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Scientific paper

Therapeutic Potential and *In silico* Evaluation of Phytochemicals in the Leaves of *Eucalyptus Globulus*, *Jasminum Officinale* and *Solanum Nigrum*

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Abstract

In this study, the plants *Eucalyptus globulus* (*E. globulus), Jasminum officinale (J. officinale*), and *Solanum nigrum* (*S. nigrum*) are investigated for their antibacterial, antioxidant, and therapeutic properties. The extraction solvents (aqueous, methanol, ethanol, and butanol) were used for phytochemical screening, antibacterial activity while aqueous extracts were specifically used for antioxidant analysis. The quantitative determination showed that the highest phenolic and tannin content was found in *J. officinale*, while highest flavonoid and alkaloids levels were found in *E. globulus* among the tested species. The disc diffusion method was followed for assessing the antibacterial activity against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). All extracts of *E. globulus* leaves showed antibacterial activity against *E. coli* and *S. aureus*. The aqueous extracts on FTIR showed quercetin, benzoic, salicylic, gallic, ferulic, and ascorbic acid. Furthermore, *in silico* analysis to assess the interaction of selected bioactive compounds, quercetin and benzoic acid, found in *E. globulus*, were docked with haemagglutinin and neuraminidase, as these influenza virus surface proteins play an important role in the virus's ability to infect host cells. Salicylic, gallic, ferulic, and ascorbic acid from *J. officinale* and *S. nigrum,* were docked with GABA receptor-associated proteins, which are important in synaptic transmission and plasticity.

Keywords: Disc diffusion method; anti-bacterial; DPPH radical scavenging assay; haemagglutinin; neuraminidase; GABA receptor-associated protein.

1. Introduction

Plants provide the basis of intricate, conventional medical systems that have been used for many years, and scientists are still developing innovative remedies for humanity today. Plants are commonly used to treat infections and other conditions.[1] Nearly two-thirds of people worldwide utilize medicinal plants for primary healthcare. When compared to conventional medications, medicinal plants have fewer side effects, are readily available, and cost less.[2]

Antibiotic resistance has been created in microorganisms due to the haphazard usage of antimicrobial drugs. Several antimicrobial drugs are needed to treat infectious diseases. One strategy is to examine the potential antibacterial properties of local herbal remedies. Medicinal plants serve as a substantial reservoir of new antifungal and antibacterial chemotherapeutic drugs.[3] Medicinal plants are good antioxidants, anti-diabetic agents, antibacterial agents, anti-cancer agents, detoxifying agents, antifungal agents, and neuro-pharmacological agents.[4]

Jasmine belongs to the olive family and is a genus of shrubs/vines in this family.[5] *J. officinale* stems have been used to treat chronic inflammatory conditions like colitis, ulceration, angiitis, and enteritis, as well as for the alleviation of insomnia.[6] The entire plant is traditionally used to treat skin conditions, tumors, and chronic ulcers. The al-

kaloids, salicylic acid, ascorbic acid, and resin found in the entire plant are used to cure fevers, skin conditions, and ulcers[7]. *Eucalyptus* is a genus that possesses various shrubs and flowering trees. Eucalyptus leaves possess antioxidant, anti-inflammatory, and antibacterial properties. *Eucalyptus* is used to treat ailments such as rheumatism, skin diseases, upper respiratory tract infections, diabetes, snakebites, and diarrhea.[8,9] *S. nigrum* is an annual herbaceous herb.[10] Whole plants are used for coughs, burns, snake bites, rabies, wound healing, and enhancing sleep.[11,12]

The discovery of new therapeutic agents has been based on medicinal plants. Based on their reported pharmacological characteristics and documented traditional uses, this study investigates the bioactive potential of *J. officinale*, *E. globulus,* and *S. nigrum*. They have been traditionally used to treat respiratory diseases and sleep disturbances. Although several phytochemicals from these plants have demonstrated preliminary antiviral and sedative effects, more research is needed to confirm their relevance to illnesses such as influenza and insomnia [13,14]

2. Materials and Methods

2. 1. Plant Collection

Fresh and disease-free plant samples of *E. globulus*, *J. officinale,* and *S. nigrum* were collected from dry and shady areas of GCU botanical garden, Lahore, Pakistan.

2. 2. Plant Extract Preparation

The plant extract preparation was started by washing the leaves 2–3 times with running tap water and drying them completely under shade. The plant leaves were dried and grounded to a fine powder. 20 g of powdered leaves from each plant species (*E. globulus*, *J. officinale,* and *S. nigrum)* were soaked in 200 mL of each solvent (ethanol, methanol, butanol, and distilled water). The mixtures with a concentration of 0.1 g/ml were periodically stirred, while being stored at room temperature (25 ± 2 °C) for 72 hours followed by filtration using Whatman No. 1 filter paper. The dried extracts were kept at 4 °C in sterile, labelled containers for phytochemical, antioxidant, and antibacterial analysis.[16–18]

2. 3. Phytochemical analysis

Both qualitative and quantitative tests were performed for phytochemical analysis.

(a) Qualitative analysis

For the determination of bioactive components present in plant leaves, different tests were performed, including alkaloids, flavonoids, phenolics, terpenoids, anthraquinones, carbohydrates, proteins, coumarins, emodins, saponins, steroids, tannins, anthocyanins, leucoanthocyanin, quinones, cardiac glycosides, and phlobotannins.[19,20]

(b) Quantitative Analysis

The total phenolics, flavonoids, tannins, alkaloids, and antioxidant activity were quantified using standard spectrophotometric methods. Measurements were taken in triplicates. Total Phenolic Content (TPC) was measured using the Folin-Ciocalteu method with gallic acid as a reference. 0.5 mL of extract was wixed with 0.6 mL of Folin-Ciocalteu reagent, then 1.5 mL of 20% Na_2CO_3 was added and incubated for 90 minutes in the dark. The absorbance was measured at 765 nm.[21]

Total Tannin Content (TTC) was calculated using the Folin-Ciocalteu method with a tannic acid standard. 0.1 mL of extract which was treated with 0.5 mL Folin reagent and 1 mL of 35% Na_2CO_3.The absorbance was measured at 725 nm after 30 minutes.[22]

Total Flavanoid Content (TFC) was calculated using the aluminium chloride method with quercetin standard. To 0.5 mL of extract, 1 mL water, 75 µL of 5% $NaNO_2$, 75 µL of 10% $AlCl_3$, and 0.5 mL of 1M NaOH was added, and incubated for 15 minutes. The absorbance at 510 nm was measured.[22]

Alkaloid content was determined by extracting 1.25 g of powder in 50 mL of 10% acetic acid in ethanol for 4 hours. The filtrate was concentrated to 1/4 and precipitated with NH_4OH. The residue was filtered, dried, weighed, and the alkaloid content was determined.[23]

The *in vitro* approach was used to measure the antioxidant activity (DPPH free radical scavenging assay) 100–200 µL extract was mixed with 1 mL of 0.4 mM DPPH. Incubated for 30 minutes in the dark. The absorbance was measured at 517 nm.[24]

2. 4. Antibacterial Activity

To determine antibacterial activity against *S. aureus* and *E. coli,* the disc diffusion method was used. Sterile 6 mm filter paper discs were impregnated with plant extracts at doses of 25, 50, 75, and 100 µL. 50 µl broth was spread on nutrient agar plates. The discs that had been impregnated with various extracts quantities were incubated at 37 °C for 24 hours. The inhibition zones were recorded.[25] As positive control the antibiotics Tetracycline was used, which zone of inhibition was 15 mm.

