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Research Article

CHEMICAL PROFILING OF *BUCHANANIA LANZAN* SPRENG ESSENTIAL OIL AND ITS BIOLOGICAL ACTIVITIES

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ABSTRACT

Objective: The present study was designed to evaluate the chemical composition of the essential oil of *Buchanania lanzan* Spreng extracted from the seeds and to evaluate *in vitro* antimicrobial antioxidants and molecular docking studies of the major bioactive compounds of essential oil.

Methods: The essential oil was obtained by hydrodistillation of the *B. lanzan* seeds and analyzed by gas chromatography-mass spectrometry (GC-MS). Antibacterial activity was evaluated against *Pseudomonas aeruginosa, Salmonella typhi*, *Vibrio cholerae*, *Staphylococcus aureus,* and *Streptococcus pneumoniae* clinical isolates by disk diffusion method and resazurin assay determined the minimum inhibitory concentration. The *in vitro* antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide (H_2O_2) scavenging assay; the essential oil major bioactive compounds are Androstan-3-ol, Campesterol, and *γ-Sitosterol* were docked against bacterial protein DNA gyrase.

Results: GC-MS analysis exhibited the presence of 19 bioactive compounds. The essential oil showed that significant antibacterial activity was noticed against *V. cholerae* and *S. typhi* with the highest zone of inhibition 15.67–1.20 and 13.83–0.33, respectively. Antioxidant activity in DPPH and H_2O_2 scavenging assays with IC_{50} values of 134.23 and 191.24, respectively. The molecular docking of Androstan-3-ol and *γ-Sitosterol* with bacterial DNA gyrase unveiled a good binding affinity of −6.4 kcal/mol and −6.3 kcal/ mol, respectively.

Conclusion: It could be concluded that the essential oils potential sources of antibacterial, antioxidant activities, and molecular docking of bioactive components. The results of this study provide partial scientific support for the traditional application of essential oils to cure diarrhea and also major bioactive compounds responsible for important biological activities.

Keywords: *Buchanania lanzan*, Gas chromatography-mass spectrometry, Antimicrobial, Antioxidant, Molecular docking.

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INTRODUCTION

Buchanania lanzan Spreng, a member Anacardiaceae family, very sparsely distributed in hot and dry deciduous forests of India [1]. Seed kernel is edible, nutritious, and vernacularly known as Chironji in Hindi. The tree can be easily identified by its dark grey crocodile bark with a red blaze. It is distributed in tropical deciduous forests of the Central Western Ghats of Karnataka and it is one of the endangered plants in Western Ghats of India listed in red data book [2], and Southern Eastern Ghats of Tamil Nadu and the hill ranges of Central India, mostly in the States of Chhattisgarh, Jharkhand, Madhya Pradesh, and Uttar Pradesh trees are found Besides India, other tropical Asian countries, Australia and the Pacific islands trees are distributed [3].

Herbal medicine is considered an important and a potential source of bioactive compounds to treat contagious diseases and many medicinal herbs screed for their antioxidant, antimicrobial, anticancer, anti-inflammatory, and antidiabetic activities which makes them useful for the production and development of new chemotherapeutic drugs. In folk medicine, *B. lanzan* fruits are used as a therapy for inflammatory diseases, including diabetes mellitus and arthritis [4]. Several studies have revealed that the crude extract of leaves *B.* lanzan possesses immunostimulant antioxidant, antidiarrheal, antispasmodic, antimicrobial, anti-inflammatory, and hepatoprotective properties. The roots of the plant have been reported to possess astringent properties and are also used in the management of diarrhea [5,6].

In Karnataka, India, the Western Ghats region mainly uses it to control cholera and infective diarrhea aqueous extract of seed. Leaf paste is also used for the management of wounds and also as a digestive, expectorant, and purgative [7]. The Chironji kernels contain about 52% oil. Which oil is used as a substitute for olive and almond oils, it has great medicinal value, especially the kernels, which are used as an expectorant and tonic. The oil extracted from kernels is used for treating skin diseases [8]. Investigation also indicated the usage of the seed as a cardiotonic, astringent drug [9].

