Preliminary antibacterial evaluation of the chemical compositions in *Herba pogostemonis* oil

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Abstract: In the present study, the antibacterial tests of *herba pogostemonis* oil were studied by using molecular-docking technology and antibacterial test *in vitro*. The 3 three-dimensional (3D) structures of the 5 compared compositions and 26 compositions from *herba pogostemonis* oil were established by using surflex-dock software (8.1). Molecular-docking was carried out between the 31 chemical compositions (ligands) and the 5 enzymes (receptors) by using surflex-dock function. By comparing the scoring result of 26 compositions in *herba pogostemonis* oil with 5 compared components, we can infer antibacterial activity about 26 compositions in *herba pogostemonis* oil. On the other hand, six frequently-used pathogenic bacteria were selected for antimicrobial test *in vitro*, *herba pogostemonis* oil and its two major compositions: (-)-*herba pogostemonis* alcohol and pogostone, which their contents exceeded 60% in *herba pogostemonis* oil samples, were selected antibacterial agents. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) were also determined. Molecular-docking technology and antimicrobial test *in vitro* all were proved that *herba pogostemonis* oil had strong antibacterial effects. Particularly, pogostone and (-)-herba pogostemonis alcohol have potent antimicrobial activity.

Keywords: *Herba pogostemonis* oil; Molecular-docking; Scoring function; Antibiotic experiment *in vitro*; MIC and MBC

INTRODUCTION

The method of simulating a geometric model of molecular and intermolecular forces by chemometrics methods in order to identify and forecast receptor-ligand complex structure is called molecular-docking. Molecular-docking has become a more mature method in drug study. It was originally proposed when studied chemical problems in the biological system based on the numerator level. As early as 1789, E.Fisher used "key and lock" as a metaphor for enzyme-substrate in his paper, and he named it identification (Fisher *et al*., 2002). At the same time, the receptor theory introduced by Langley is believed that most drugs must combine with some particular molecules on the cell membrane, and these particular molecules were named receptor (Rang, 2006). So the concept of receptor in receptor theory and in molecular-docking is essentially the same which lays theoretical foundation for molecular- docking approach. Molecular-docking techniques have shown great promise as a new tool in the discovery of novels mall molecule drugs for targeting proteins (Pham *et al*., 2006; Jain, 2004).

Herba pogostemonis [*Pogostemon cablin* (Blanco)Benth.] is a Traditional Chinese Medicine (TCM) that has been widely used in Philippines, Malaysia, India and China. The dry leaves of *herba pogostemonis* on steam distillation yield an essential oil called the *herba pogostemonis* oil. *Herba pogostemonis* oil is hence an important ingredient in many fine fragrance products like perfumes, as well as in soaps and cosmetic products (Singh *et al*., 2002). It is also on the FDA's (Food and Drug Administration) list of substances approved for human consumption, in section 172.510, as a natural additive for food flavoring (Donelian *et al*., 2009).

Moreover, *herba pogostemonis* oil in the plant is widely used in TCM as it offers various types of pharmacological activities (Hu *et al*., 2006). It has also been reported to strengthen the immunity activity and resistance to bacterial action *et al.,* (Wu *et al*., 2004). The composition of the *herba pogostemonis* oil is complex like many essential oils, which consist of the major components such as patchoulol alcohol and pogostone *et al*., The action mechanism of major pharmacologic components in *herba pogostemonis* oil as an antibacterial agent has not been reported. To identify the possible biochemical path ways involved and to assess the therapeutic potential of *herba pogostemonis* oil in bacterial infection, we evaluated the effects of this herb by using molecular-docking technology and antibiotic experiment *in vitro.*

In this study, 5 biological macromolecule enzymes: Penicillin binding proteins (PBPs), dihydrofolate synthetase (DHPS), dihydrofolate reductase (DHFR), DNA gyrase and RNA polymerase, which were needed by bacteria in the process of biosynthesis, were selected as target molecules. 5 chemical compositions included

