Phytochemical Screening and Anti- Inflammatory Docking of Potential Plant *Vitex Negundo*

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Abstract*— The rise in drug resistance shown by human pathogenic bacteria become a clinical crisis from all over the world. In order to control the situation, it is essential to develop alternative drugs for the treatment of infectious diseases. Recent advancements in the field of green synthesis have enabled the development of alternative drugs. The work described in this paper details the phytochemical screening and pharmacological study of Vitex negundo leaf extracts, which is mentioned in Ayurveda as a potential medicine to cure several diseases. Identification, Collection & Authentication of plants, Drying & Storing of the plants for studies, Extraction, Qualitative Phytochemical Screening reveals the presence of phytochemical constituents like carbohydrates, alkaloids, tannins, saponins, and flavonoids. Microbiological investigation revealed that Staphylococcus aureus and E. coli was highly inhibited by methanolic extract of vitex negundo. We used molecular docking to better understand the mechanism underlying the extract's anti-inflammatory effects with a high docking score.

Index Terms- Drug Resistance, Vitex negundo, Staphylococcus aureus, methanolic extract

I. INTRODUCTION

Nature always stands as golden mark exemplify the outstanding phenomenon of symbiosis . The biotic element of nature are all interdependent [1]. Plant are indispensable to man. The history of herbal medicine is as old as human civilization. Herb is used in the pharmaceutical therapy of disease curing. Historically, medicinal plant parts have been used as part of a global folk healthcare approach to administer pharmaceutical treatments [2].In traditional medicine, physical and mental illnesses are prevented, diagnosed, improved, or treated using "knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures." At http://www.who.int/topics/ traditional_ medicine/en/, the World Health Organization provides information. Although there are many distinct traditional medical systems, all of them share a holistic outlook on life, an emphasis on health rather than disease, and an equilibrium of the mind, body, and environment. These systems' philosophies and practices are shaped by the conditions, environment, and geographic area in which they originated (WHO 2005). All systems of traditional medicine include the use of herbs as a fundamental component, with the focus typically being on the individual's overall condition rather than the specific illness or disease the patient is experiencing.

In most parts of the world, the development and mass manufacturing of chemically manufactured medications throughout the past century has changed health care. Nonetheless, a sizable portion of the populace in poor nations still receives their primary treatment from herbal remedies and traditional healers. Up to 90% of people in Africa and 70% of people in India get their medical care from traditional medicine. Roughly 40% of all medical care provided in China is provided by traditional medicine, and over 90% of general hospitals contain traditional medicine departments (WHO 2005). Traditional medicine is still used in developed nations, though, and during the past 20 years, the public's interest in complementary therapies has grown significantly in industrialized nations as well.

Certain microbes have the ability to spread disease, the majority do not, and to view all microbes as harmful would be to misunderstand our dependence on and relationship with them. The majority of bacteria on the world, as will be described, are found in the soil and the ocean and never come into contact with humans, much less spread illness.The idea of "selective toxicity," which refers to how to harm or suppress the infectious bacterium without harming the host, iscrucial to understanding how illnesses are treated for those who do. All the many categories of microorganisms will be discussed in order, beginning

with bacteria and viruses as they are the main sources of diseases due to general principles that apply to all of them Similar diversity may be found in the human body, which serves as a home to a wide variety of microorganisms. The precise term for this type of association is commensal, meaning that most of the time the organisms benefit from it without endangering the host. This contrasts with partnerships that are mutually beneficial or parasitic, in which one party benefits at the expense of the other. Some body components are home to a multitude of organisms, whereas others—such as the majority of internal organs, bones, blood, and the central nervous system—are typically sterile. An invasive infection results if germs manage to infiltrate these typically sterile regions. Antimicrobial agents work to stop infections and illnesses brought on by microorganisms. medicine becomes more common in cases where it is unable to treat an illness, such as advanced cancer or emerging infectious diseases. Additionally, traditional remedies are often thought to be harmless, natural, and non-toxic.

