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Molecular docking and *in vitro* analysis of phyto-extracts from *B. serrata* for antibacterial activities

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Abstract:
The bioactives of *Boswellia serrata* have a role in ulcer healing therapies. Eleven bioactive compounds were obtained by GC-MS among which Cholan-24-oic acid, 3,12-bis (acetyl oxy) has a high molecular weight of 490.6719 with a retention time of 26.729. Twenty wound samples were collected aseptically from the labs and hospitals in and around the Namakkal districts of Tamilnadu, India. The antibacterial potential of *E.coli* showed a maximum inhibition of 27 mm against Tetracycline at 30µg. The ethanolic extract of the *B. serrata* shows a susceptibility of 19mm towards *E. coli* at 60µg concentration in MIC. Molecular docking results show the binding energy of Cholan-24-oic acid, 3,12-bis(acetyloxy) -8.6 (kcal/mol) followed by Pyrene, hexadecahydro- -6.7 (kcal/mol), and 5(1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8S-cis)- 6.4 (kcal/mol) for further consideration.

Keywords: Plant extract; GCMS; bioactive compounds; Molecular docking

Background:
The diseased field is replaced by rejuvenating content due to the result of the immune system and for attaining new epithelialization [1]. Infection ranges from a simple wound to septicemia and the pathogenicity is treated with novel drugs from plants that fulfill the targets [2]. In spiritual formalities, the *Boswellia* groves and their lubricant are used as bouquets [3]. The resin of the *Boswellia serrata* acts like a lytic of tumors (ganglions healer) [4]. Leukopenic power helps in treating autoimmune and genetic disorders it is also known as olibanum. The ingredients in the *Boswellia serrata* supports and quickens not only the repairing process but also urge the formation of the strengthened scars [5]. MAE usage of solvents is very minimal, in-expensive, low consumption of time, high yield, best for extracting lipids and glycans from the various origin so it is entitled as 'green technique [6]. Therefore, it is of interest to document the molecular docking and in vitro analysis of phytoextracts with *B. serrata* for antibacterial potentials

Materials and methods:
Collection of resins:
Fresh bronzed or bottle green resins brought from the local market of Rasipuram are then washed in Milli-Q water air-dried and grounded into powder using an electric mixer.

Extraction:
For extraction the weighed powdered resin was mixed with the solvents such as ethanol and aqueous at a different ratio in a 250 ml Erlenmeyer flask and placed over the circulating disk in the oven [7]. Parameters like temperature, time are maintained as per the protocol. Filtered aqueous phase air-dried as per the formula

the dry weight of each crude is examined and maintained the crude at -5 degree C for future process.

Gas Chromatography-Mass Spectrometry (GC–MS):
The system used is Agilent GC 7890A/ gas chromatograph MS detector MS5975C, US and samples dissolved in dichloromethane Gas chromatography linked to a mass spectrometer (GC–MS) equipped with fused silica capillary column and an Agilent DB5MS, (Column Length: 30m/0.25mm internal dia/0.25micron film thickness.

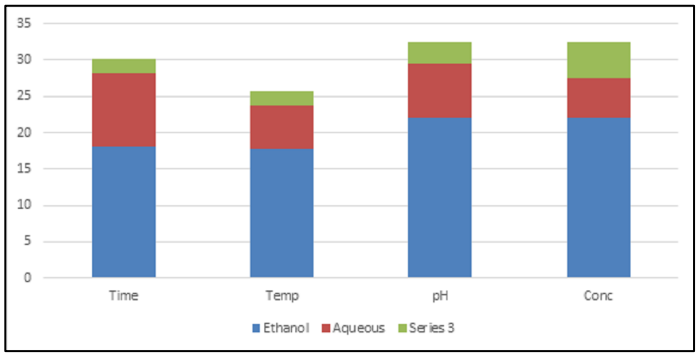

Figure 1: Yield of the crude ethanol and the aqueous

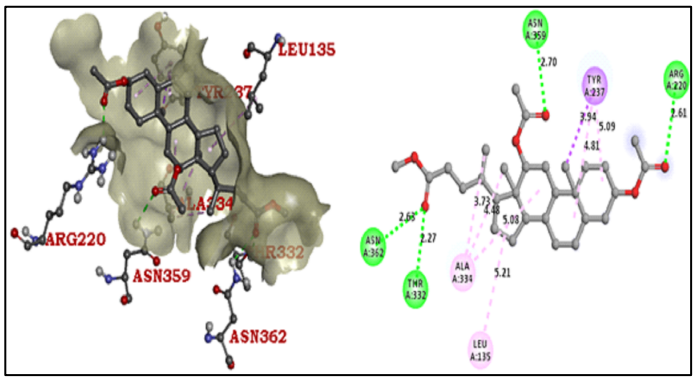

Figure 2: AmPC *E. coli* - CID 21140628 Docking Pose & Interaction Plot (-8.5 Kcal/mol)