2. 5. Bioinformatic Analysis

The aqueous extracts of *E. globulus*, *J. officinale,* and *S. nigrum* were used to identify different functional groups of bioactive compounds by FTIR.[26] The compounds were found using the Pubchem database (https://pubchem.ncbi.nlm.nih.gov/). The bioactive compounds in *E. globulus* found by FTIR were used for *in silico* investigation by molecular docking against the target proteins influenza virus hemagglutinin (PDB ID: 4WE5) and neuraminidase (PDB ID: 7U4F) and bioactive compounds in *J. officinale* and *S. nigrum* against GABA receptors associated protein

(1KOT). The Galaxy web (https://galaxy.seoklab.org/) was used for the determination of ligand and protein interaction. The protein structure was retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org/)

2. 6. Statistical Analysis

All analyses were analyzed as triplicates, and for statistical analysis, SPSS (version 32) was used, and the one-way ANOVA test was performed to check the significance ($p < 0.01$) of the results.

3. Results and Discussion

There is a dire need to develop novel antibacterial medicines, as the excessive usage of antibiotics leads to antibiotic resistance.[27] Plants are a big source of phytochemicals and possess numerous biological properties.[28] The use of solvents also plays a crucial role in the extraction process of phytochemicals.[30] Different types and content of secondary metabolites that are recovered from plants are influenced by the solvents employed in the extraction process.[31] Phenols, flavonoids, and tannins are polar phytochemicals that dissolve easily in polar or semi-polar solvents, making them more effective for extracting these components from dried plant materials.[33]

3. 1. Antimicrobial Activity of Extracts

The leaf extracts of *E. globulus* showed antibacterial activity against both *E. coli* and *S. aureus* (Fig. 1–3). All extracts of *J. officinale* leaves showed zones of inhibition against *E. coli* and *S. aureus*, while the leaf extracts of *S. nigrum* showed no activity against *E. coli* and *S. aureus*. *S. nigrum* methanol extracts showed the highest activity against *E. coli* (Fig. 1–3).

With the increase in concentration, an increase in inhibition zones was observed that has been reported.[34] Butanol extracts proved to be the most effective due to their intermediate polarity.[35] This enables it to extract a diverse spectrum of bioactive components, including moderately polar phytochemicals, which may contribute to the observed antibacterial properties. As an organic solvent, it enhances the dissolving of both polar and non-polar molecules, hence improving the extraction of a wide range of active components.[36] Methanol and butanol extracts of *E. globulus* leaves showed more inhibition zones against *S.*

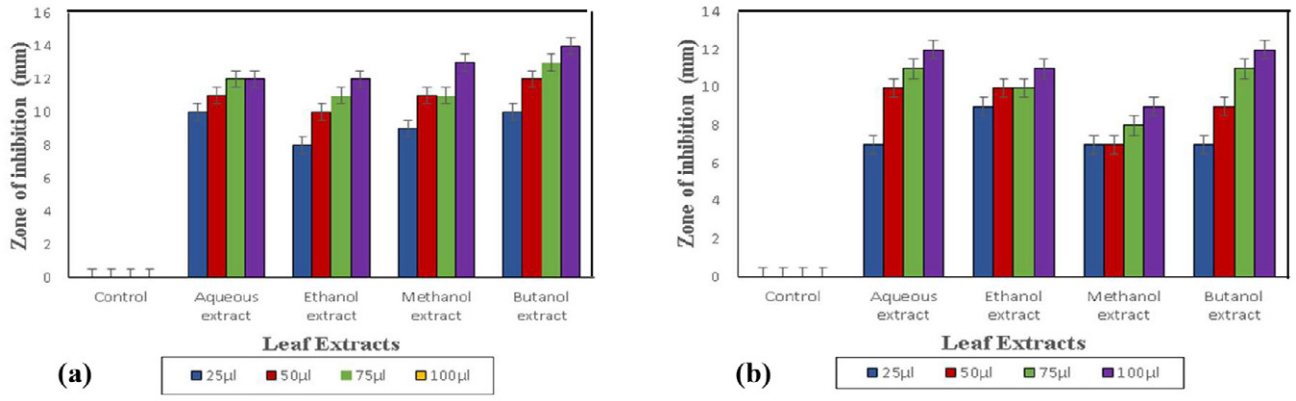

Fig. 1. (a) Antibacterial activity of *E. globulus* leaf extracts against *E. coli* **(b)** Antibacterial activity of *E. globulus* leaf extracts against *S. aureus.*

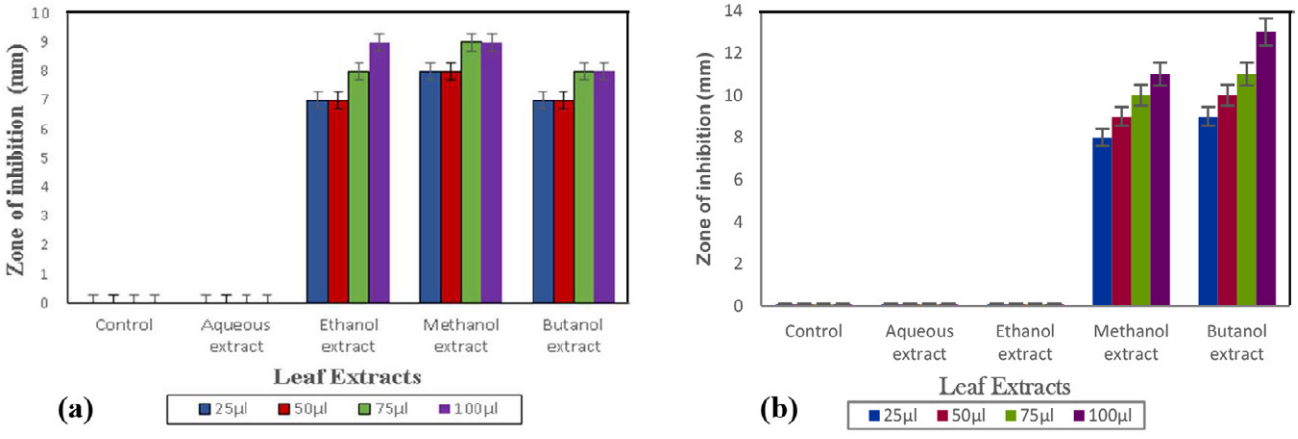

Fig. 2. (a) Antibacterial activity of *J. officinale* leaf extracts against *E. coli* **(b)** Antibacterial activity of *J. officinale* leaf extracts against *S. aureus.*

