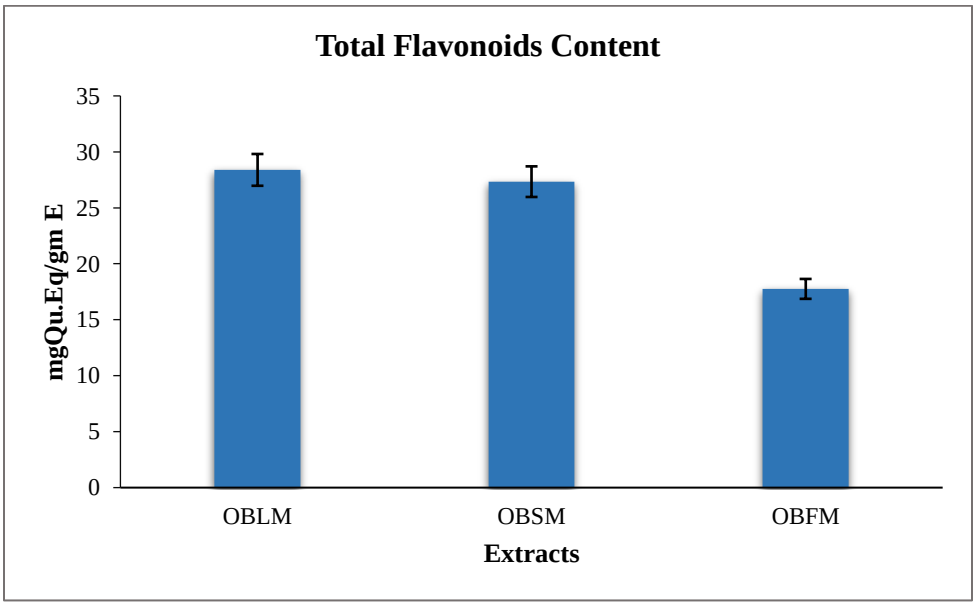

Figure 6. Graphical representation of Total Flavonoid Content (TFC) of *Onosma bracteatum*

The results show highest phenolic content in methanolic extract of *Onosma bracteatum* leaves (191.4265 mg GA.Eq/gm E of extracts). The calibration curve of Gallic Acid for TPC is shown in figure below

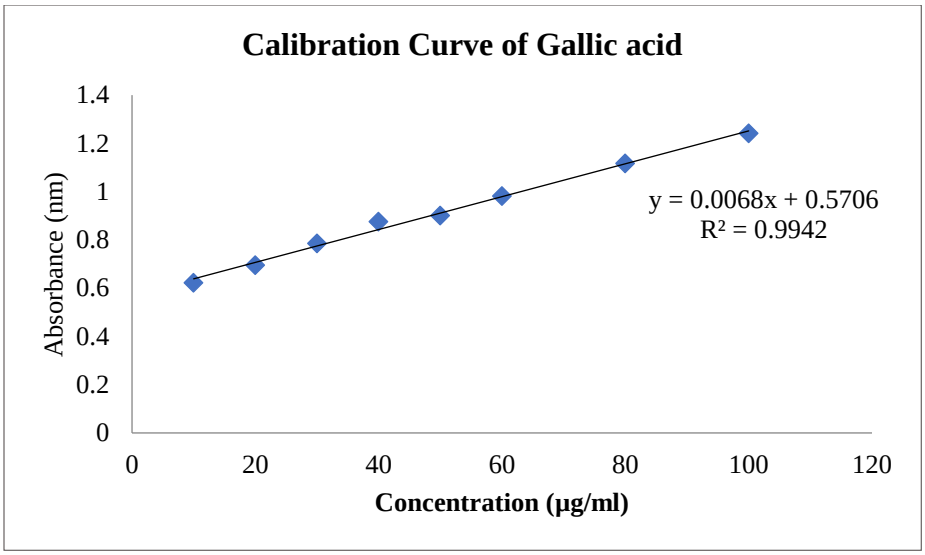

Figure 7. Calibration curve of Gallic Acid for TPC

A graphical representation of the TPC of *Onosma bracteatum* is shown in fig. below.