A herb is mostly a plant component that is used for its flavor or medicinal qualities. One category of dietary supplement is herbal medicine. A herb is typically a plant's leafy green or flowering portion. such as: Vitex negundo Linn., commonly known as Nirgundi, is a member of the Verbenaceae family. primarily The blue-flowering cultivar is called Nirgundi or Sephaali, while the white-flowering variant is called Sinduvaara. In addition to flavonoids like vitexin, which is a flavanol glycoside other than casticin, and the glycosides luteolin-7-glucoside and a-Dglucoside of tetrahydroxy monomethoxy flavones, the leaves of *Vitex negundo* contain irridoid glycosides such negundoside [3-10].It thrives in humid places occurs in wastelands and mixed open forests and has been reported to occur in Afghanistan, India, Pakistan, Sri Lanka, Thailand, Malaysia, eastern Africa and Madagascar. It is grown commercially as a crop in parts of Asia, Europe, North America and West Indies, also finds use as a food crop and a source of timber. Vitex negundo L. was found to have anti-amnesic [7] , anti-arthritic [8], anti-cancer [20], anti-convulsant [9] , anti-eosinophili [10],anti-filariasis [11] , anti-fungal [12], anti-inflammatory [13] , anti-microbial [14], anti-pyretic [15], anti-thypoid [16], anti-ulcer[17], anti- venom[18], anti-viral [19], larvicidal [20] and hepatoprotective activity [21] . On the basis of review the present study was undertaken to evaluate the anthelmintic activity and antimicrobial of *Vitex negundo L*

A computational method for predicting ligand binding affinities to receptor proteins is called molecular docking. While it may find applications in the field of nutraceutical research, it is a powerful instrument in the drug development process. Nutraceuticals are bioactive compounds found in food sources that have the potential to treat and prevent disease.New treatments tailored to individual diseases may be developed with the aid of identified molecular targets. This review looked at the use of molecular docking in the investigation of dietary supplements and illness treatment In recent years, molecular docking has emerged as a crucial component of in-silico drug development. This method entails forecasting the atomic-level interactions between a tiny chemical and a protein1. This makes it possible for scientists to investigate how tiny compounds, such nutraceuticals, behave within a target protein's binding region and comprehend the basic biochemical mechanism underpinning this interaction.A number of free and commercial computational tools and methods are available for molecular docking procedures. These tools and programs have been created and are being utilized in the academic and pharmaceutical research fields.have recently documented a rise in interest in using this technique in the field of food science. In particular, molecular docking is used in disease management to validate the molecular targets of nutraceuticals.Nutraceuticals are organic compounds that have medicinal effects on human health and are frequently obtained through nutrition. These have become more and more well-liked in recent years due to their ability to prevent and control chronic diseases such as diabetes, cancer, cardiovascular disease, and neurological disorders. Molecular docking studies are employed in the realm of nutraceutical research to provide important information prior to in vitro investigations26. Examining the most relevant molecular docking applications to evaluate the potential health- promoting benefits of nutraceuticals is the aim of this review. AutoDock Vina, Discovery Studio, Surflex, AutoDock GOLD, Glide, MCDock, MOE-Dock, FlexX, DOCK, LeDock, rDock, ICM, Cdcker, LigandFit, FRED, and UCSF Dock are a few

of the most widely used docking tools. These applications have been ranked highly, with AutoDock Vina, Glide, and AutoDock GOLD having the highest marks [17].

Plant profile
VITEX NEGUNDO LINN.
Taxonomical classification[16]
Kingdom: Plantae- Plants

Subkingdom: Tracheobionta – Vascular plants Super division: Spermatophyte – Seed plants Division: Magnoliophyta – Flowering plants Class: Magnoliopsida – Dicotyledons Subclass: Asteridae Order: Lamiales Family: Verbenaceae – Verbena family Genus: Vitex Linn.