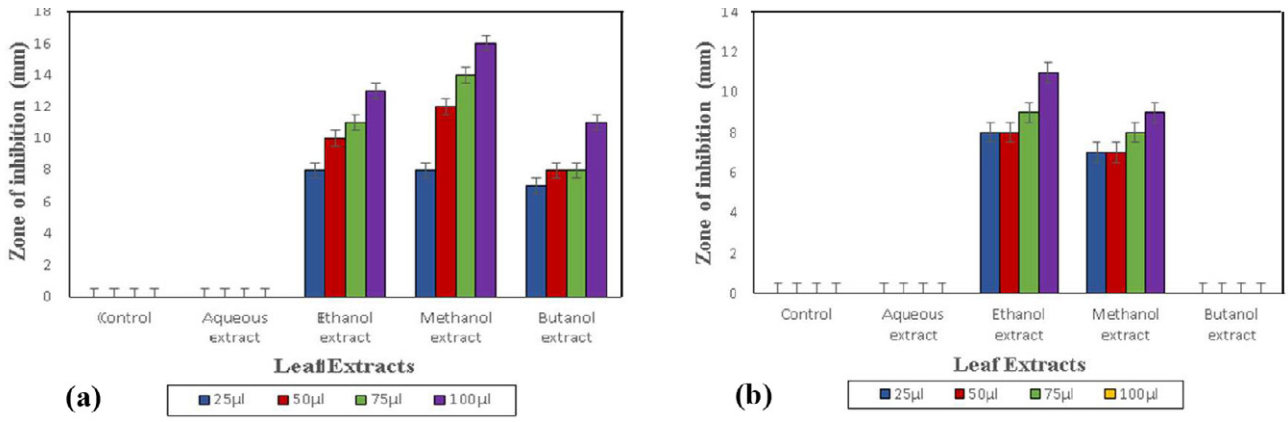

Fig. 3. (a) Antibacterial activity of *S. nigrum* leaf extracts against *E. coli* **(b)** Antibacterial activity of *S. nigrum* leaf extracts against *S. aureus.*

aureus and *E. coli,* which has been reported in different publications.[8,18,37] It was also reported that similar leave- and root- aqueous extracts showed higher activity against *E. coli* and *S. aureus* as compared ethanol extract.[38] The highest zones in butanol might be due to its high polarity as compared to other solvents.[39] Ethanol extract of *J. officinale* inhibited both strains, similar to the results of [7] and,[40] which reported that DCM and methanol extract of *J. officinale* whole plant showed the higher inhibition zones as compared to aqueous, ethanol, methanol and butanol extracts of leaves. The results of this study follow the work of different research groups,[41, 42] who reported that aqueous extract of *S. nigrum* leaves showed no inhibition zones, while methanol extracts showed high inhibition zones against *E. coli* and *S. aureus*. Our results are contradictory to the work of,[11] who reported that aqueous extract showed inhibitory activity against both *E. coli* and *S. aureus*. Butanol extract from leaves of *S. nigrum* did not show activity against *S. aureus*.

3. 2. Qualitative Analysis of Extracts

Qualitative phytochemical analysis of leaf extracts of *E. globulus*, *J. officinale* and *S. nigrum* was performed for the identification of secondary metabolites present in them. Extracts showed that alkaloids, flavonoids, saponins, steroids, carbohydrates and tannins are present in these extracts, while phlobotannins, emodins, anthocyanins and leucoanthocyanins were not found.[43–45] Qualitative analysis of different solvent extracts of *E. globulus*, *J. officinale* and *S. nigrum* was performed. Phytochemical tests showed that alkaloids, carbohydrates, coumarins, cardiac glycosides, steroids, phenol, protein, tannin, quinones and terpenoids were the bioactive components identified in all plant extracts.[46–48] The results of this work correlate with the work of different research groups.[18,49] Furthermore, it has been reported that anthocyanins, anthraquinones, phlobtinins and leucoanthocyanins are not present in any parts of *E. globulus, J. officinale* and *S. nigrum*. These phytochemicals make plants good antioxidants, enhancing antibacterial, anti-inflammatory, anti-influenza, anti-diabetic, and anti-sedative properties.[50,51]

3. 3. Quantitative Analysis of Extracts

Quantitative phytochemical analysis of the leaf extracts of *E. globulus*, *J. officinale*, and *S. nigrum* were performed (Fig. 4). Total phenol content, flavonoid content, tannin content, alkaloids, and antioxidant activity of each extract were calculated. The results showed that maximum alkaloid content (60%) was present in *E. globulus* as compared to other extracts; maximum tannin content (0.38 mg TAE/mL) was found in *J. officinale*, max. phenolic content was found in *J. officinale* (0.64 mg GAE/mL), and the highest flavonoid (11.39 mg QAE/mL) content was present in *E. globulus* leaves.

The highest amount of flavonoid was found in the leaves of *E. globulus* (11.39 mg/mL) and the least in *S. nigrum* (1.725 mg/mL). Total phenolic content and tannin content were highest in *J. officinale* (0.64 mg/mL, 0.38 mg/mL) and least in *S. nigrum* leaves (0.15 mg/mL, 0.209 mg/mL). Alkaloid content was found to be highest in *E. globulus* (60%) and least in *S. nigrum* (46%). These results are compatible with the results of,[37] which showed similar values of the total flavonoids and phenolic content in the leaves of *E. globulus*. The aqueous extract of *E. globulus* showed higher flavonoid, phenol, tannin, and alkaloid content as compared to the methanol extract, which has been reported.[8,52] Methanol extract of *J. officinale* leaves showed lower flavonoid, phenol, and tannin content that has already been reported by[53] and is in agreement with.[54] Methanol extract of *S. nigrum* leaves showed more flavonoid, phenol, tannin, and alkaloid content as compared to aqueous extract.[41]

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was used to measure the antioxidant activity of aqueous extracts. Aqueous extracts were chosen to assess antioxidant activity since they are commonly used in traditional medicine and contain polar phytochemicals such as phenols and flavonoids, both of which contribute considerably to antioxidant activity. Furthermore, aqueous extracts are