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benzylpenicillin (act on PBPs), sulfadiazine (act on DHPS), trimethoprim (act on DHFR), ciprofloxacin (act on DNA gyrase) and rifaximin (act on RNA polymerase), which are generally acknowledged as very good antibacterial drugs, were selected as compared components. The 3D structures of the 5 compared components and 26 compositions in *herba pogostemonis* oil were built by using the surflex-dock software (8.1) which is a software of molecular-docking developed by professor Ajay N.Jain (Jain, 1996; Welch *et al*., 1996; Ruppert *et al*., 1997). And the 3D structures of 5 biological macromolecule enzymes are derived from Protein Data Bank (PDB). Molecular-docking was carry out between the 31 chemical compositions (ligands) and the 5 enzymes (receptors) by using surflex-dock function. Further, the antibacterial effects of 31 chemical compositions were investigated by the scoring function after molecular-docking had been finished. By comparing the scoring results of 26 compositions in *herba pogostemonis* oil with 5 compared components, we can infer antibacterial activity about 26 compositions in *herba pogostemonis* oil.

On the other hand, six frequently-used pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus proteus*, *Shigella dysenteriae*, *Typhoid bacillus*, *Staphylococcus aureu*) were selected for antibiotic experiment in vitro, *herba pogostemonis* oil and its two major compositions: (-)-*herba pogostemonis* alcohol and pogostone, which their contents exceeded 60% (g/g) in *herba pogostemonis* oil samples (Kraft *et al*., 2005), were selected antibacterial agents. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) were determined in order to verify the result of molecular-docking.

MATERIALS AND METHODS

Materials

Herba pogostemonis samples were collected from Guangdong place in October 2010, China. Professor Shui-Ping Yang, College of Resources and Environment, Southwest University, identified the raw medicinal herbs, and the voucher specimens deposited at the Herbarium of Southwest University (Voucher No.2011019). Gram-negative (*Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Bacillus proteus* ATCC18663, *Shigella dysenteriae* ATCC18664, *Typhoid bacillus* ATCC18665), Gram-positive (*Staphylococcus aureus* ATCC2925) were offered by Centre for microbial diagnosis of Chongqing Medical University. Penicillin G (sodium salt) was purchased from M&H manufacturing (Samutprakarn, Thailand). Mueller-Hinton Broth (MHB) was purchased from Difco laboratories (Detroit, MI, USA). (-)-herba pogostemonis alcohol was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Pogostone was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade and purchased from Promega Chemicals Co. (Madison, WI, USA).

Methods

Extract of herba pogostemonis oil

Based on the Chinese Pharmacopoeia 2010 edition, *Herba pogostemonis* oil was extracted as follow: 200g air-dried P*ogostemon cahlin* (Blanco) Benth powder were immersed in water (800 ml) and then continuously was heated by use of a fixed power heater with a maximum delivered power of 500W and temperature variation to be ±1^{o}C. The essential oil which is entrained by azeotropic distillation was freed. The vapour then passes through a condenser outside the power heater where it condensed. The distillate is collected continuously with a Clevenger-type apparatus (Pe´rino-Issartier *et al*., 2010) and stored at 4^{o}C until used.

Establish of 3D structure library about compositions in herba pogostemonis oil and 5compared components

The structures of 5 compared components and 26 chemical compositions in *herba pogostemonis* oil are shown in fig. 1 (Hu *et al*., 2002; Srikrishn *et al*., 2005; Deguerry *et al*., 2006) and their 3D structures were built by using surflex-dock software (8.1).