II. MATERIAL AND METHODS: [17]

2.1Identification, Collection & Authentication of plants

The plant vitex negundo was collected from adjoining area of Bolangir(Odisha) in the month of November. The plant was identified as vitex negundo Schult. (Asclepiadaceae), Ref. No:CNH/I-I/17/2010/Tech.II/197 by Botanical Survey of India (BSI), Kolkata.

2.2Drying & Storing of the plants for studies

The aerial parts were dried under shade and powdered by the help of mechanical process. The coarse powder have stored in an air tight container for further studies.

2.3Extraction

2.3.1Hot successive extraction

The shade dried coarse powder of aerial part was subjected to continuous hot extraction (Soxhlet extractor) with ethanol. The extract was dried by Rotary evaporator, weighed and percentage of yield was calculated in terms of air-dried crude powdered materials. The extracts were then stored in a desiccator for experimental work.

2.3.2Qualitative Phytochemical Screening

The of *vitex* negundo part were subjected for the following phytochemical screening to identify various active constituents.

Materials

Pet. Ether, Ethanol & Water extracts. Reagents: Mayer's, Wagner's, Hager's, Dragendorff's, Fehling's A & B, Barfoed's, Millon's, α-naphthol, Conc. Sulphuric acid, 10 % Ammonia, Pyridine, Sodium nitropruside, Acetic anhydride, Ferric chloride, 10 % Lead acetate, 10 % Ammonium hydroxide solution.

Methods

Carbohydrates

Two hundred milligram of extract was dissolved in 50 ml of water and filtered. The filtrate was exposed to the following tests.

Molish's test: Two drops of alcoholic solution of α-naphthol was added in 2 ml of filtrate and the mixture was shaken well .Then one ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand for some time. A violet ring was obtained which indicate the presence of carbohydrates.

Fehling's test: One ml of filtrate was boiled on water bath with one ml each of Fehling solutions A and B. A red precipitate was obtained which indicate the presence of sugar.

Barfoed's test: One millilitre of Barfoed's reagent was added in1 ml of filtrate and heated on a boiling water bath for about 2 min. A red precipitate was obtained which indicates the presence of sugar.

Benedict's test: About 0.5 ml of Benedict's reagent was added in point 5 ml of filtrate.

The mixture was heated on a boiling water bath for two min. A characteristic colour precipitate was obtained which indicates the presence of sugar.

Selwinoff's test for hexose sugar: About 3 ml of Selwinoff's and 1 ml of test solution was heated on a boiling water bath for two min. A red colour was obtained which indicates the presence of sugar.

Glycosides

Fifty milligram of extract was hydrolysed with concentrated hydrochloric acid for 2 hrs on a water bath and filtered. Then the hydrolysate was subjected to the following tests.

Anthraquinone glycoside

Borntrager's test: Ten millilitre of chloroform was added in 2 ml of filtered hydrolysate and shaken properly. Then chloroform layer was separated and 10% ammonia solution was added to it. Pink colour was obtained which indicates the presence of glycosides.

Cardiac glycoside

Legal's test: One ml of pyridine and 1ml of sodium nitropruside was added to the extracts. Pink to red colour was obtained which indicates the presence of cardiac glycosides.

Keller -Killiani test or Test for reducing sugar (Digitoxose): Glacial acetic acid, one drop 5% Fecl3 and conc. H2SO4 were added to 2 ml of extract. Reddish brown color appears at junction of two liquid layersand upper layer appears bluish green.

Saponin

Fifty milligram of extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for fifteen min. A layer of foam indicates the presence of saponin

Alkaloids

Two hundred milligram of solvent free extract was stirred with 50 ml of dilute hydrochloric acid and filtered. Then the filtrate was tested carefully with various alkaloidal reagents as follows.

Mayer's test: Two drops of Mayer's regent was added in a 2-3 ml of filtrate by the side of the test tube. A white precipitate was obtained which show the presence of alkaloids.

Wagner's test: Two drops of Wagner's regent was added in a 2-3 ml of filtrate by the side of the test tube. A reddish brown precipitate was obtained which indicate the presence of alkaloids.