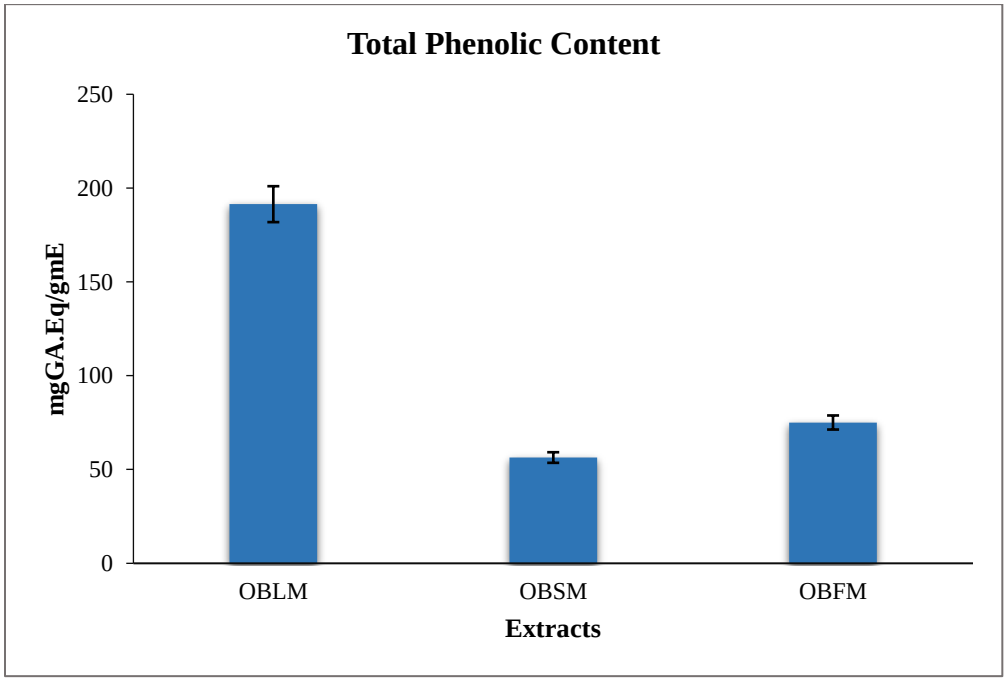

Figure 8. Graphical representation of Total Phenolic Content (TPC) of *Onosma bracteatum*

Antioxidant activity of *Onosma bracteatum* is determined by DPPH(1,1-diphenyl-1-picryl-hydrazyl) Assay. The calibration curve of ascorbic acid for DPPH is shown in the figure below.

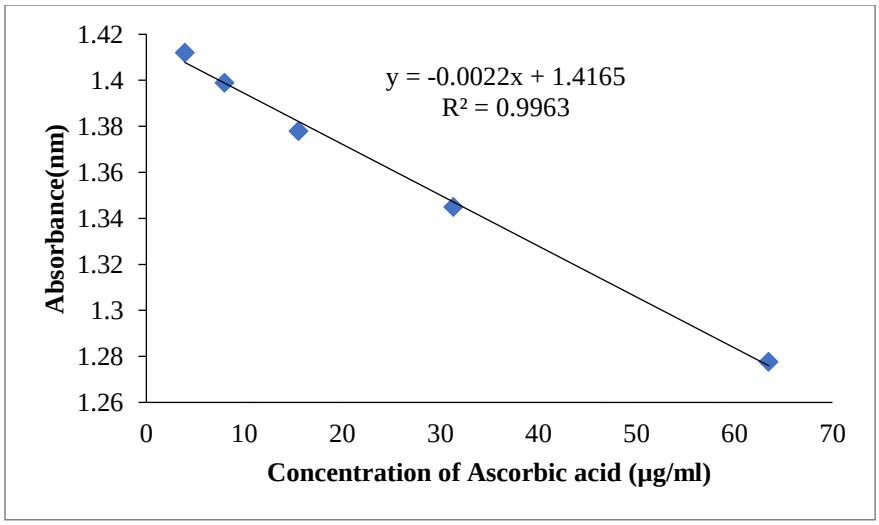

Figure 9. Calibration curve of ascorbic acid for DPP

The graphical representation of the antioxidant activity of *Onosma bracteatum* by DPPH method is shown in figure below.