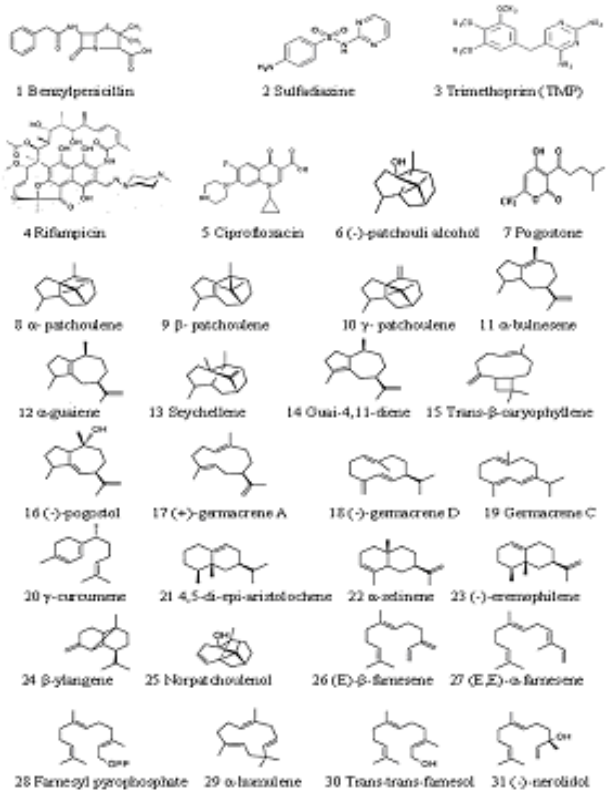

Fig.1: Chemical structures of 5 compared components and 26 chemical compositions in *herba pogostemonis* oil.

Select of five antibacterial targets and source of their 3D structure

5 biological macromolecule enzymes: PBPs (fig. 2A), DHPS (fig. 2B.), DHFR (fig. 2C.), RNA polymerase (fig. 2D.) and DNA gyrase (fig. 2E), which were needed by bacteria in the process of biosynthesis, were selected to serve as target molecules. Their 3D structures were downloaded from PDB which was established in the autumn of 1971 (Kirchmair *et al*., 2008) (Campagna-Slater *et al*., 2010; Mrozek *et al*., 2009.).

The reason about selection of 5 biological macromolecule enzymes as antibacterial targets were as follows: Cell wall, cell membrane, cell cytoplasm, nucleoplasm (which was aggregated of DNA and RNA, do not have complete nuclear structure, so also called nucleoid) and folic acid were all basic structure of bacterial cell. There were some biosynthetic targets in the structures, and if these targets were combined competitively by drug small molecules, the biosynthesis of a complete bacteria cell structure could not be accomplished, which helped to get antimicrobial effect. In this study, we chosen five typical targets such as: (1) Penicillin binding proteins (PBPs, PDB numberd:3OCL) are bacterial enzyme that catalyze the final steps in cell wall biosynthesis and are the lethal targets of β-lactam antibiotics such as benzylpenicillin (The 1st chemical composition in fig. 1) , which acted on PBPs and caused bacterial cell walls dissolved (Stefanova *et al*., 2010; Kawai *et al*., 2010). (2) DHFR (PDB number:3INV) is a key enzyme related with folic acid metabolism too. DHFR is a proven target for antibacterial agents, with diaminopyrimidine based inhibitors of DHFR, such as trimethoprim (the 3rd chemical composition in fig. 1), used clinically with relative success for decades as a monotherapy and in combination with other agents (Agarwal *et al*., 2010). (3) DHPS (PDB numberd 3NRS) can promote bacteria folic acid metabolism. Folic acid whose chemical name was "pteroylglutamic acid" was combined with *p*-Aminobenzoic (PABA) and glutamic acid under the catalysis of DHPS, FAH_2 is synthetized by PABA and dihydropteridine pyro phosphate. However, FAH_2 is one of the necessary substances in the process of bacteria combining. Only under exist of folic acid, nucleic acids can be synthesized, and at last the bacteria grow. The drug such as sulfadiazine (the 2nd chemical composition in fig. 1) with similar chemical structure of PABA can displace the site of PABA to hinder the folic acid biosynthesis. Finally, bacteria pass away because of lack of folic acid (Agarwal *et al*., 2010). (4) The bacterial topoisomerases (DNA gyrase and topo IV, with PDB number: 3M4I and 3LTN, respectively) are multisubunit enzymes that play essential roles in DNA replication and are validated targets for clinically useful antimicrobial drugs. One protein subunit (GyrA orParC) participates in protein-DNA interactions and other (GyrB or ParE) in ATP hydrolysis. The quinolone antibiotics (e.g., ciprofloxacin, the 5th chemical composition in fig. 1) directly affect the gyrase/topoisomerase- DNA interaction by trapping the proteo-nucleic acid complex at the site of GyrA or ParC (Saíz-Urra *et al*., 2011; Bansal *et al*., 2011). (5) RNA polymerase (PDB number: 2RF4) is essential enzyme involved in protein biosynthesis in bacteria, which have emerged as interesting targets in antibacterial research. RNA polymerase represents a potential drug target. A large number of promising lead compounds have been identified using as the inhibitors of RNA polymerase, Rifaximin (The 4th chemical composition in fig. 1) is known to act upon such a target (an inhibitor of isoleucyl-tRNA synthetase) (Yang *et al*., 2010; Stalder *et al*., 2011).