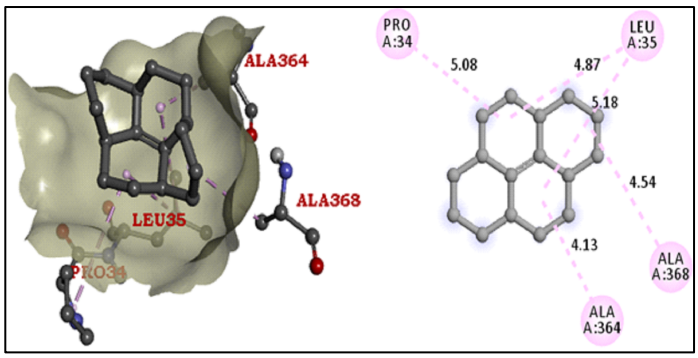

Figure 3: AmPC *E. coli* - CID 75524 Docking Pose & Interaction Plot (-6.7 Kcal/mol)

Wound and skin samples:
Encircling the Namakkal district approximately twenty samples were collected from the different ulcer, diabetic and sore patients **[8, 9]**. Using sterile swabs swabbed the pus, wound, and other exudates then which are packed aseptically in a transport media containing polypropylene container, sealed and marked the history of the specimen and stored at -4 degree C brought to our laboratory for research work, received from various sophisticated labs and hospitals.

Isolation and identification:
In various selective media, the collected commensals are inoculated and incubated at room temperature. After 24 hrs, the natural edges, texture color, and odor of the colonies are visualized, for further phenotypic identification a few drops of primary stain sprinkled on the smear in the slide then washed using H_2O after few seconds the slide was flooded with mordant (iodine) mean-while a quick water wash was done. A few minutes later, the slide is rinsed with the decolorizer (alcohol) and then the slide is shown under the tap water. At last gram +ve and gram-ve are identified by the counterstain safranin which is spread on the slide and excess stains are removed by showering the smear in water and droplets are isolated by wrapping in soft tissue paper **[10]**. Gaseous bubble formation, pink, violet, purple cherry red color appearance, and production of nonorganic acids are the positive signs in biochemical tests of some pathogens to identify their metabolic and enzymatic characteristics **[11]**.

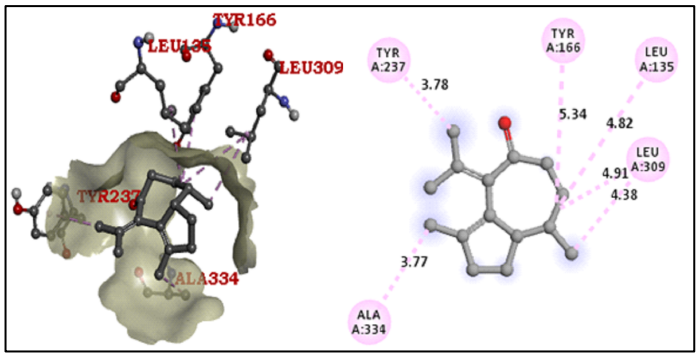

Figure 4: AmPC *E. coli* - CID 91735354 Docking Pose & Interaction Plot (-6.4 Kcal/mol)

Antibiotic sensitivity:
Isolates of gram-positive and gram-negative spread uniformly all over the MHA plate, along with a circular disk loaded with antibiotics are kept aseptically in the center of the plate and then incubated. Simultaneously, **[10]** another plate loaded with antibiotic disks without the inoculation of the pathogen was maintained as control kept for incubation and observed. After 24 hrs, MDR, PR, and sensitivity against a broad spectrum of antibiotics are measured.