To the best of our knowledge, there has been no published detailed information about the chemical composition and biological activities of the essential oil of *B. lanzan.* Western Ghats of India, therefore, the objectives of the present study were to investigate the composition of the essential oil obtained from the *B. lanzan* seeds oil as well as evaluate its chemical constituents *in vitro* antibacterial, antioxidant activities, and molecular docking studies.

MATERIALS AND METHODS

Plant material collection and extraction

Seeds of *B. lanzan* Spreng were collected in April from Bhadra reserve forest of Western Ghats, Karnataka, India. The botanical sample was identified by Prof V Krishna and a voucher specimen (KUBPHS128) is deposited in the Biotechnology research lab at Kuvempu University. The 1000 g seeds were primarily washed with tap water and subsequently with distilled water. Seeds were shade dried used for further work.

Isolation of essential oil

The essential oils were hydro-distilled by Clevenger apparatus according to the method described by Khalid *et al.* [10]. Briefly, 1000 g of dry seeds subjected to hydrodistillation in a Clevenger-type apparatus for an average duration of 3 h. The distillation was repeated to yield the maximum volume of oil. The extracted essential oil was dehydrated by

anhydrous sodium sulfate and stored in a small opaque flask at room temperature for further experiments.

Gas chromatography-mass spectrometry (GC-MS) analysis

The essential oil of the seed was subjected to GC-MS analysis using Shimadzu GCMS-QP2010S instrument with GC-MS software and the compounds were separated using Rtx -5, capillary column (0.25 mm, 0.25 μm), a split ratio of 1:25, injector temperature was 300°C, the column temperature was maintained at 60°C, followed by 10 min at 300°C. About 1 μl of the sample was injected into the column by the split mode. GC-MS was performed by an electron ionization system, with ionization of 70 eV. Helium gas (99.999%) was used as carrier gas at a flow rate of 1 ml/min, a scan interval of 0.5 s and fragments from 45 to 450 Da. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, Interpretation of GC-MS chromatogram was conducted using the two databases of NIST05 and WILY7 mass spectral libraries which provide the best information about the identification of components [11,12].

Antibacterial activity

Bacterial strains

The clinical isolates of Gram-negative bacterial strains *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Vibrio cholerae.* Moreover, Gram-positive bacterial strains – *Staphylococcus aureus* and *Streptococcus pneumonia* – were obtained from the Institute of Medical Sciences College, Shivamogga, Karnataka.

Agar well diffusion method

Determination of the antibacterial activity of essential oil was evaluated by agar disk diffusion method [13]. The oil dissolved in 10% dimethyl sulfoxide (DMSO) and tested individually against a panel of clinically pathogenic microbial strains selected. A loop full of bacterial strain was subcultured in a conical flask and incubated at 37°C for 24 h in nutrient broth media day before experimenting. One hundred microliters of bacterial culture (105 cells/ml) were inoculated on nutrient agar culture plates to determine their viability. By making wells of 6 mm diameter for loading the seed oil in various concentrations (20, 40, and 80 mg) and standard drug ciprofloxacin 20 μl into respected wells and were kept for incubation at 37°C. After 24 h, a zone of inhibition was recorded

Determination of minimum inhibitory concentration (MIC)

The MIC of the essential oil was evaluated by modified resazurin (7-Hydroxy- 3H-phenoxazin-3-one 10-oxide) microtiter plate assay described by Palomino *et al*. [14]. Fifty microliters of a test sample containing 50 μg of oil (1 mg/ml [w/v]) solutions in 10% (DMSO, v/v) and 50 μg of standard antibiotic (1 mg/ml [w/v]) solutions in 10% DMSO. Fifty microliters of nutrient broth were added to all wells (microtiter plate). Two-fold serial dilutions were performed using a pipette such that each well had 50 μl of the test material in serially descending concentrations. Thirty microliters of 3.3 times stronger high sensitivity broth and 10 μl of resazurin indicator solution (prepared by dissolving 27 mg resazurin in 4 ml of sterile distilled water) were added to each well. Finally, 10 μl of bacterial suspension was added to the appropriate wells to achieve a concentration of approx. 5 × 106 CFU/ml.