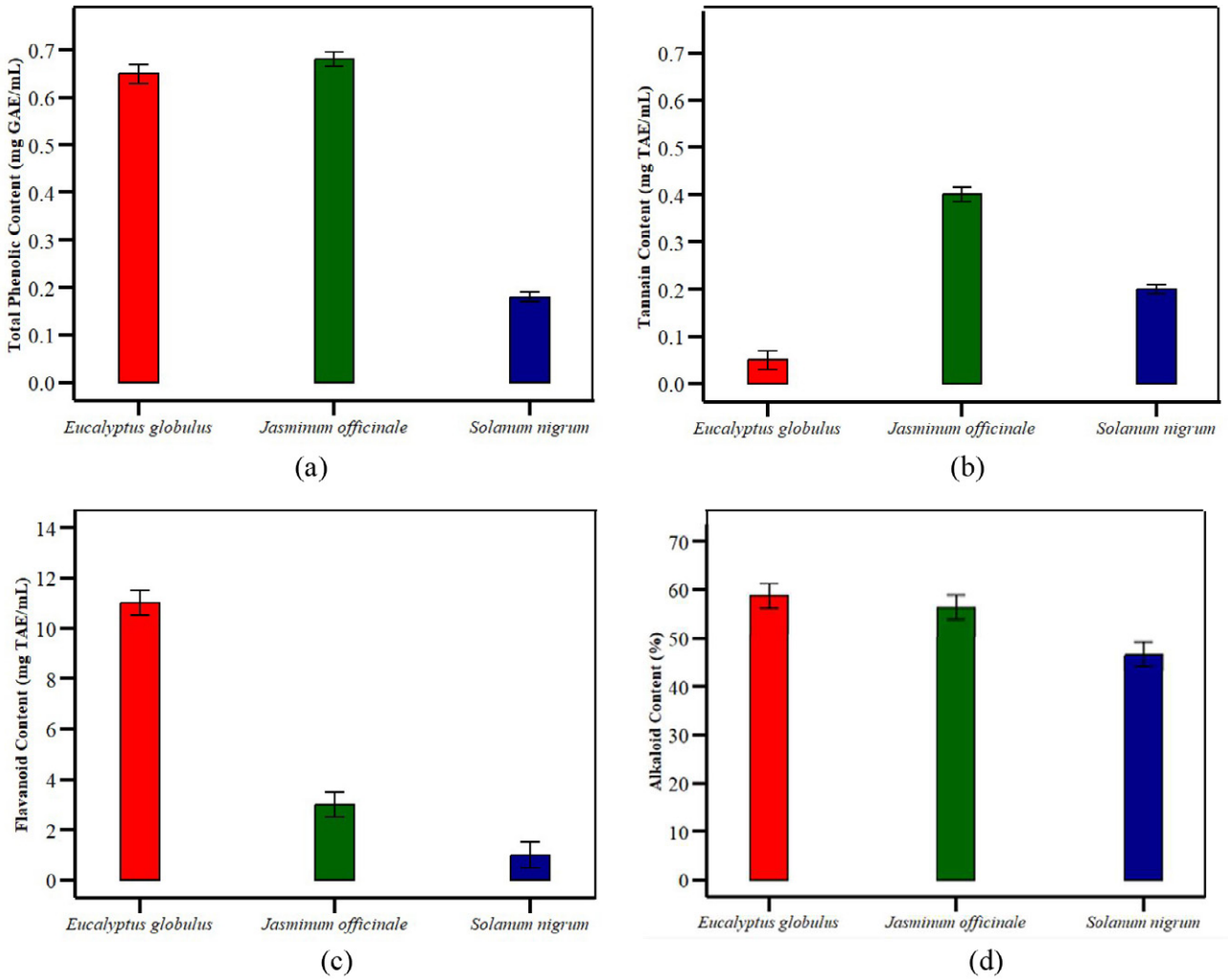

Fig. 4. (a) Determination of total phenolic content in aqueous leaf extracts of *E. globulus, J. offcinale* and *S. nigrum* **(b)** Tannin content in aqueous extracts leaves of *E. globulus, J. offcinale* and *S. nigrum*. **(c)** Flavonoid content in aqueous extracts of leaves of *E. globulus, J. offcinale* and *S. nigrum*. **(d)** Alkaloid content in leaves of *E. globulus, J. offcinale* and *S. nigrum*.

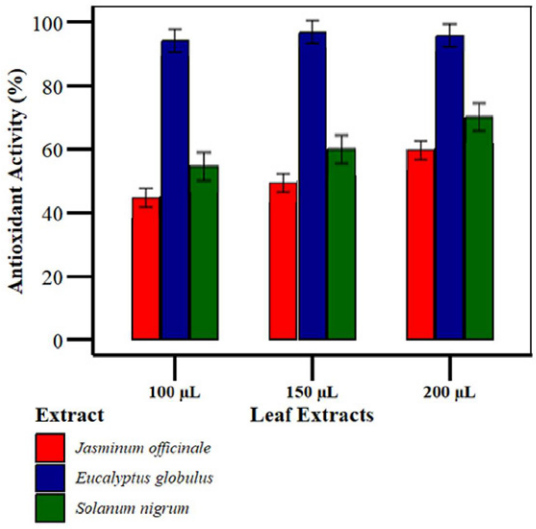

Fig. 5. Determination of the antioxidant activity of *J. offcinale*, *E. globulus* and *S. nigrum* leaves.

easier to use in the DPPH assay since they do not require organic solvents or evaporation stages, making them ideal for early antioxidant screening. An increase in radical scavenging activity shows a low absorbance value.[55] The antioxidant activity increased with the increase in concentration.[56] The results of this study are consistent with previous findings, which reported that the antioxidant activity was lower in leaves than in stems of *E. globulus*.[52] Another study found higher antioxidant activity in methanol extract of *E. globulus* leaves, when compared with its aqueous extract. For *J. officinale*, the leaves exhibited the highest antioxidant activity, as previously reported.[53] (Fig. 5). Comparative evaluation of solvent extracts is a promising subject for future research.

3. 4. FTIR Analysis

The aqueous extracts of *E. globulus*, *J. officinale,* and *S. nigrum* were used for FTIR analysis to determine dis-

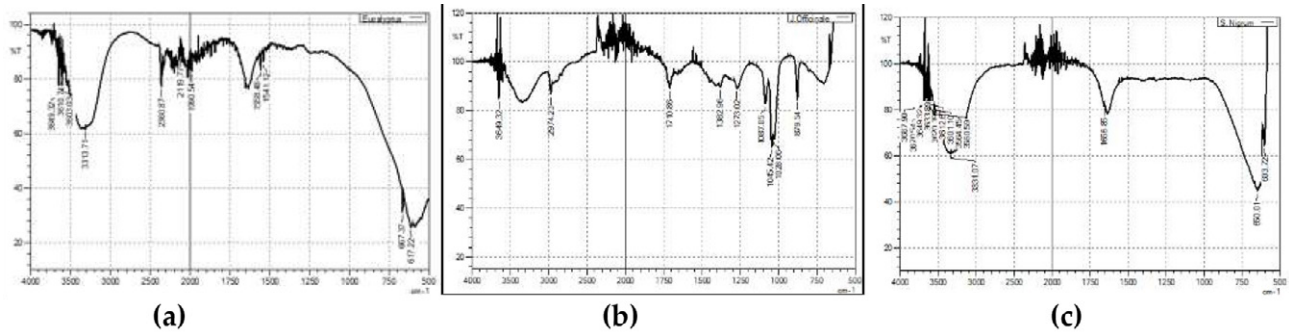

Fig. 6. FTIR spectrum of *E. globulus* leaves (a), *J. officinale* leaves (b), and *S. nigrum* leaves (c)

tinctive functional groups in the phytochemical profile (Fig. 6, Table 1). This analysis revealed functional groups such as C=C, COO^-, C=O, C–N, and N–H, recognized through distinctive absorption peaks. These groups are frequently found in classes of bioactive chemicals such as flavonoids, phenolics, and carboxylic acids. These functional groups are found in many beneficial substances, including quercetin, benzoic acid, salicylic acid, rutin, and ascorbic acid. While FTIR cannot determine the specific identity of these compounds, spectrum similarities indicate their possible presence, backed up by literature and bioinformatic analysis on known phytochemicals in these plants.[57] This guided the selection of quercetin, benzoic acid, salicylic acid, and ascorbic acid for molecular docking investigations. Thus, FTIR gave functional proof of their presence, but compound-level validation is outside the purview of this investigation.