Antibacterial activity:
A sterile cork Borell of 6 mm is used to make a well on the MHA plate for diffusion **[12]**, **[13]**. Then crude of aqueous and ethanol at different concentrations (20µg, 40µg, and 60µg) is loaded on the well to examine the antagonistic activity of the crude against the inoculated wound gram+ve and gram-ve isolates and kept for incubation. After 24 hrs, the zone of inhibition or minimal inhibitory concentration is noted by observing the halo around the well.

Molecular docking:
Small preliminary work is done for selecting the protein molecule by downloading (www.rcsb.org) or PDB format. Editing is done in the format via pymol or word pad tool **[14]**. The protein chain in the document begins with the letter "TER"and this shows the chain is terminated and the file is saved, ready for docking **[15]**. For the execution of docking install "autodock suite-4.2.5.1-i86Windows.exe downloaded from the website (http://autodock.scripps.edu/) Mol soft and chimera is used to draw the ligand structures. The molecules, ligands, and amino acid interaction and their energies are predicted by the software tools **[16]** until they are present in the grid box. The active site, binding site, and other essential regions of the molecules are predicted

after setting the grid box. All 'PDP' files of protein and ligands are moved into the 'work folder' for further execution of docking.

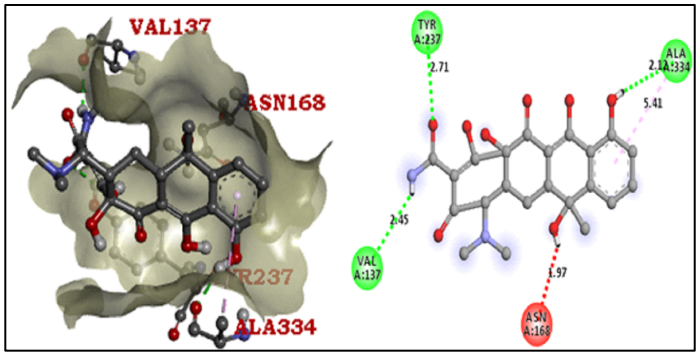

Figure 5: AmPC *E. coli*- CID 54675776 Docking Pose & Interaction Plot (-8.0 Kcal/mol)

Ligand preparation:

The GC-MS identified bioactive compounds of the plant extract *B. Serrata* were chosen for the current study using i) Ethyl 2-chloro propionate ii) alpha-Asarone iii) 5-Dodecyne iv) 5-Isopropenyl-2-methyl-7-oxabicyclo v) o-Mentha-1(7),8-dien-3-ol vi) Carbonic acid, 2-chloroethyl 2,2,2-trichloroethyl ester vi) Benzene, 1-[(2-chloroethyl)sulfonyl 2-nitro vii) 3-chloro-4-nitrophenol viii) Cholan-24-oic acid, 3,12-bis(acetyloxy) ix) Pyrene, hexadecahydro- viii) 5(1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8S-cis)- and the antibiotic reference drugs used for molecular docking were Gentamycin, Meropenem, Tetracyclin, and Vancomycin. The three-dimensional (3D) structures of all the selected cyano compounds were retrieved from the pub chem compound database https://pubch em.ncbi.nlm.nih.gov/ in the SDF file which was then converted into PDB format for docking study [**17**, **18**].

Result and Discussion:

The colony morphology of the pathogens wound isolates is cohesive, raised off–white, mucus and shiny texture like colonies are observed in the MSA, nutrient agar, blood agar, EMB agar and Mcconkey agar this indicates the isolates are *E.coli*, and *S.aureus*, whether they are gram +ve or gram-ve is identified along with the biochemical study they are briefly described in T**able 1.** Among the broad spectrum of antibiotics such as Tetracycline, Meropenem, Vancomycin, and Gentamycin, the gram –ve bacteria *E.coli* show resistance to tetracycline and vancomycin because the zone of inhibition is in the range of 6-14 mm. But in the case of *S.aureus*, it also shows resistance to vancomycin, because the ZOI is 16 mm. Then the antibiogram profile with other antibiotics is listed in **Table 2.** In MIC the ethanolic and aqueous extract of *B. serrata* resins show susceptibility towards gram-negative bacteria of *E.coli* with a zone of inhibition of 19mm at 60µg concentration but *S. aureus* showed a zone of inhibition in the range of 15 and 17mm. The other concentrations and their minimal inhibitory concentration level are shown in **Table 3.**