***In vitro* antioxidant activity**

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Different concentrations (100, 200, and 300 μl) of essential oil were added to a tube containing 3 ml of an ethanolic solution of DPPH (0.004%). The mixture obtained was incubated in the dark for 30 min and the absorbance read at 517 nm using a spectrophotometer. Ascorbic acid (0.005 M) was taken as the standard antioxidant. The DPPH inhibition percentage was calculated according to the following equation: % inhibition = $[(A_{blank} \times A_{sample})/A_{blank}] \times 100$. Where A_{blank} is the absorbance of the negative control and A_{sample} the absorbance of the essential oil. Antioxidant activity of the *B. lanzan* seed essential oil was expressed as an inhibitory concentration (IC_{50}), which is the amount of essential oil required to cause a 50% decrease in initial DPPH concentration. Linear regression was used to calculate the IC_{50}. All tests were performed in triplicate.

Hydrogen peroxide (H_2O_2) scavenging assay

H_2O_2 scavenging activity of the oil was determined by the method of Ruch *et al*. [15]. Different concentrations of the essential oil (100, 200, and 300 μg) were mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm. Ascorbic acid was used as a standard compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using the following equation: Percentage of inhibition = $[(A_{control}-A_{test})/A_{control}] \times 100$, where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance of the extract reaction. IC_{50} value was calculated using the formula $IC_{50} = [(\Sigma C/\Sigma I) \times 50]$, where ΣC is the sum of extracts concentrations used to test and ΣI is the sum of the percentage of inhibition at different concentrations.

***In silico* molecular docking studies**

Lipinski "Rule of five" is commonly used as a filter for drug-like properties [16]. The *in silico* pharmacokinetic properties and absorption, distribution, metabolism, and elimination and toxicity analysis were predicted using DataWarrior. DataWarrior tries to assess the toxicity risk by finding substructures within the chemical structure being indicative of a toxicity risk within one of said four major toxicity classes.

The chemical structure of GC-MS identified compounds; namely, Campesterol, Gamma.-Sitosterol, and Androstan-3-ol, 9-methyl-, acetate, (3.beta., 5.alpha.) and the standard drug ciprofloxacin were drawn using Chem Bio Draw tool (Chem Bio Office Ultra 14.0 suite) assigned with proper 2D orientation, and structure of each was checked for structural drawing error. The energy of each molecule was minimized using ChemBio3D. The energy minimized ligand molecules were then used as input for AutoDock Vina to carry out the molecular docking. The protein data bank (PDB) coordinates file with the name "2XCT.pdb" was used as a receptor molecule [17]. All the water molecules and the heteroatoms were removed from the receptor. The graphical user interface program MGL tool was used to set the grid box for molecular docking. The grid was set so that it surrounds the region of interest in the macromolecule. The grid box volume was set to 8, 14, and 14 Å for x, y, and z dimensions, respectively, and the grid center was set to 3.194, 43.143, and 69.977 for x, y, and z center, respectively, which covered the amino acid residues in the considered active pocket. The docking algorithm provided with AutoDock Vina was used to search for the best-docked conformation between ligand and protein. During the docking process, a maximum of ten conformers was considered for each ligand. Molecular docking was performed on the Corei5 Intel processor CPU with 6 GB DDR3 RAM. AutoDock Vina was compiled and run on a Windows 8.0 professional operating system [18]. LigPlot+ and PyMol educational version was used to deduce the 2D and 3D interaction between the ligands and the receptor [19]. The ligands are represented in green color, H-bonds with their respective distances are represented by red, and the interacting residues are represented in ball and stick model representation.

Statistical analysis

Statistical analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software and Microsoft excels to determine the mean and standard deviation of the zone of inhibition values.

RESULTS AND DISCUSSION

GC-MS analysis

The hydrodistillation of the seeds of *B. lanzan* (1000 g) provided an essential oil in a yield of 10% (v/w relative to air-dried material weight). The components of the essential oil were determined by GC-MS analysis. A total of 19 peaks were identified (Table 1 and Fig. 1)

GC-MS plays a key role in the analysis of unknown components of plant origin. In general, the plant materials are highly complex, which makes GC-MS well suited for their analysis because of its high sensitivity. GC-MS ionizes