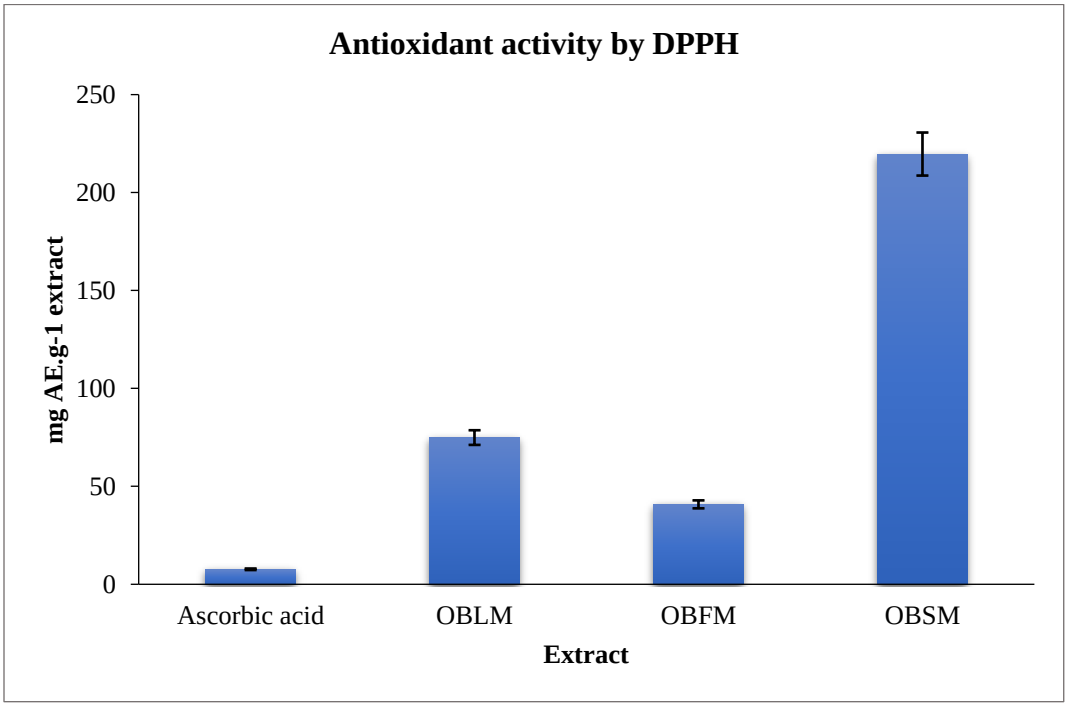

Figure 10. Graphical representation of the antioxidant activity by DPPH

The antibacterial properties in plants attributed to phytochemicals, namely saponins, alkaloids, glycosides, and flavonoids compounds. The results shown indicate the zone of inhibition increases in concentration.

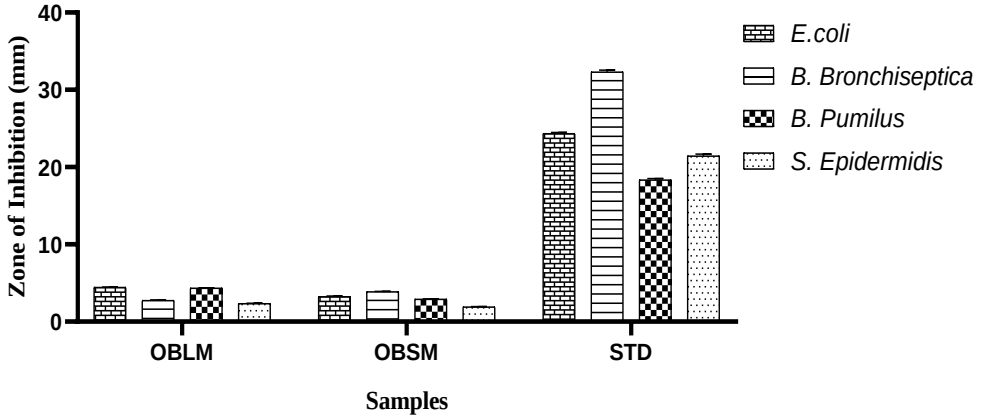

Figure 11. Antibacterial Activity against *E. coli, B. Bronchiseptica, B. Pumilus and S. Epidermidis*

It was shown that the cold-water extract of *O. bracteatum* exhibited significant antibacterial properties against *S. aureus*. While there was no or little activity against other strains, the zone of inhibition measured at 15.3mm. *O. bracteatum* hot water extract showed strong antibacterial activity against S. *aureus and B. subtilis.*