The crude of the resin obtained by ethanolic extraction shows high yield than compared with the aqueous extraction by maintaining different parameters like time (5, 10, 15, 20), temperature (200W, 300W, 500W, and 700W), pH (6, 7, 8, 9) concentration (100, 100, 100, 100). Therefore, the crude ethanolic resin obtained at 15minutes at the temperature of 700W provides a good yield and is shown in **Figure 1**. The yield is determined by implementing the dry weight formula shown below:

Dry wt% =Wt. of the dry extract x 100/ Wt of the resin PWD

The GC-MS analysis explored eleven bioactive compounds in the ethanolic extract. The molecular formula, molecular weight, retention time and area % of the compounds are presented in **Table 4**. Among the observed bioactive compounds the Cholan-24-oic acid, 3,12 bis(acetyloxy) show a binding affinity of -8 (kcal/mol) and the reference antibiotic tetracycline also has the same binding affinity (-8 (kcal/mol) with ligand Ampc *E. coli*. Arg220, Thr332, Asn359, Asn362, Leu135, Tyr237, Ala334 are the active site residues in the beta-lactamase protein molecule. The binding score and 3D graphical structure are all shown below with their CID 21140628, CID75524, CID 91735354, and CID 54675776 **(Tables 5 to 6 and Figures 2 to 5).** The ingredients which are having most effective tumor lysing ache solving WBC production minimizing, fungal resisting inflammation controlling bursal complication resolving types available in saturated forms these are all obtained as per the international protocol experimentally and inhibitory effect explored after treatment with GCMS and docking almost all systems of the physiology CVS, rheumatic, RS, COPD, GI, IBS, CNS, PN along with these especially in the RS very many ailments like genetical, congenital, geriatrics pediatrics, youths (infertility) are all under its control. Genetical hypogonadism, geriatrics, sexual disorders, pediatrics turner's syndrome [**19**]. In cosmetology, the bioactive compounds of *B. serrata* are helpful in the management of hair loss and diseases of the nails [**20**], [**21**].

Table 1: Microscopic and Biochemical characterization of the isolates

Gram Staining	Motility	Carbohydrate Fermentation								Oxidase	Urease
		G	L	S	I	MR	VP	Cit	Cat		
G +ve Cocci in clusters	Non-motile	P	P	P	N		P	P	P	N	P
G –ve Rod	motile	P	P	P	P	P	N	N	P	N	N

G; Glucose, L; Lactose, S; Sucrose, I; Indole, MR; Methyl red, VP; Voges Proskauer, Cit; Citrate utilization, CAT; Catalase

Table 2: Antibiotic sensitivity against the nosocomial pathogens (ZOI in mm)

Isolates	Tetracycline 30µg		Meropenem 30µg		Vancomycin 5µg		Gentamycin 10µg	
	ZOI	Inf	ZOI	Inf	ZOI	Inf	ZOI	Inf
Staphylococcus aureus	28 ±1	S	23 ±1	S	16 ±1	R	21 ±1	S
E.coli	14 ±1	R	27 ±1	S ±1	6 ±1	R	19 ±1	S

ZOI – Zone of Inhibition, Inf – Inference, S – Sensitive, R – Resistance, I – Intermediate

Table 3: Antibacterial activity of *B. serrata* extract against the pathogens

S. No	Pathogen	Zone of Inhibition					
		Ethanolic extract			Aqueous extract		
		20µg	40 µg	60µg	20µg	40 µg	60µg
1	Staphylococcus aureus	7 mm	11 mm	15 mm	11mm	14mm	17 mm
2	E.coli	14 mm	17 mm	19 mm	14mm	17 mm	19mm