Table 1: The chemical composition of *B. lanzan* seeds essential oil

S. No.	R. Time	I-Time	F-Time	Area	Area%	Name
1.	10.182	10.092	10.242	52339	0.02	Cycloisosativene
2.	10.368	10.300	10.467	76830	0.02	Tetradecane
3.	14.647	14.558	14.842	244624	0.08	Tetradecanoic acid
4.	16.351	16.258	16.433	147645	0.05	Hexadecanoic acid, methyl ester
5.	16.748	16.625	16.433	57163443	12.83	l-(+)-Ascorbic acid 2,6-dihexadecanoate
6.	17.011	16.950	17.617	17951782	5.68	Hexadecanoic acid, ethyl ester
7.	18.454	18.292	18.817	87702747	27.73	Oleic acid
8.	18.620	18.567	19.342	40570894	18.07	9, 12-Octadecadienoic acid (Z.Z)
9.	18.855	18.817	19.983	14605881	4.62	Octadecanoic acid, ethyl ester
10.	19.784	19.700	21.000	2887588	0.91	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester
11.	20.875	20.767	21.333	608215	0.19	Oleoyl chloride
12.	21.267	21.183	21.558	5913865	1.87	9-Octadecenoic acid, 1,2,3-propanetriyl ester,
13.	22.837	22.758	22.850	3314596	1.05	Bi-1-cycloocten-1-yl
14.	22.906	22.850	22.942	15161637	4.79	Azulene, 1,2,3,
15.	22.994	22.942	23.133	24662290	7.80	Phenol, 3-pentadecyl-
16.	24.719	24.642	24.633	8631712	2.73	Androstan-3-ol, 9-methyl-, acetate, (3.beta., 5.alpha.)-
17.	27.798	27.672	28.375	659825	0.21	Campesterol
18.	28.167	28.025	29.108	845532	0.27	Stigmasterol
19.	28.925	28.817	31.150	3689705	1.17	Gamma.-Sitosterol

*RI: Relative retention indices to C8-C29 n-alkanes on Rtx-5 capillary column, *compounds listed in order of elution from Rtx-5 capillary column. *B. lanzan: Buchanania lanzan*

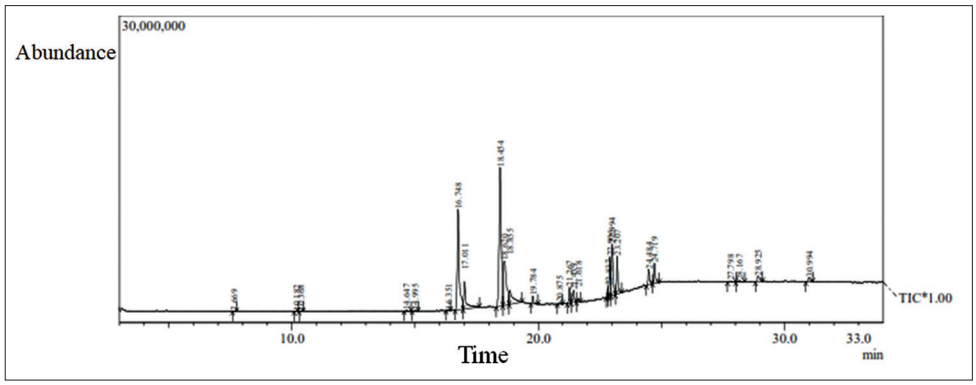

Fig. 1: Gas chromatography-mass spectrometry chromatogram of *Buchanania lanzan* seeds essential oil

compounds and measures their mass numbers. It provides additional information on the structure of these profiles. The overall evaluation of the compounds present in the seed oil was analyzed using GC-MS. A total of 19 compounds were identified in the extract. The results revealed the presence of l-Ascorbic acid, 2, 6-dihexadecanoate, 9,12-octadecadienoic, octadecanoic acid, ethyl ester, oleic acid, hexadecanoic acid, and ethyl ester acid (Z, Z)- as the major compound (70.13%). Among the identified phytochemicals, N-hexadecanoic acid was reported to show antioxidant and antimicrobial activities 9, 12-Octadecadienoic acid (Z, Z)- has the property of antimicrobial, anticancer, anti-inflammatory, antifungal, antioxidant, and immunomodulatory properties [20,21]. Oleic acid has the property of antimicrobial, hypercholesterolemia, dermatogenic, anti-inflammatory, and antitumor activities. β-sitosterol is used for heart disease and high cholesterol. Oleic is also used for boosting the immune system and for preventing colon cancer, as well as for gallstones, the common cold, and flu [22,23].