Table 1. Functional groups detected by FTIR analysis found *Eucalyptus globulus*, *Jasminum officinale* and *Solanum nigrum*

Plants extracts	Frequency range	Functional group
Eucalyptus globulus	1541 cm^{-1}	Carboxyl group (COO^-) and
	637 cm^{-1}	C=C stretching C–H stretch and C≡C–H bend
Jasminum officinale	1273 cm^{-1}	C–N stretch
	1382 cm^{-1}	C–N stretch
	1636 cm^{-1}	C=O stretch
	3313 cm^{-1}	N-H functional group
Solanum nigrum	667 cm^{-1}	C–H stretch and C≡C–H bend
	1656 cm^{-1}	C=C stretching
	3313 cm^{-1}	N–H functional group

3. 5. Bioinformatic Analysis of Bioactive Compounds

Molecular docking studies were conducted against specific protein targets that correspond with the traditional or pharmacological use of the plants in order to offer a deeper understanding of the therapeutic relevance of the phytochemicals found. The antiviral properties of *E. globulus* are well known, particularly in relation to respiratory tract infections. Thus, the Influenza A virus's surface glycoproteins, haemagglutinin and neuraminidase, were selected to investigate possible antiviral interactions. On the other hand, *S. nigrum* and *J. officinale* have ethnomedical significance in neurological conditions like anxiety and insomnia. In order to study neuropharmacological interactions, GABA receptor-associated protein, a protein that regulates neurotransmitters in the central nervous system was chosen. In order to guarantee that the docking simulations represent therapeutic pathways that are biologically plausible, these choices were made.

Molecular docking was performed of the determined bioactive components against the two proteins haemagglutinin and neuraminidase in *E. globulus*, and GABA receptor associated protein in *J. officinale* and *S. nigrum* against.

Molecular docking studies were conducted to evaluate the interactions of quercetin and benzoic acid ligands with neuraminidase (PDB ID: 7U4F) and haemagglutinin (PDB ID: 4WE5). The analysis revealed various hydrophobic interactions and hydrogen bonds between the ligands and target proteins. The best docking models, which displayed hydrogen bonding patterns, hydrophobic contacts, ligand-binding sites, and overall protein–ligand interactions, were selected (Tables 2–3; Figs. 7–9). *In silico* screening was performed to identify potential new drug leads and elucidate possible mechanisms of action. Additional docking of identified bioactive compounds was carried out with haemagglutinin (4WE5), neuraminidase (7U4F), and the GABA receptor-associated protein (1KOT), revealing hydrogen bonding and hydrophobic interactions within the receptor binding pockets.[58] An increase in negative binding energy indicated greater stability of the protein–ligand complexes.[59]

The Influenza A virus is an infectious respiratory disease.[60] Hemagglutinin and neuraminidase are surface glycoproteins of the Influenza A virus[61] and functional targets for anti-influenza therapy.[62] FTIR spectra confirmed the presence of quercetin, methane and benzoic acid in *E. globulus*. These bioactive components were docked with 4WE5 and 7U4f. Methane did not bind with 4WE5 and 7U45, while quercetin and benzoic acid actively bind with

the proteins, as reported by.[63] Quercetin is a compound which belongs to the flavonoids class.[64] It possesses antimicrobial and anti-inflammatory properties.[65] Benzoic acid is a compound composed of a benzene ring along with carboxylic acid. It possesses antibacterial, antiviral and food preservation properties. Compounds of benzoic acid have anti-influenza virus properties.[66] Quercetin binds with neuraminidase via hydrogen bonding and hydrophobic interaction with Glu 413, Trp 87, Arg 85, Cys 124, Phe 410, Glu 229, Cys 280 and Ser 228 (Table 2), which are the amino acids that bind with the head of neuraminidase ranging from 91–469. Whereas benzoic acid did not show hydrogen bonding and hydrophobic interactions with Neuraminidase. Quercetin binds with haemagglutinin via hydrogen bonding with Thr 65 and Tyr 100 and hydrophobic interaction with Tyr 105, Arg 109 and Ile 67 (Table 3). Whereas, benzoic acid binds with haemagglutinin via hydrogen bonding with Arg 109 and hydrophobic interactions with Ile 67, Tyr 100, Tyr 105 and Val 102 (Table 3), which are the amino acids present in the topological domain extending from 17–530.[67] Neuraminidase cleaves terminal sialic acids and prevents the escape of the virus from host cells.[61] Haemagglutinin binds with sialic acids, attaches the virus on the cell surface and penetrates the virus into host cells.[68] Benzoic acid and quercetin prevent the virus from penetrating and escaping from its host cell.[69] The result showed that benzoic acid and quercetin bind with the active catalytic site of the domain and suppresses the neuraminidase and haemagglutinin activity.[70,71] Quercetin showed strong binding abilities with haemagglutinin and neuraminidase. As Quercetin bound with both surface proteins of the Influenza A virus, it could be a potent anti-influenza compound.

Interactions of ligands salicylic acid, gallic acid, ferulic acid and ascorbic acid with protein GABA receptor associated protein (PDB ID: 1KOT). Different hydrogen bonds and hydrophobic interactions were formed between the protein and ligands. The best model was chosen that shows the hydrogen bonding, hydrophobic interaction, ligand binding sites and protein ligand interaction (Table 4 and Fig. 10–13).

Table 2. Binding of Quercetin with Neuraminidase

Ligand	7U4F AA	Types of bonds	Length (A)	Ligand AA binding sites
Quercetin	Glu 413	HB	3.35	OE1-O7
	Trp 87	HI		
	Arg 85	HI		
	Cys 124	HI		
	Phe 410	HI		
	Glu 229	HI		
	Cys 280	HI		
	Ser 228	HI		

Table 3. Binding of quercetin and benzoic acid with haemagglutinin protein

Ligand	Haemagglutinin AA	Types of bonds	Length (A)	Ligand AA binding sites
Quercetin	Thr 65	HB	2.95	OG1–O6
	Tyr 100	HB	2.87	OH–O6
	Tyr 105	HI		
	Arg 109	HI		
	Ile 67	HI		
		HI		
		HI		
		HI		
Benzoic aicd	Arg 109	HB	3.15	NH2–O2
	Ile 67	HI		
	Tyr 100	HI		
	Tyr 105	HI		
	Val 102	HI		