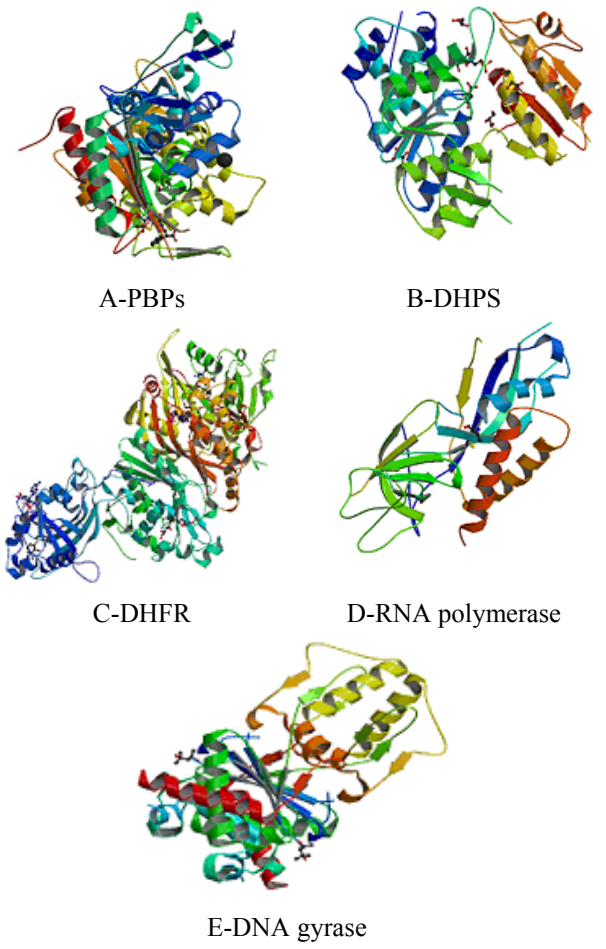

Fig. 2: The three-dimensional (3D) structures of 5 enzymes (receptors)

Scoring function of surflex-dock

Molecular-docking was carried out between the 31 compositions (ligands) and the 5 enzymes (receptors) by using surflex-dock function. Further, the antibacterial effects of 31 compositions were investigated by scoring function after molecular-docking had been finished. Surflex-Dock was a software of molecular-docking which used a unique and experiential scoring function and a novel search engine (Based on molecule similarity), which docked ligand molecule to the binding sites of

protein (receptor). In other words, the higher experiential scoring function, the better antibacterial effect, so the conformation of complex made from target spot (protein)-chemical components (ligand) was more stability (Jain, 2003).