Antibacterial activity

Antimicrobial resistance is very common to continue to develop drug resistance by employing various mechanisms to survive in the lethal environment created by antimicrobials [24]. Attention has been given more toward the extracts and biologically active compounds isolated from plants. Hence, much attention has been given to these natural products as new therapeutic agents. The essential seed oil exhibited a varying level of antibacterial activity against the tested pathogenic microbial strains. The results of the disk diffusion assay followed by modified resazurin assay indicated that the seed oil exhibited the highest inhibitory activity against Gram-negative bacteria *V. cholerae* with a significant inhibition zone of 15.67±1.20 mm as compared to the standard antibiotic drug ciprofloxacin. While a moderate zone of inhibition was observed against *S. aureus* (13.17±0.33) and *S. typhi* (13.83±0.88), and data summarized in Table 2. The final concentration was measured based on the percentage of inhibition of bacterial strains and the data are summarized in Table 3.

The fact that the seed oil was active against the organisms tested (Gram-negative and Gram-positive) may suggest a broad spectrum of activity. There is a developing interest in relating the phytochemical constituents of a medicinal plant with its pharmacological activity. Phytochemicals are non-nutritive plant chemicals that may have defensive or disease preventive antimicrobial activities. Because of their structural differences from those of the more studied antimicrobial reasons, their mode of action may too differ [25,26]. It is still not clear by which mechanism the fatty acids acting as antimicrobial agents. However, the main objective is the cell membrane of bacteria and other mechanisms may involve the membrane. Checking growth effect is related to an amphiphilic property of fatty acids permitting them to interact with cell membranes generating temporary or permanent pores of various sizes. With the high concentration, a detergent such as free fatty acids being able to dissolve cell membranes and hence releasing or disrupting a larger portion. Free fatty acids also stimulus energy production in the cell membrane by disturbing the electron transport chain and oxidative phosphorylation [27,28].

The MIC essential oil of *B. lanzan* against the microorganism tested is shown in Table 3. The MIC is also helpful in ascertaining the level of resistance of a particular bacterial strain and thus serves as a pointer

to the use of certain antimicrobial agents. The essential oil shows significant inhibitory activities against the two multidrug-resistant reference strains of *V. cholerae* and *S. aureus* was 66.821% and 52.790% at 80 mg/ml showed more potent activities than other strains of bacteria. Further, establish the findings obtained in our previous study [29] of *B. lanzan* oil were more effective against Gram-negative bacteria. The high fatty acid content, as shown in Table 3, excellent antimicrobial activity recorded in this work because previous documentation by these researchers has attested to the fact that high fatty acid components might be responsible for the antibacterial, anti-inflammatory, and antiviral potentials. In addition to this, the disparities are seen in the antimicrobial properties with some bioactive compounds such as alkaloids, tannins, terpenoids, ether, and phenolic compounds like flavonoids, which are considered to be bacteriostatic and bactericidal as reported in our previous study. Findings from this study point out that the antibacterial components that justify the traditional and medicinal usage of the plant to guard against infections caused by both Gram-positive and Gram-negative bacteria.

***In vitro* antioxidant assay**

The effect of antioxidants on DPPH is due to their hydrogen-donating ability. DPPH radical scavenging activity of the seed oil was compared with ascorbic acid. It was observed that seed essential oil had shown efficient activity. In DPPH radical scavenging activity in a dose-dependent manner in the range of 67.83±0.41% to 81.05±0.53%. The IC_{50} value for oil and standard was 134.23 and 109.46 µg. In H_2O_2 scavenging assay dose-dependent range is 47.87±0.07 and 56.91±0.51%. Moreover, IC_{50} values of oil and standard are 191.24 and 134.36 µg, respectively. It was evident that the oil shows proton donating ability and this could serve as free radical inhibitors or scavengers, acting possibly as primary oxidants. The result is shown in Table 4.