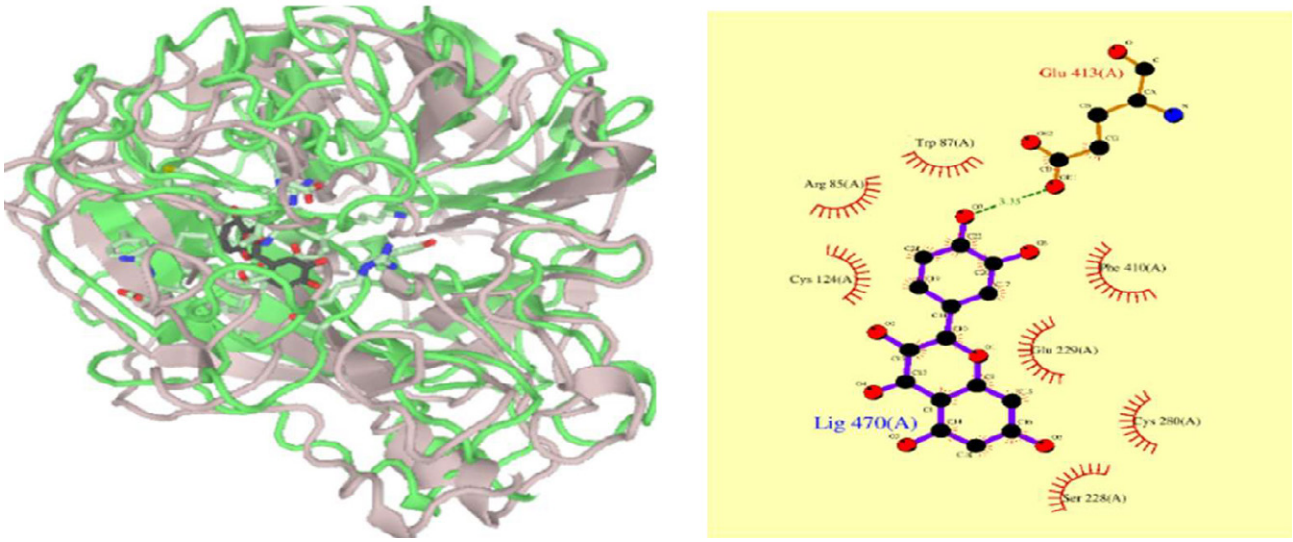

Fig. 7. Results of docking of quercetin ligand with neuraminidase

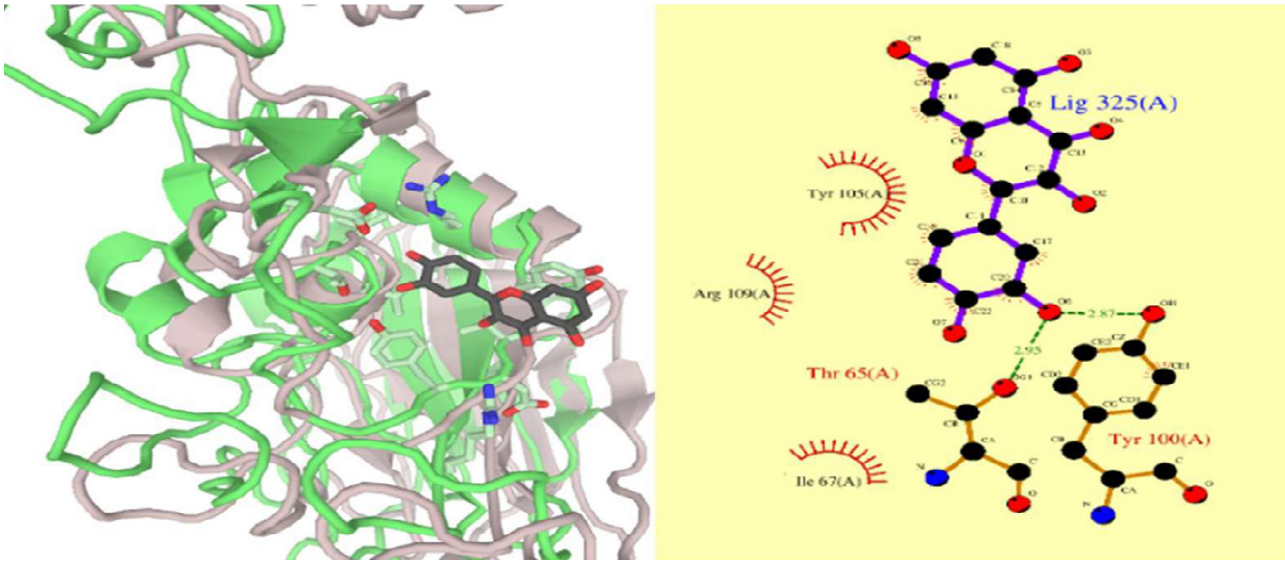

Fig. 8. Results of docking of quercetin ligand with haemagglutinin

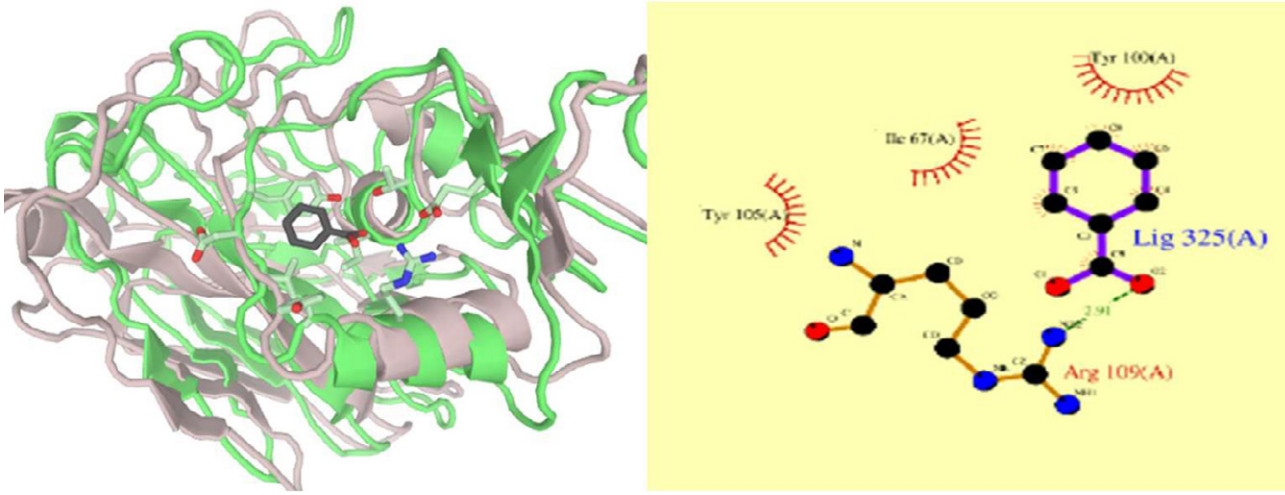

Fig. 9. Results of docking of benzoic acid with haemagglutinin

Table 4. Binding of different salicylic acid, ferulic acid, gallic acid and sscorbic acid with GABA receptor associated protein

Ligand	GABA receptor associated protein (AA)	Types of bonds	Length (A)	Ligand AA binding sites
Salicylic acid	Asn 84	HB	3.11	ND2–O1
	Val 85	HI		
	Ile 86	HI		
Ferulic acid	Lys 40	HB	2.86	NZ–O1
	Ser 112	HB	2.84	
	Glu 114	HI		
	Val 116	HI		
	Gly 118	HI		OG–O3
Gallic acid	Asp 113	HB	2.86	OD2–O1
	Lys 40	HI		
	Glu 114	HI		
	Val 116	HI		
Ascorbic acid	Lys 238	HB	3.05	NZ–O3
	Ile 236	HI		
	Ile 260	HI		
	Leu 111	HI		
	Leu 177	HI		
	Ser 115	HI		

GABA receptor-associated proteins are neurotransmitters that play a role in the regulation of the sleep cycle.[72] A new method of pharmacologically influencing receptor activation and neurotransmitter action at the synaptic junction is the modification of GABA receptor-associated protein binding to its interacting partners.[73] FTIR spectra of *J. officinale* confirmed the presence of salicylic acid, gallic acid, ferulic acid, epicatechin and rutin.