Hager's test: Two drops of Hager's reagent (saturated aqueous solution of picric acid)

was added in a 2-3ml of filtrate by the side of the test tube. A prominent yellow precipitate was obtained which indicate the presence of alkaloids.

Dragendorff's test: Two drops of Dragendorff's regent was added in a 2-3 ml of filtrate by the side of the test tube. A prominent yellow precipitate was obtained which indicates the presence of alkaloids.

Anthraquinone glycoside

Borntrager's test: Ten millilitre of chloroform was added in 2 ml of filtered hydrolysate and shaken properly. Then chloroform layer was separated and 10% ammonia solution was added to it. Pink colour was obtained which indicates the presence of glycosides.

Cardiac glycoside

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Saponin

Fifty milligram of extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for fifteen min. A layer of foam indicates the presence of saponin.

2.4Gas Chromatography - Mass Spectroscopy (GC-MS)

Materials

Trace 1300 GC, Tsq 8000 Triple Quadrupole MS with a column TG 5MS (30 m × 0.25 mm, 0.25 µm).

Methods

Vitellaria negundo ethanol extract was subjected to GC-MS analysis using a Trace 1300 GC, Gas Chromatography Liquid, gas, and solid analyses are carried out using mass spectrometry. In most cases, the sample is injected straight into the GC for liquids and gases. Solids are analyzed by pyrolysis, solvent extraction, or outgassing (desorption). During desorption studies, analytes are collected on a cryogenic trap and the temperature is regulated between 40 and 300 ºC under helium flow. A 1.25" x 4" cylinder serves as the sample chamber. Another sample method for materials analysis that cannot be introduced directly into the GC-MS is pyrolysis. A repeatable method of breaking down the molecule is to apply heat directly to the sample. Smaller compounds that make it to the GC after that are examined using GC-MS. Probe temperatures as high as 1400ºC can be employed with this technique. Furthermore, there are several different sample preparation and sampling techniques that have uses depending on the sample type and species of interest. These techniques include derivatization, static headspace analysis, purge and trap, and SPME (solid phase microextraction). [18,19]

2.5In vitro anti-microbial screening

The disc diffusion strategy is the method that is most frequently used. Basically, the process is seeding agar with the test bacterium and then covering it with a disc that has been impregnated with an antibiotic. The antibiotic will first absorb moisture and then diffuse outward across the agar medium, creating a gradient in the antibiotic's concentration.701 In order to allow the organism to grow freely and unhindered, the concentration of antibiotics is highest near the edge of the disk and gradually lowers away from it.72 When an antibiotic is used during incubation to stop bacteria from growing, a clean zone or ring develops around the disc. [14]

2.6 Molecular Docking Introduction: . [20]

Molecular docking has been an essential part of in-silico drug research in recent years. With this approach, the atomic-level interactions between a small molecule and a protein are predicted1. This enables researchers to examine the behavior of small molecules, such nutraceuticals, within the binding area of a target protein and to understand the fundamental metabolic process underlying this interaction. For molecular docking processes, a variety of commercial and free computational toolsand approaches are available. The domains of academia and pharmaceutical research have developed and are making use of these tools and systems.

Procedure :

Molecular docking is a computer-based method that predicts the structure of a ligand-receptor complex. It involves two main steps:

1. Sampling the ligand: Use sampling algorithms to find the most energetically favorable conformations of the ligand within the protein's active site.

2. Ranking the conformations: Use a scoring function to rank the conformations.

III. RESULT AND DISCUSSION

Qualitative Phytochemical Screening of *V. nirgundo* aerial part extract

Table3.1

Sl. No	Phytochemical test	Alcoholic
1. Alkaloids		
1	Mayer'stest	+
2	Wagner'stest	+
3	Hager'stest	+
4	Dragendorff'stest	+
2. Carbohydrates		
1	Molish's test	+
2	Fehling'stest	+
3	Barfoed'stest	+
4	Benedict'stest	+
5	Selwinoff'stestforhexose sugar	_

4. Saponins		
1	Foamtest	+

Antibacterial activity
Result & Discussion

The interpretation of susceptibility test findings obtained with compounds not included in the chart is based on the existence or lack of a distinct zone of inhibition. The results are regarded as qualitative until such time as interpretative zones are determined.