DPPH radical scavenging is considered to be a good *in vitro* model widely used to assess the antioxidant efficacy of single compounds as well as for different plant extracts within a very short period [30]. The essential oil compounds of *B. lanzan* activity were increased by increasing the concentration of the oil. It showed significant antioxidant activity with the IC_{50} value of 134.23 µg/ml. DPPH radicals have stable nitrogen that can accept a hydrogen atom or electron from the scavenger molecule, that is, antioxidant, results in the reduction of unpaired valence electron at one atom of nitrogen bridge in DPPH leading to the change of purple color to yellow with a decrease in absorbance at 517 nm. The color change indicates the scavenging potential of the essential oil.

The IC_{50} value for H_2O_2 scavenging assay shows 191.24 µg/ml good results because phenolic compounds present in the oil are good electron donors, they may accelerate the conversion of $H_2O_2 \rightarrow H_2O$. ROS, including free radicals such as superoxide anion radicals, hydroxyl radicals, and non-free radicals such as H_2O_2 and singlet oxygen along with various forms of active oxygen, is involved in various physicochemical processes in the body and aging [31]. Many reports indicated that hexadecanoic acid and oleic acid active antimicrobial, cytotoxic, and antidiarrheal agent activities of different medicinal plants [32,33]. Thus, the observed antibacterial and antioxidant effects of seed oil are due to the presence of terpenoid compounds.

Molecular docking study

In association with *in vitro* antimicrobial activity, it is useful to carry out *in silico* studies to predict the orientation and binding affinity at the active site of the receptor. The molecular docking of GC-MS identified ligand molecules – Campesterol, Gamma.-Sitosterol, and Androstan-3-ol, 9-methyl-, acetate, (3.beta. 5. alpha.) – With bacterial enzyme DNA

Table 2: Antibacterial activity of essential oil of *B. lanzan* seeds

		Zone of inhibition (mm)				
Concentration in (µl)		***P. aeruginosa***	***S. typhi***	***S. pneumoniae***	***V. cholerae***	***S. aureus***
BSO	20	7.47±0.81	7.63±0.51	8.20±0.36	8.87±0.78	7.70±0.66
	40	9.17±0.31	10.77±0.55	9.80±0.61	10.83±0.60	10.17±0.31
	80	12.17±0.33	13.83±0.88	12.33±0.44	15.67±1.20	13.17±0.33
Ciprofloxacin	20	20.97±0.50	20.90±0.10	21.43±0.70	21.20±1.13	20.33±0.38

BSO: *Buchanania lanzan* seed oil, Values are mean±standard error (n=3) of three different samples, ZI the diameter of inhibition zone (mm), *P. aeruginosa: Pseudomonas aeruginosa, S. typhi: Salmonella typhi, S. pneumonia: Streptococcus pneumonia, V. cholerae: Vibrio cholerae, S. aureus: Staphylococcus aureus. B. lanzan: Buchanania lanzan, S. pneumonia: Streptococcus pneumoniae*

Table 3: MIC values of essential oil of *B. lanzan* seeds

		MIC				
Concentration in (µl)		***P. aeruginosa***	***S. typhi***	***S. pneumoniae***	***V. cholerae***	***S. aureus***
BSO	10	27.395	22.665	37.281	42.368	27.395
	15	33.24	41.277	42.735	56.417	37.24
	20	42.790	48.039	46.377	66.821	52.790

BSO: *Buchanania lanzan* seed oil, % of Inhibition = OD of Control- OD of Test/OD of Test. MIC: Minimum inhibitory concentration (mg/ml). *P. aeruginosa: Pseudomonas aeruginosa, S. typhi: Salmonella typhi, S. pneumonia: Streptococcus pneumonia, V. cholerae: Vibrio cholerae, S. aureus: Staphylococcus aureus. B. lanzan: Buchanania lanzan, S. pneumonia: Streptococcus pneumoniae*

Table 4: DPPH and H_2O_2 scavenging assay

S. No.	Activity	Concentration of BSO in µg/ml	% of Inhibition	IC_{50} µg/ml	Standard IC_{50} in µg/ml
					Ascorbic acid
1.	DPPH assay	100	67.83±0.41	134.23	109.46
		200	74.61±0.49		
		300	81.05±0.53		
2.	H_2O_2 scavenging assay	100	47.87±0.07	191.24	134.36
		200	52.09±0.35		
		300	56.91±0.51		