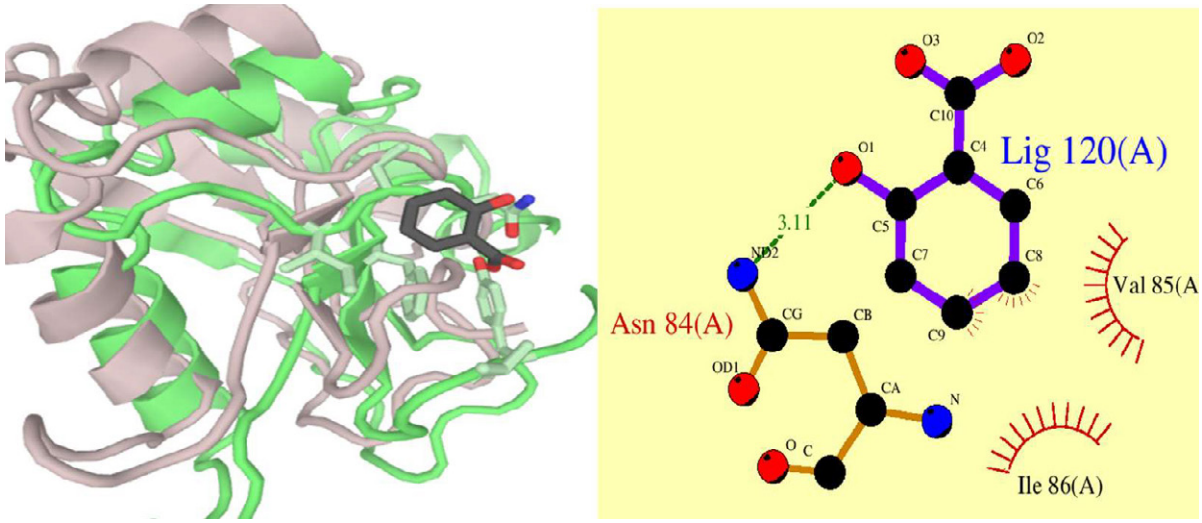

Fig. 10. Results of docking of salicylic acid ligand with GABA receptor associated protein

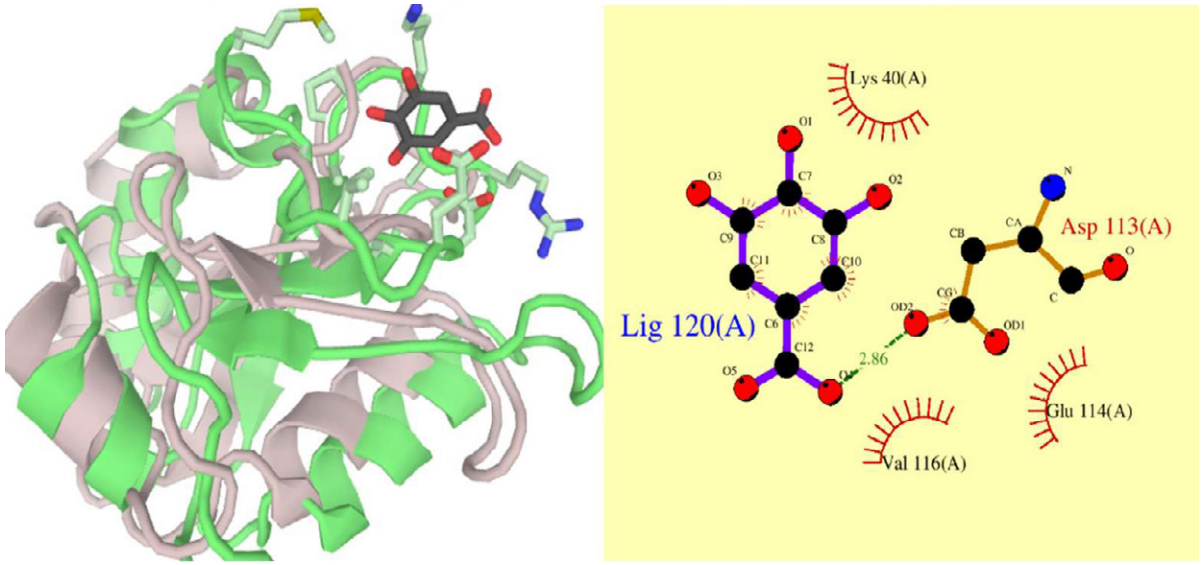

Fig. 11. Results of docking of gallic acid ligand with GABA receptor associated protein

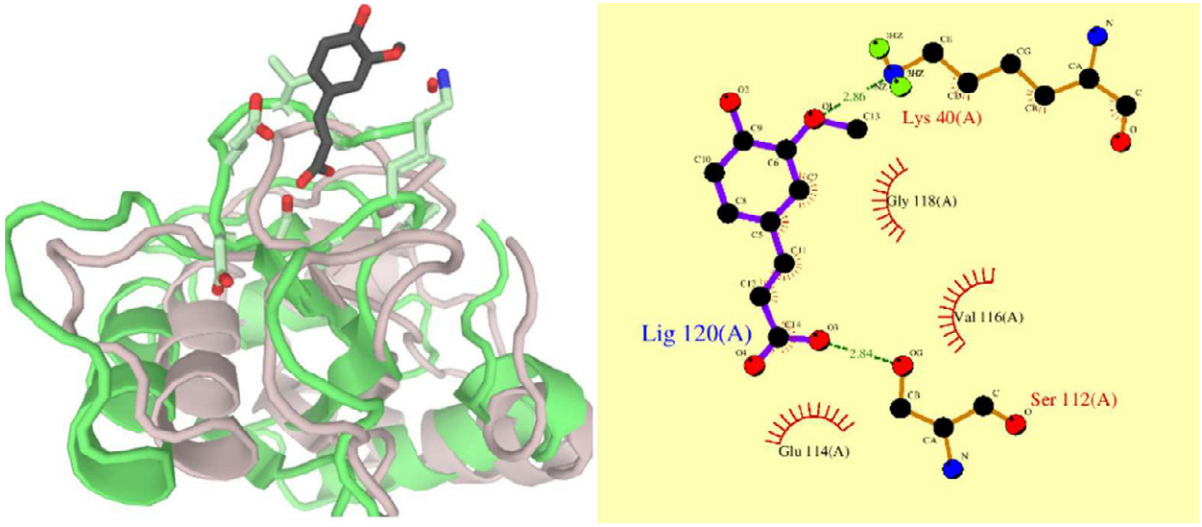

Fig. 12. Results of docking of ferulic acid ligand with GABA receptor associated protein