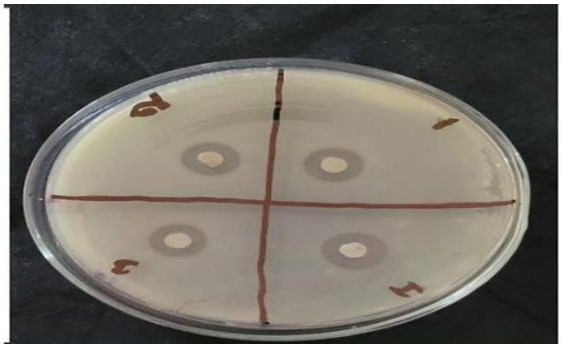


Figure 3.1 Disc Diffusion Method

Table No3.2

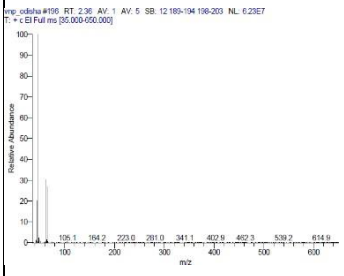
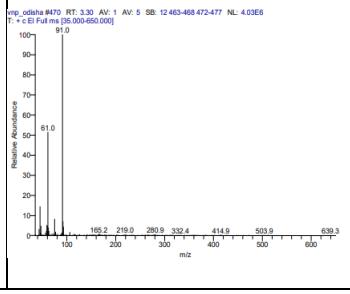
Antibacterial activity of Methanolic extract			
Treatment	Dose (µg/mg)	Zone of inhibition (mm)	
		E.coli	s. aureus
Negative control (DMSO+ Microorganism)	200 µl	0	0
	400 µl	0	0
	500 µl	0	0
	800 µl	0	0
Positive standard(ciprofloxacin)	25 µg	0	08±0.19
	50 µg	8±0.14	09.±0.14
	75 µg	11±0.29	13±0.22
	100 µg	16±0.11	15±0.16
Methanolic extract (test-1)	200 µg	0	0
	400 µg	0	0
	600 µg	0	0
	800 µg	0	04±0.38

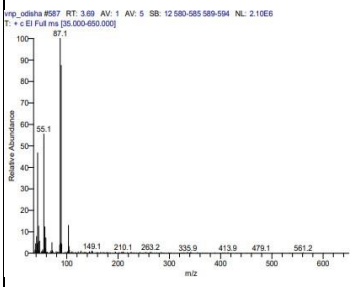
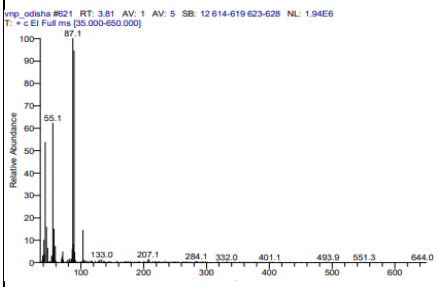
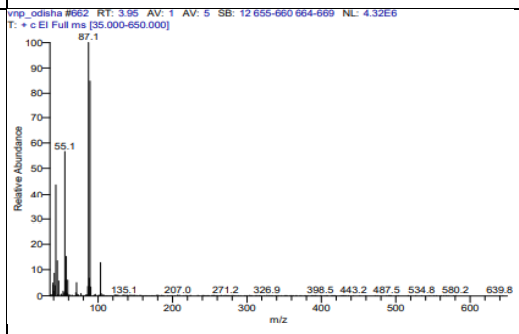
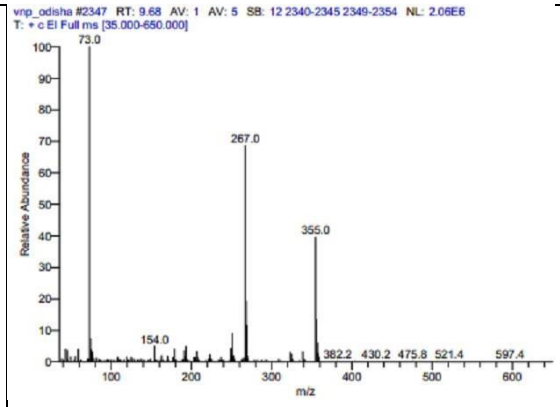
GC-MS ANALYSIS