All values were expressed in mean±SEM (n=3), BSO: *Buchanania lanzan* seed oil, DPPH: 2,2-diphenyl-1-picrylhydrazyl, H_2O_2: Hydrogen peroxide scavenging assay SEM: Standard error of the mean, IC_{50}: Inhibitory concentration. *B. lanzan: Buchanania lanzan*, H_2O_2: Hydrogen peroxide

gyrase are shown in Fig. 2. Among them, the compound Androstan-3-ol exhibited better docking efficiency with DNA gyrase. It forms two hydrogen bonds with amino acids Asp437 and Gly459 in the active site of the target protein with bond length 2.81 and 3.01, respectively, with the least binding affinity −6.4 kcal/mol and hence is considered as the best dock conformation (Table 5). Compound Gamma.-Sitosterol forms one hydrogen bond with Asp437 amino acids with bond length 2.70, and campesterol forms only one hydrogen bond with the amino acid Asp510 with bond length 3.18 Å. However, all these docked molecules exhibited more hydrophobic interaction than the standard drug ciprofloxacin. The root-mean-square deviation (RMSD) has often been used to measure the quality of reproduction of a known binding pose by molecules with ligands. All docked molecules have zero RMSD values, as shown in Table 6.

The *in silico* docking of seed oil compound, Androstan-3-ol, with the bacterial protein DNA gyrase showed a higher binding affinity as well as hydrogen bonding and good hydrophobic interaction with the receptor. Among these 3 ligands, Androstan-3-ol showed the highest binding affinity and hydrophobic interaction with the amino acids of the active pocket. DNA gyrase is an essential bacterial enzyme that catalyzes the introduction of negative (−) supercoils into chromosomal and plasmid DNA. Gyrase was discovered soon after it was clear that *in vitro* recombination of bacteriophage λ required a negatively supercoiled DNA substrate. DNA gyrase cleaves and religates DNA to regulate DNA topology and is a major class of antibacterial and anticancer drug targets [34]. The 5 ligand molecules exhibited antibacterial activity by hindering the function of DNA gyrase.