Study on bacteriostatic test in vitro (Determination of MIC and MBC)

The mini mum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) was measured by broth dilution method using Mueller-Hinton Broth (MHB) (Devi *et al*., 2010; Ogata *et al*., 2000). The MIC of *herba pogostemonis* oil, (-)-herba pogostemonis alcohol and pogostone were determined turbidimetrically (Genesys 20 spectro-photometer; Thermospectrum), as reported elsewhere (Wangthong *et al*., 2010). In short, *herba pogostemonis* oil, (-)-herba pogostemonis alcohol and pogostone were dissolved in MHB. They were adjusted to the desired concentration in a final volume of 20µl and added into 2ml of sterile MHB, mixed and serially diluted prior to inoculation with 15µl of freshly prepared bacteria suspension (10^7 CFU/ml in MHB). The positive control was performed using 12, 6, 3, 1.5, 0.75, 0.37, 0.18 and 0.09mM (final concentration) Penicillin G and blank control tubes contained only MHB and the sample solvent as appropriate. After mixing, the tubes were incubated at 37^{o}C for 24h in an incubator (Mermmet model 800).The tubes were then examined after 24h for visible signs of growth and for turbidity by absorbance at 600nm. The lowest concentration of each sample that inhibited the bacterial growth was taken as the MIC. The MBC, or the lowest concentration of sample that kills 99.9% of bacteria, was determined by assaying the live organisms of those tubes from the MIC that showed no growth as previously described (Avadietal, 2004). A loopful of bacterial broth from each of the tubes showing no growth was inoculated onto MHB plates and examined for signs of growth (colonies) after 24h of incubation at 37^{o}C. All experiments were performed in triplicate.

RESULTS

The results of molecular-docking

As shown in table 1. In 5 compared components, benzylpenicillin, sulfadiazine, trimethoprim, ciprofloxacin and rifaximin acted on corresponding targets at PBPs (fig. 3A), DHPS (fig.3B), DHFR (fig 3C.), RNA Polymerase (fig 3D), the bacterial topoisomerases (DNA gyrase, fig 3E), respectively. Their scoring were 7.22, 7.33, 6.82, 6.94 and 6.78, respectively. In the meantime, 26 compositions in *herba pogostemonis* oil all acted on above 5 targets. The results showed that no composition was higher than 5 targets. However, as shown in table 1. We found that most chemical compositions in *herba pogostemonis* oil always characterized their low drug action and multi-target effect. We took the (-)-herba pogostemonis alcohol (from fig. 3F to fig. 3J) and pogostone (From fig. 3K to fig. 3O) as examples, who are the most two compositions in *herba pogostemonis* oil, the (-)-herba pogostemonis alcohol mainly caused bacterial cell walls defects as same as benzylpenicillin. However, we thought that it has certain effective at the other targets which can be seen by scoring result of the (-)-herba pogostemonis alcohol with 3.57 (PBPs, Docking fig. 3F), 3.47 (DHFR, Docking fig. 3G), 4.47 (DHPS, Docking fig. 3H), 4.22 (DNA gyrase, Docking fig. 3I) and 4.91 (RNA Polymerase, Docking fig. 3J) respectively. Similarly, coring result of the pogostone were 4.69 (PBPs, Docking fig. 3K), 3.54(DHFR, Docking fig. 3L), 4.23 (DHPS, Docking fig. 3M), 3.75 (DNA gyrase, Docking fig. 3N) and 3.77 (RNA Polymerase, Docking fig. 3O) respectively. However, on account of this pellet-model of multi- target effeet, *herba pogostemonis* oil had strong antimicrobial effects.

Antimicrobial test in vitro

As shown in table 2, six frequently-used pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus proteus*, *Shigella dysenteriae*, *Typhoid bacillus*, *Staphylococcus aureu*) were selected for antimicrobial test in vitro, *herba pogostemonis* oil and its two major compositions: (-)-herba pogostemonis alcohol and pogostone, whose contents exceeded 60%(g/g) in *herba pogostemonis* oil samples(13), were selected antibacterial agents. The results of MIC and MBC showed that *herba pogostemonis* oil, (-)-herba pogostemonis alcohol and pogostone all have good antibacterial activities. These proved that the method and result of molecular- docking were feasible and reliable (See table 2).