Table 4: Plant Phytocompounds Identified Through GCMS Analysis

S. No	Compound	Retention time	Molecular Formula	Molecular weight	Area %
1	Ethyl 2-chloropropionate Propanoic acid, 2-chloro	10.875	C5H9ClO2	136.58 g/mol	2.75
2	Alpha-Asarone	14.397	C12H16O3	208.25 g/mol	2.23
3	5-Dodecyne	20.041	C12H22	166.3 g/mol	10.51
4	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	21.085	C10H16O2	168.23 g/mol	3.07
5	o-Mentha-1(7),8-dien-3-ol	21.352	C10H16O	152.23 g/mol	2.67
6	Carbonic acid, propargyl 2,2,2-trichloroethyl ester	22.063	C6H5Cl3O3	231.5 g/mol	1.26
7	Benzene, 4-chloro-1-[(2-chloroethyl) sulfonyl]-2-nitro-	22.13	C8H7Cl2NO4S	284.12 g/mol	1.07
8	3-Chloro-4-nitrophenol	26.318	C6H4ClNO3	173.55 g/mol	1.62
9	Cholan-24-oic acid, 3,12-bis(acetyloxy)-, methyl ester, (3ĥ,5H,12ĥ)	26.729	$C_{29}H_{46}O_6$	490.6719	8.88
10	Pyrene, hexadecahydro-tetracyclo[6.6.2.0<4,16>.0<11,15>]hexadecane	26.84	C16H26	218.38 g/mol	12.13
11	5(1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8S-cis)-	26.84	C15H22O	218.33 g/mol	12.13

Table 5: Docking Interaction Table for AmPC *E.Coli* ligand complexes

S. No	Complex Name	Bonded Interactions	Non Bonded Interactions	Docking Score (-K.Cal/mol)
1.	CID 136928	-	Asp280,Met281,Leu290	-4.9
2.	ID 119093338	Ser298	Met281,Ile307	-5.6
3.	CID 10807	Ser80,Ala334	-	-4.1
4.	CID 636822	Arg312,His330	Met281,Ile299,Ile299	-5.4
5.	CID 140583	-	Ala334,Val227,Tyr237	-4.5
6.	CID 10465500	Asp280,Ser298	Ser303,His330,Gly302,Met281	-6.3
7.	CID 14489	Ser80,Ala334	Tyr166,Leu135,Leu309	-4.6
8.	CID 565280	Ser80,Asn305, Ala334	Leu135	-5.3
9.	CID 564552	Ser80,Ala334	Leu135,Tyr300	-5.7
10.	CID 87646995	Ser80,Asn168	-	-4.4
11.	CID 80935	Asn168,Lys331, Thr332,Asn362	Tyr237,Ala334	-6.3
12.	CID 10283	His330	Met281,Ala308	-5.4
13.	CID 21140628	Arg220,Thr332, Asn359,Asn362	Leu135,Tyr237,Ala334	-8.5
14.	CID 75524	-	Pro34,Leu35,Ala364,Ala368	-6.7
15.	CID 91735354	-	Leu135,Tyr166,Tyr237,Leu309,Ala334	-6.4
16.	CID 5959	Gln136,Asn305,Thr332,Asn362,Asn168	Tyr237,Ala334	-6.6
17.	CID 5329	Asn168, Ala334	Tyr237,Tyr166,Leu309,Ser80	-7.1
18.	CID 5578	Ala231, Pro138	Asn168,Tyr237	-6.1
19.	CID 37569	Val137,Leu135,Asn168,Ser80,Asn305,Ala334	Lys83	-7.3
20.	CID 441130	Ser80,Asn168,Asn305	Asn362	-7.5
21.	CID 54675776	Val137,Tyr237,Ala334	Asn168	-8
22.	CID14969	His226,Glu212,Val227,Ala224, Asp139	Val225,Trp217,Ala224,Trp217	-6.9

Table 6: Docking Score of Phytocompounds And Antibiotic Reference Drug Against AmpC

Compounds	Binding affinities (kcal/mol) with AmpC from E.coli
Ethyl 2-chloropropionate	-4.1
Alpha-Asarone	-5.4
5-Dodecyne	-4.5
5-Isopropenyl-2-methyl-7-oxabicyclo	-5.3
o-Mentha-1(7),8-dien-3-ol	-5.7
Carbonic acid, 2-chloroethyl 2,2,2-trichloroethyl ester	-4.4
Benzene, 1-[(2-chloroethyl)sulfonyl 4-nitro	-6.3
3-chloro-4-nitrophenol	-5.4
Cholan-24-oic acid, 3,12-bis(acetyloxy)	-8.5
Pyrene, hexadecahydro-	-6.7
5(1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8S-cis)-	-6.4
Gentamycin	-7.3
Meropenem	-7.5
Tetracyclin	-8
Vancomycin	-6.9

Conclusion:

We show the good binding features of the bioactive compound as Cholan-24-oic acid, 3,12 bis(acetyloxy) from *B. serrata* with AmpC for further consideration in the context of antibacterial potential and wound healing.

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Conflict of interests: The authors declare no conflicts of interest.

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