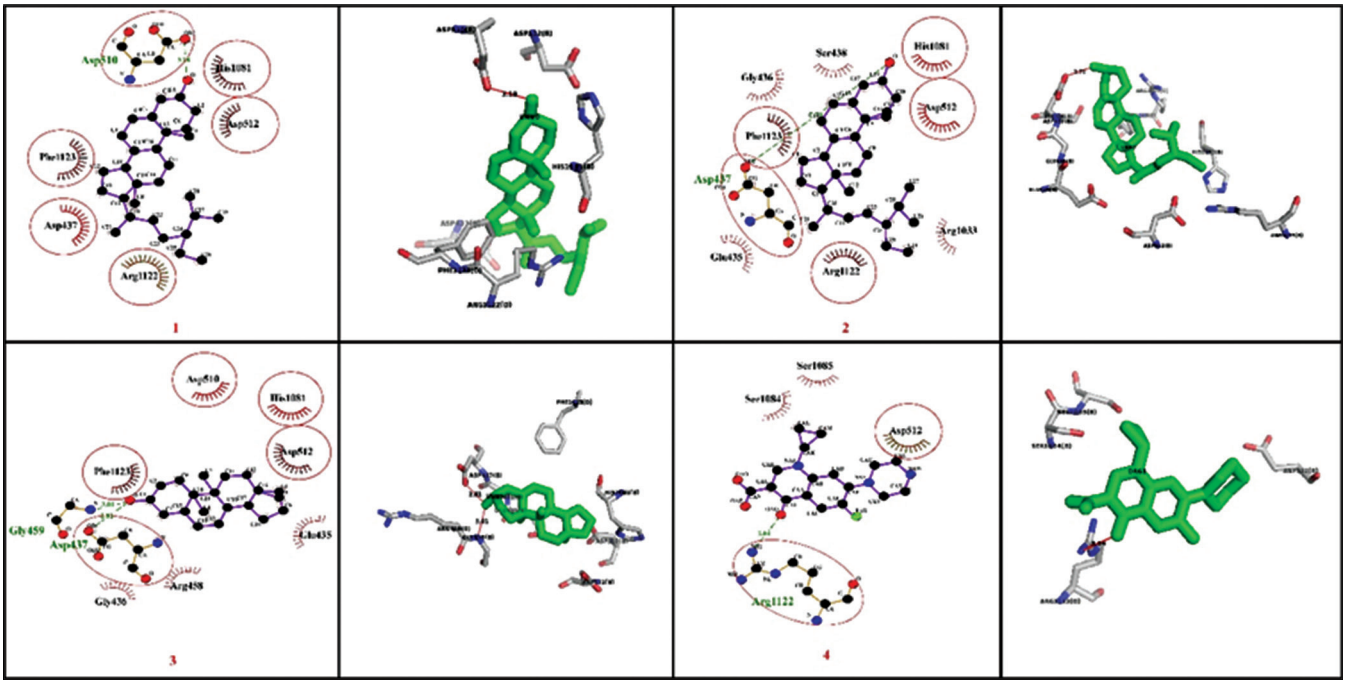

Fig. 2: Two-dimensional and three-dimensional protein-ligand interaction DNA gyrase with the ligands 1.campesterol, 2.gamma.-sitosterol acid, and 3.androstan-3-ol 4.Ciprofloxacin

Table 5: Molecular docking values of the compound obtained from GC-MS analysis

S. No.	Ligand	Affinity (kcal/mol)	H-Bonds	H-Bond Length (Å)	H-Bond with	Hydrophobic interactions
1.	Campesterol	−5.3	1	3.18	2XCT:Asp510::1:O	Asp437, Asp512, His1081, Arg1122, Phe1123
2.	Gamma.-Sitosterol	−6.2	1	2.70	2XCT:Asp437::2:O	Glu435, Gly436, Ser438, Asp512, Arg1033, His1081, Arg1122, Phe1123
3.	Androstan-3-ol,	−6.4	2	2.81 3.01	2XCT:Asp437::3:O 2XCT:Gly459::3:O	Glu435, Gly436, Arg458, Asp510, Asp512, His1081, Phe1123
4.	Ciprofloxacin	−6.0	1	3.04	2XCT:Arg1122::CIP:OAQ	Asp512, Ser1084, Ser1085

GC-MS: Gas chromatography-mass spectrometry

Table 6: *In silico* ADMET and drug-likeness prediction using data warrior

S. No.	Compound	CLogP	CLogS	H-Acceptor	H-Donors	TPSA	Ligand efficiency	Drug likeness
1.	Campesterol	7.400	−6.399	1	1	20.23	−0.17794	−8.190
2.	Gamma.-Sitosterol	7.855	−6.669	1	1	20.23	0.1527	−4.475
3.	Androstan-3-ol,	5.1252	−5.26	2	0	255.12	0.0945	−3.627

H-Acceptor: Hydrogen Acceptor, H-Donors: Hydrogen Donors, TPSA: Topological polar surface area. ADMET: Absorption, distribution, metabolism, and elimination and toxicity

CONCLUSION

In this study, we investigated the chemical composition and the biological activity of the *B. lanzan* seeds essential oil. Nineteen constituents were identified. *B. lanzan* seed essential oil exhibited promising antidiarrheal activity against the human bacterial strain *V. cholerae.* It shows 15.67±1.20 zone of inhibition. DPPH radical scavenging, H_2O_2 scavenging assay of essential oils, also revealed the significant results *in vitro* antioxidant property. A molecular docking study was carried for Campesterol, Gamma.-Sitosterol, and Androstan with bacterial enzyme DNA gyrase Androstan were found to be more effective compared to the other two compounds. The result of the present study, the ethnomedical claim of *B. lanzan* Spreng oil shows promising results to antimicrobial and antioxidant properties. Further studies should be performed to understand well the main mechanism of these activities and to determine which of the isolated compounds may be responsible for these activities.

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AUTHORS' CONTRIBUTIONS

Mr. Ajith S experimented and performed and statistical analysis; Prof. V. Krishna contributed to designing and supervision of the work along with drafting the article. Wrote the manuscript with support from Ravikumar S and Vinay N M.

CONFLICTS OF INTEREST

All authors declare that there are no conflicts of interest.

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