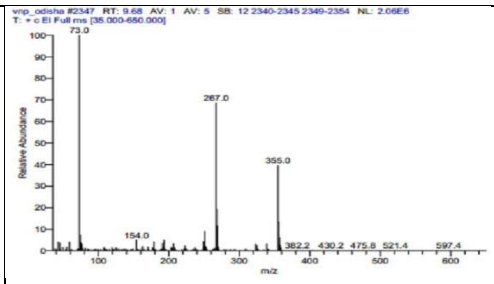
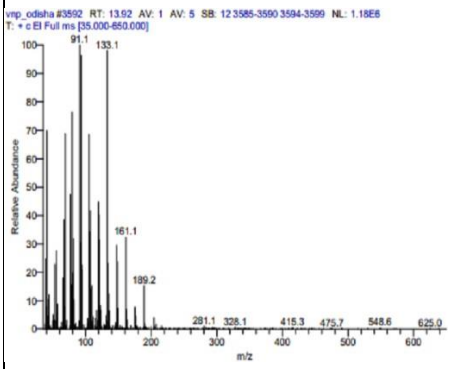
GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

Table No 3.3

Sl. No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
01	2.36	Oxalyl chloride	C2Cl2O2	126.92	78.97
02	3.30	Benzene, 2-methoxy-1-(2- nitroethenyl)-3-(phenylmethoxy)-	C16H15NO4	285.29	1.52
03	3.69	2,2-Dimethoxybutane	C6H14O2	86.18	0.75
04	3.81	2-Methoxy-3-methyl-butyric acid, methyl ester	C7H14O3	146.18	1.53
05	3.95	2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]ethyl acetate	C10H20O6	236.26	2.55
06	7.21	Cyclotetrasiloxane, octamethyl-	C8H24O4Si4	296.61	4.16
07	9.68	Benzoic acid, 2,6- bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	C16H30O4Si3	370.66	1.23
08	13.92	Caryophyllene	C15H24	204.35	1.88

Oxalyl chloride	
Benzene, 2-methoxy-1-(2-nitroethenyl)- 3-(phenylmethoxy)-	

2,2-Dimethoxybutane	
2-Methoxy-3-methyl-butyric acid, methyl ester	
2-[2-[2-(2- Hydroxyethoxy)ethoxy]ethoxy]ethyl acetate	
Cyclotetrasiloxane, octamethyl-	

Benzoic acid, 2,6- bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	
Caryophyllene	

Anti-inflammatory docking

The body uses ammation as a component of its immunological reaction to stimuli. It is helpful initially because it starts the mending process. But it's concerning since inflammation has the ability to self-replicate, causing new inflammation to arise in reaction to pre-existing inflammation.To batter understand the mechanism of the extract's Anti-inflammatory effect, we preformed Molecular docking . All compounds were showed good docking score.[92]

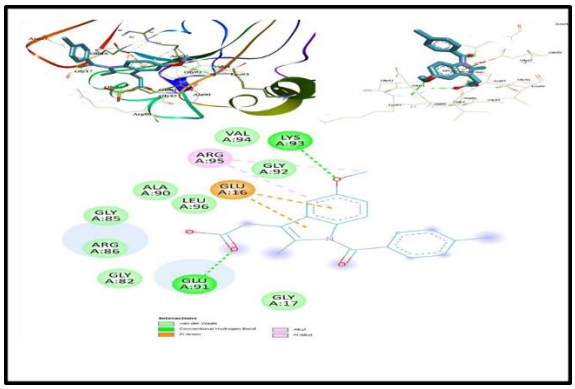


Fig. 3.2: 3D and 2D structures demonstrating the affinity for interaction between indomethacin and the protein Human Peroxiredoxin 5's active site (PDB ID: 1HD2).