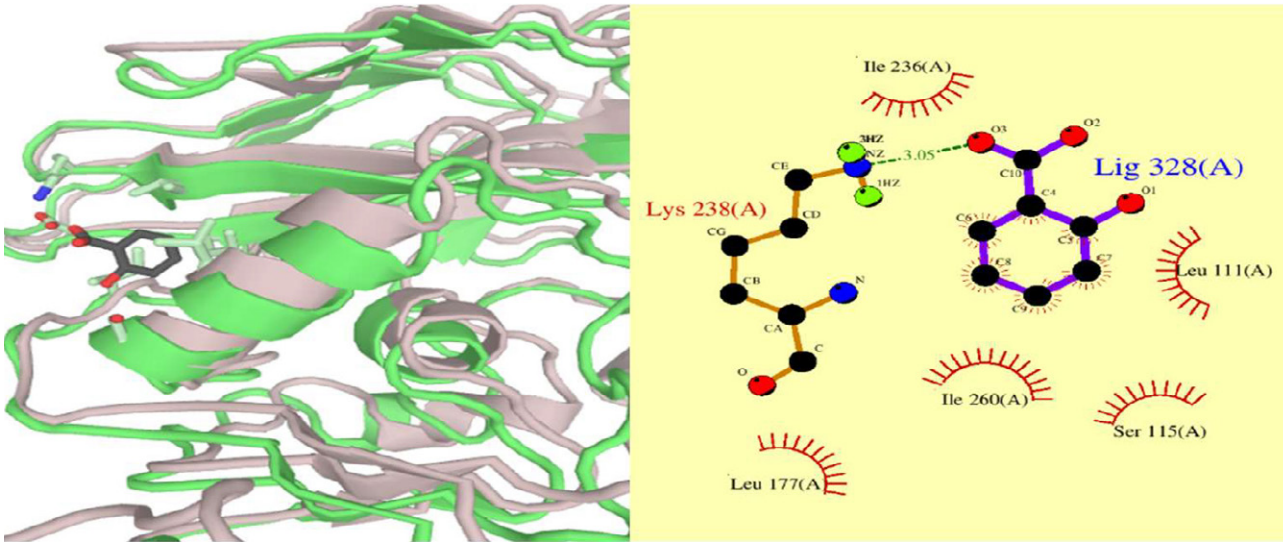

Fig. 13. Results of docking of ascorbic acid ligand with GABA receptor associated protein

Spectrum of *S. nigrum* supported the existence of ascorbic acid, rutin, *p*-cymene and 3,4-dihydroxybenzoic acid. These bioactive components were docked with GABA receptor-associated protein. Epicatechin, rutin, *p*-cymene, and 3,4-dihydroxybenzoic acid did not bind with the GABA receptor-associated protein. While ferulic acid, salicylic acid, gallic acid and ascorbic acid bind via hydrogen bonding and hydrophobic interactions. Ferulic acid and salicylic acid are compounds belonging to the phenol family. Gallic acid belongs to the hydrolysable tannins family. Ascorbic acid, also known as vitamin C, is a potent antioxidant agent. They possess anti-bacterial, anti-inflammatory and hypnotic properties. Studies have shown that a low intake of vitamin C can lead to sleep disorders. One may be able to prolong sleep and lessen sleep disturbances by increasing the consumption of this antioxidant.[74] Salicylic acid binds via hydrogen bonding with Asn 84 and hydrophobic interaction with Val 85 and Ile 86 with GABA receptor associated protein (Table 4), which the amino acids that bind with gephyrin *E* domain are ranging from 36–117. GABA receptor-associated protein and gephyrin are dependent on each other, as if one of the proteins is down-regulated, the other one is also down-regulated.[75] Gallic acid binds via hydrogen bonding with Asp 113 and hydrophobic interaction with Lys 40, Glu 114 and Val 116 with GABA receptor associated protein (Table 4), which are the amino acids that bind with gephyrin *E* domain ranging from 36–117. Ferulic acid binds with protein via hydrogen bonding (Lys 40, Ser 112) and hydrophobic interaction (Glu 114, Val 116 and Gly 118) (Table 4), which are the amino acids that bind with *C*-terminal domain of GABA receptor gamma 2 domain and gephyrin *E* domain ranging from 36–68 and 36–117, respectively. Ascorbic acid binds via hydrogen bonding with Lys 238, and hydrophobic interaction with Ile 236, Ile260, Leu 111, Leu 177 and Ser 115 (Table 4), which are the amino acids that bind with *N*-terminal domain of tubulin, *C*-terminal domain of GABA receptor gamma 2 and gephyrin *E* domain ranging from 1–22, 36–68 and 36–117, respectively. The result showed that salicylic, gallic, ferulic and ascorbic acid bind with the agonist binding site of domains and will stimulate the release of GABA receptors and inhibit the catabolism of GABA receptor associated protein. Ferulic acid showed more hydrogen bonds as compared to gallic acid and salicylic acid. Ascorbic acid also formed more than one hydrogen bond. These bioactive components bind with neurotransmitter receptors and strengthen the GABAergic system, which increases the efficacy of hypnotic activity.[76] Models that show good binding energy are suggested to interact with the enzyme active sites, indicating better stability with the GABA receptor-associated protein. These bioactive compounds showed good affinity by forming hydrogen bonds and hydrophobic interactions, and they could be a good alternative to medicines to treat influenza and regulate the sleep cycle.

4. Conclusions

In this work, biological activities of medicinal plants were assessed, which demonstrates the potential use of isolated components from plants as alternative therapies or as models for the synthesis of novel compounds. Furthermore, it was concluded that these plant extracts possess an appreciable content of phytochemicals as well as good antioxidant and antibacterial potential. The butanol extract of these plants showed the highest antibacterial potential. The findings show that the bioactive components found in these plants may have the potential to treat influenza-related symptoms and sleep disruptions, indicating that they are worth further exploration as complementary therapies. The combined *in silico* and *in vitro* approach

brings up new possibilities for developing novel therapies to treat various illnesses. This study can be a guideline for researchers in the field of pharmacology and pharma industries to develop novel therapeutic agents.

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Ethical statements

All the research was approved by the ethical committee of Lahore College for Women University and no human or animal was involved in this research.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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Povzetek

Rastlinam in njihovim ekstraktom *Eucalyptus globu*lus (*E. globulus*), *Jasminum officinale* (*J. officinal*e) in *Solanum nigrum* (*S. nigrum*) so določilie njihovo protibakterijsko, antioksidativno aktivnost in terapevtske lastnosti. Za fitokemijsko presejanje in protibakterijsko aktivnost so bila uporabljena ekstrakcijska topila (voda, metanol, etanol in butanol), medtem ko so bili za analizo antioksidativne aktivnosti uporabljeni izključno vodni izvlečki. Kvantitativna določitev je pokazala, da je imel *J. officinale* najvišjo vsebnost fenolov in taninov, medtem ko je imel *E. globulus* najvišje ravni flavonoidov in alkaloidov med preizkušenimi vrstami. Za oceno protibakterijske aktivnosti proti *Escherichia coli* (*E. coli*) in *Staphylococcus aureus* (*S. aureus*) je bila uporabljena metoda difuzije po disku. Vsi izvlečki listov *E. globulus* so pokazali protibakterijsko delovanje proti *E. coli* in *S. aureus*. FTIR analiza vodnih izvlečkov je pokazala prisotnost kvercetina, benzojske, salicilne, galne, ferulne in askorbinske kisline. Poleg tega je bila izvedena *in silico* analiza za oceno interakcije izbranih bioaktivnih spojin: kvercetina in benzojske kisline iz *E. globulus* sta bila sidrana v hemaglutinin in nevraminidazo, saj imata ti površinski beljakovini virusa influence ključno vlogo pri zmožnosti virusa, da okuži gostiteljske celice. Salicilna, galna, ferulna in askorbinska kislina iz *J. officinale* in S. nigrum so bile sidrane na proteine, povezane z GABA receptorji, ki so pomembni pri sinaptičnem prenosu in plastičnosti.