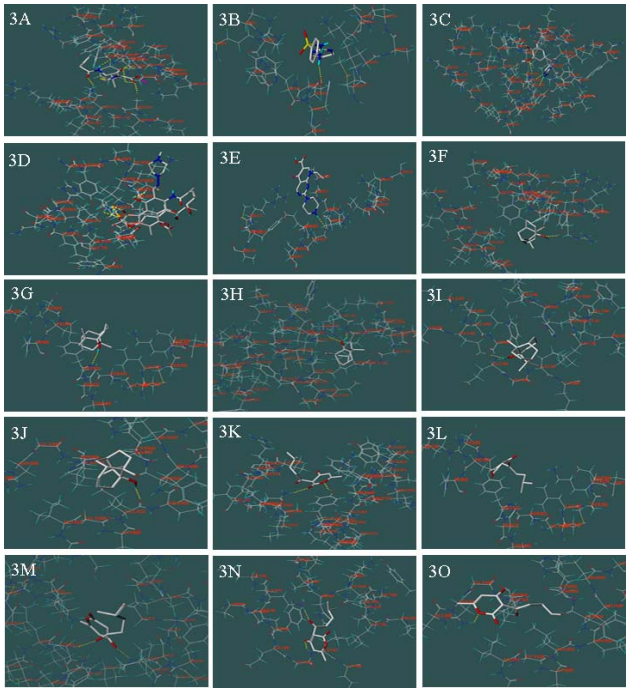

Fig.3: Molecular-docking between 31 chemical compositions and 5 enzymes (receptor) respectively

(3A) between benzylpenicillin and PBPs; (3B) Between sulfadiazine and DHPS; (3C) Between trimethoprim and DHFR; (3D) Between rifaximin and RNA polymerase; (3E) Between Ciprofloxacin and DNA gyrase; (3F) Between (-)-herba pogostemonis alcohol and PBPs; (3G) Between (-)-herba pogostemonis alcohol and DHPS; (3H) Between (-)-herba pogostemonis alcohol and DHFR; (3I) Between (-)-herba pogostemonis alcohol and RNA Polymerase; (3J) Between (-)-herba pogostemonis alcohol and DNA gyrase; (3K) Between pogostone and PBPs; (3L) Between pogostone and DHPS; (3M)Between pogostone and DHFR; (3N)Between pogostone and RNA Polymerase; (3O) Between pogostone and DNA gyras

Table 1: The scoring function result of 31 chemical constructions

Number		Component (ligand)	Target molecules (receptor)				
			PBPs	DHFR	DHPS	DNA gyrase	RNA Polymerase
compared components	1	Penicillin	7.22	-	-	-	-
	2	Trimethoprim	-	6.82	-	-	-
	3	Sulfadiazine	-	-	7.33	-	-
	4	Ciprofloxacin	-	-	-	6.94	-
	5	Rifaximin	-	-	-	-	6.78
The chemical compositions in patchouli oil	6	(-)-patchouli alcohol	3.57	3.47	4.47	4.22	4.91
	7	Pogostone	4.69	3.54	4.23	3.75	3.77
	8	α-patchoulene	1.56	4.13	6.02	4.44	2.92
	9	β-patchoulene	4.45	4.85	4.14	2.83	2.03
	10	γ-patchoulene	0.43	2.76	5.17	2.92	2.05
	11	α-bulnesene	4.58	3.11	5.42	3.07	2.14
	12	α-guaiene	-5.52	4.51	4.15	5.02	2.96
	13	Seychellene	4.79	3.34	5.24	0.59	4.02
	14	Guai-4,11-diene	1.41	2.87	4.14	1.18	2.98
	15	Trans-β-caryophyllene	0.23	5.50	2.58	4.37	3.01
	16	(-)-pogostol	2.92	3.71	5.45	2.04	3.24
	17	(+)-germacrene A	5.13	3.90	5.16	4.04	3.06
	18	(-)-germacrene D	-4.25	4.81	4.51	2.75	2.89
	19	Germacrene C	4.57	3.83	5.74	4.99	3.02
	20	γ-curcumene	2.32	3.65	3.76	3.47	3.12
	21	4,5-di-epi-aristolochene	-1.26	4.39	5.36	3.71	3.14
	22	α–selinene	3.67	3.79	4.53	2.17	5.13
	23	(-)-eremophilene	2.84	2.19	2.26	5.63	4.09
	24	β-ylangene	5.25	5.52	4.63	4.89	3.20
	25	Norpatchoulenol	4.68	5.16	4.45	3.92	3.18
	26	(E)-β-farnesene	2.28	-2.19	4.17	4.51	4.81
	27	(E,E)-α-farnesene	3.21	-11.47	3.41	3.62	3.26
	28	Farnesyl pyrophosphate	5.22	0.38	2.08	4.55	3.28
	29	α-humulene	3.74	5.21	0.47	4.88	2.68
	30	Trans-trans-farnesol	1.08	-3.09	5.37	3.51	3.26
	31	(-)-nerolidol	3.98	3.99	4.17	5.34	3.52