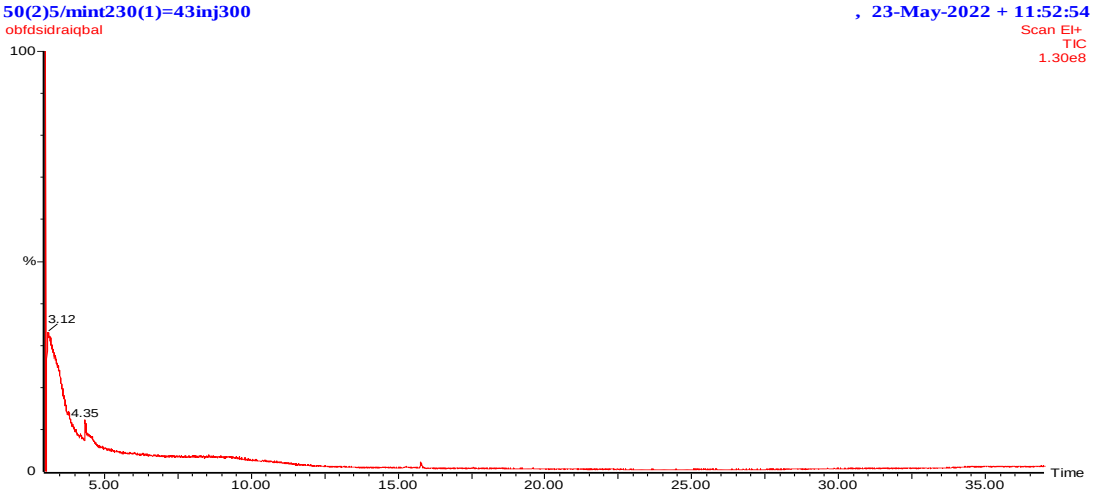

Figure 12.GC-MS chromatogram for methanolic extract obtained from Flowers

4. Discussion

The plant *Onosma bracteatum* has vast importance as it has many phytochemicals constituents and many pharmacological activities. The Genus *onosma* has many species(*Onosma bracteatum* PFAF Plant Database). One of the specie bracteates has biological activity and many pharmaceutical components in its leaves, stems, and flowers. The leaves of *Onosma bracteatum* has Saponins, cardiac glycosides, Alkaloids while it does not have Anthraquinone glycosides,

tannins, and flavonoids. The stems of *Onosma bracteatum* have Saponins, cardiac glycosides, Alkaloids while it doesn't have Anthraquinone glycosides, tannins, and flavonoids. Phytochemical analysis of different fractions of *Onosma bracteatum* showed that they are rich in secondary metabolites. The results of phytochemical analysis of *Onosma bracteatum* showed that it contains alkaloids, glycosides, phenols, tannins, saponins and flavonoids. Secondary metabolites contribute to various therapeutic activities. The findings were compared to the results present in literature.

In silico docking studies are conducted to theoretically anticipate the ligand-target interaction in order to evaluate the biological activity of naturally occurring products at the molecular level. It is also crucial for investigating additional modes of action and the binding affinities of various active substances against certain enzymes. The GC/MS findings of the methanolic extract of *Onosma brateatum* flowers were used to study three substances, with lipoxygenase, nitric oxide synthase, and xanthine oxidase enzymes serving as standards. Via in silico docking investigations, the drugs' inhibitory action was investigated.

Constituents, such as alkaloids, possess various biological activities including antimicrobial activities; tannins and flavonoids as an anti-oxidant and antibacterial agent; and sapiens acts as antibacterial agents. The results of phytochemical analysis suggested that *Onosma bracteatum* contains good levels of phenols and flavonoids. Literature showed that plants containing good levels of phenols and flavonoids are highly potent. The methanolic extract of *Onosma bracteatum* leaves showed good levels of total flavonoid content and total phenolic contents.

TFC results showed that flavonoids were higher in methanolic extract of *Onosma bracteatum* leaves 28.39189 mg Qu.Eq/gm of extract. The methanolic extract of *Onosma bracteatum* of flowers show lowest total flavonoid content OBFM (17.75676 mg Qu.Eq/gm of extract). The calibration curve of Quercetin for TFC is shown in figure below.

5. Conclusion

Onosma bracteatum is a medicinal plant that contains secondary metabolites, such as phenols and flavonoids, that may have promising effects on health. The results of this study show that *Onosma bracteatum* has mild to moderate pharmacological actions, such as anti-inflammatory, antibacterial, antiviral, and antioxidant activities. These actions may be due to the ability of the secondary metabolites to modulate the immune system, inhibit the growth or replication of pathogens, scavenge free radicals, and protect the cells from oxidative stress. The finding of this study suggests that *Onosma bracteatum* can be a potential candidate of natural remedies for diseases, such as arthritis, peptic ulcer disease, and infections caused by viruses and bacteria. However, additional studies are required to validate *Onosma bracteatum*'s efficacy as well as safety in healthcare system.

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