Table 3.4: Anti-inflammatory docking

Name of the compound	Docking score
Indomethacin	-6.4
Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	-5.8
Caryophyllene	-6.1
Oxalyl chloride	-3.5

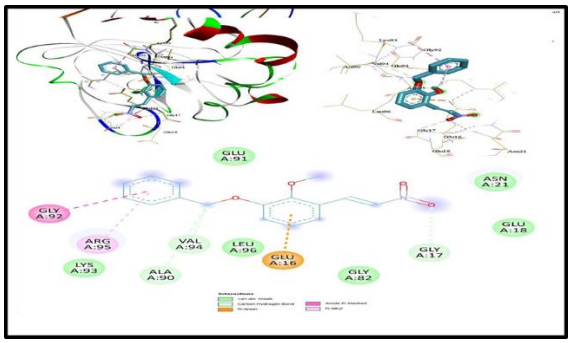


Fig. 3.2: Human peroxiredoxin 5 (PDB ID: 1HD2) protein's 3D and 2D structure demonstrates the binding affinity between benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester, and the protein's active site.

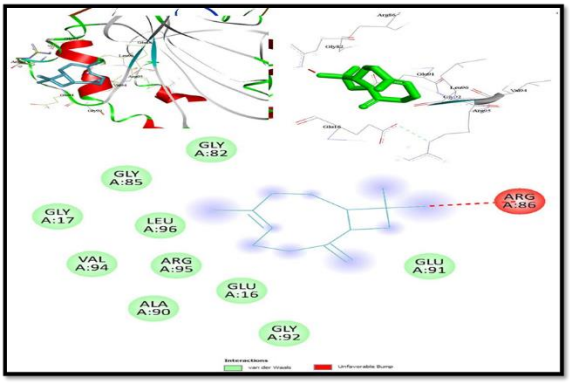


Fig.3.3: Human Peroxiredoxin 5's (PDB ID: 1HD2) active site and caryophyllene have a binding affinity that is demonstrated by a 3D and 2D structure.

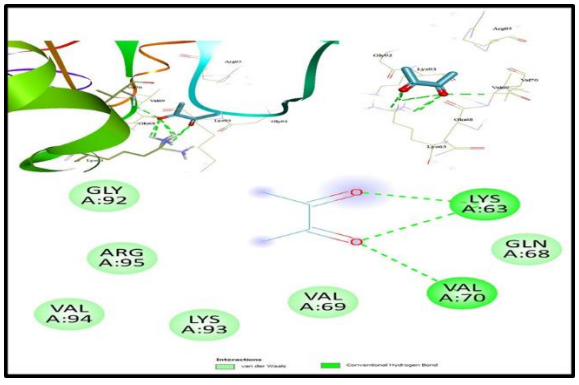


Figure 4.4: Oxalyl chloride and the active site of the protein Human Peroxiredoxin 5 (PDB ID: 1HD2) exhibit a binding affinity.

CONCLUSION

The current study's findings demonstrated the existence of a broad range of antibacterial activity against each of the bacterial infections mentioned above. As a result, it can be utilised to create new medicinal agents and as an antibacterial supplement. and had a lower level of anthelmintic action. The study's findings will be very beneficial for creating any formulation. We used molecular docking to better understand the mechanism underlying the extract's anti-inflammatory and anti- cancer effects. Every chemical displayed a high docking score. According to our research, the plant may be able to assist in the development of effective and safe drugs for a variety of ailments. More research is necessary to determine its bioactivity, and innovative drug formulations necessitate clinical studies.

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