Table 2: Anti-bacterial activities of patchouli oil and its two chemical compositions

Fungi	Patchouli oil		Patchoulol		Pogostone	
	MIC ($mg \cdot mL^{-1}$)	MBC ($mg \cdot mL^{-1}$)	MIC ($mg \cdot mL^{-1}$)	MBC ($mg \cdot mL^{-1}$)	MIC ($mg \cdot mL^{-1}$)	MBC ($mg \cdot mL^{-1}$)
Escherichia coli	4.0	2.0	1.0	2.5	0.45	0.80
Pseudomonas aeruginosa	5.5	>10.0	3.5	7.5	3.0	5.5
Bacillus proteus	7.5	>10.0	3.5	8.5	4.0	6.5
Shigella dysenteriae	6.5	>10.0	3.0	5.0	3.5	6.0
Typhoid bacillus	5.5	7.5	6.5	8.0	6.0	>10.0
Staphylococcus aureus	4.5	6.5	2.0	7.5	1.0	4.5

DISCUSSION

The method of molecular-docking is primitively used as screening of chemical drugs, but it scarcely any applied research on TCM. In this study, 5 biological macromolecule enzymes: PBPs, DHPS, DHFR, RNA polymerase and DNA gyrase, which were necessary for biosynthesis in bacteria, were selected to serve as target molecules. Their 3D structures were downloaded from PDB. 5 chemical compositions included benzylpenicillin, sulfadiazine, trimethoprim, rifaximin and ciprofloxacin, which are generally acknowledged as very good antibacterial drugs, were selected as compared components. The 3D structures of the 5 compared components and 26 compositions from *herba pogostemonis* oil were established by using surflex-dock software (8.1). Molecular-docking was carried out between the 31 chemical compositions (ligands) and the 5 enzymes (receptors) by using surflex-dock function. Further, the antibacterial effects of 31 chemical compositions were investigated by the scoring function after molecular-docking had been finished. The results showed that most compositions in *herba pogostemonis* oil always characterized their multi-target effect. On account of 26 compositions in *herba pogostemonis* oil and multi-target effects of each composition, *herba pogostemonis* oil had strong antimicrobial effects, alike the canister shot which we can call it canister shot-model of multi-target effect. This may be basic principle of TCM therapy. Molecular-docking technology and antimicrobial test in vitro all illustrated well the action mechanism of *herba pogostemonis* oil as antimicrobial drug in TCM therapy. Due to strong antimicrobial effects, particularly potent antimicrobial activity of pogostone and (-)-herba pogostemonis alcohol, *herba pogostemonis* oil has its broader therapeutic prospects in bacterial infection.

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