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Medicinal herbs production to discourage and eliminate germs that contaminate burns: In silico study

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Abstract

Civilian patients with severe burns were almost local and systemic cell host immune responses concurrent depression because of complications after microbial infections. The most abundant and harmful bacterial infections are associated with opportunistic bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*, linked to nosocomial infection with prolonged stay in a hospital. Many virulence factors excrete from both species that facilitate adherence to the burned area, localized and invasion tissue, and the emergence of antibacterial resistance. Molecular docking of molecules assimilates the interaction between a ligand and its receptor the virulence factor; this project designed to show the extent of interaction between active component extracted from black tea and mint and virulence factors (coagulase, exozyme S, the extended-spectrum of beta- lactamase and beta-lactam binding protein) using molecular docking. Aqueous extracts of black tea and mint were analyzed by GC mass and each of the bioactive components was docked against each virulence factor. Re- rank score was monitored to explain the power of interaction between ligand and receptor.Results found here that components of black tea gave more re-rank score with all virulence factors; the most active was Myricetin that needs -80.906, -80.19, -69.04, and -67.06 for PBP2 protein, exozyne S, extended-spectrum beta-lactamase, and coagulase respectively. It was noticed that Myricetin was more active against proteins excreted from *P. aeruginosa* than that of *S. aureus*. Kaempferol as a second bioactive compound that gave a positive effect regarding its re rank score -90.195 and -79.54 when docked with PBP2 and exozyme S from *P.aeruginosa.*Molecular docking revealed that the aqueous extract of both black tea and mint have high re-rank as inhibitory substances against different virulence factors of *S.aureus* and *P.aeruginosa* that could be a good candidate for treating burns injuries.

Keywords: Molecular docking, Re-Rank score, exozyme S, coagulase.

Introduction

One of the most important medicinal strategies is the substitution of chemical antimicrobials with herbal antimicrobials, which, due to their efficacy as an antibiotic agent and the radical scavenger capability, provide perfect solutions. Because of their unique properties, herbal materials have created a new and exciting field for all sciences, in particular medicine (1). Their medical uses have already led to new medical products being developed. The design of new, non-side-effects antimicrobial drugs not only creates a new study area but also can support expanding human needs (2). Herbal medicines are often preferred by comparison with synthetic antimicrobials due to their less toxic and non-substantial nature. Herbal medicines also demonstrate a high potential for antibiotics at a lower price (3). The primary measure for assessing the health benefits of herbal medicine for humans is bioavailability. The idea that natural remedies are safer compared to prescription drugs has gained traction in recent years and helped to boost phytopharmaceutical applications (4). Two traditional herbs, the black tea, and mint were concluded in many medical applications, the tea plant (*Camellia sinensis)* in the family Theaceae is native to Southeast Asia, recently is currently cultivated in over 30 countries worldwide (5). The tea species whose leaf buds and leaves are consumed worldwide as either black tea 78% or green tea 20% and as oolong 2% (6). The tea has medicinal properties in traditional Chinese and Indian medicine

systems; tea has been used as a stimulant, diuretic, astringent, and to improve heart health (7). Tea has been considered to be anti-inflammatory, anti-oxidative and anti - carcinogenic. Phytochemical compounds of alkaloids, saponins, tannins, catechin, and polyphenols make up more than 25% of the dry weight that responsible for the antibacterial activities of various tea products (8). Catechins that are well described as isoflavanoids are the simplest compounds, mainly composed of four compounds, (-)-epigallocatechin (EGC), -epicatechin gallate ECG, -epigallocatechin gallate (EGCG), and (-)-epicatechin (EC) (9).

Theaflavins (The Flavin's), and thearubigins, which are oxidation and polymerized products of simple isoflavanoids (isoflavonoids) , are the main and major molecules in black tea. Mint (Mentha) is one of the perennial aromatic herbs of the family of Lamiaceae. It was used in a number of applications, like pharmaceuticals and cosmetics, essential oils (EOs), and watery mint extracts, which have potential antioxidant properties because of phenolic compounds (10). This plant's antioxidant activity depends only on its chemical composition and can prevent cellular and live-body oxidative stress. Many studies have shown the inhibitory ability of the plant to cope with Gram-positive bacteria and, especially *S. aureus*, dependent on the type of bacteria (11).On the basis of the high antimicrobial and antioxidant ability, all types of wounds, burns and bacterial infections can be cured. Many types of branches destroy protective skin barriers that prevent microorganisms from invading, and inhabiting (12). Cell, and humoral immune decreasing burn patients are susceptible to various infections (13). The risk of invading a bacterial infection is influenced by burn depth, other host factors, and virulence factors of the bacterial community which colonises the burn area, in these patients the infections with burned wounds are easily increased to sepsis (14). *S. aureus* and *Pseudomonas aeruginosa* are the most common pathogens isolated from burn wounds (75 and 25 percent, respectively). Both bacteria compromise various factors of virulence, including adhesion, obtaining nutrients, immune system, and killing of tissue, killing of leucocytes, and ultimately bloodstream invasions (15). It also carries numerous antimicrobial resistance traits that have been acquired as well as extensive spectrum β- lactamase which makes it difficult to treat infected burn wounds by traditional antibiotics. Molecular doping is a modern scientific method that predicts the favorite molecular orientation in the form of a stable complex from one molecule to the next (16). Knowledge of the preferred orientation is important to predict whether two molecules combine strength or binding affinity. The combinations of biological molecules such as proteins, nucleic acids, carbohydrates, and lipids are a key to signal translation (17). Facilities for molecular docking studies show the existing fitness between molecules as well as their relative orientation can affect the type of signal produced. Therefore, recent research is focusing on the characterization of the effectiveness of the active component in two herb extracts (black tea and mint) against four bacterial virulence factors (coagulase , exoenzyme-S, extended- spectrum β- lactamase and beta-lactam binding protein PBP2) using molecular docking technique, future therapeutic progress and development of pharmaceutical products.

Material and Methods

The extraction of active compounds

Black tea was purchase from the local market of Baghdad Province, Iraq. While peppermint (*Mentha piperita* L) was grown and harvested from Iraqi plants during March 2021. The plant cleaned from other herbs and rinsed with plenty of water then gently dried on paper towels. Meanwhile, plant was collected and dried using paper bags holding a dark place equipped to 25^{o}C for nearly 30 days (18).

The dried leaves of both black tea and peppermint were grounded a fine powder and sieved through a 0.4 mm mesh panel. Preparation of tea and mint aqueous extracts using water, ethanol and was carried out according to the mentioned process. Dried samples (500 g) of mint and black tea were extracted with 100 ml of ethanol or water and were kept a water path equilibrated to 60^{o}C for 20 minutes. Vacuum filtration assembly was used to recover the extract and rotary evaporate was applied to dry the extracted material. The final powder was weighed and stored in a sealed bag at 4^{o}C until used (19).

Gas chromatography-mass spectrometry (GC-MS) analysis

The biological active compounds were detected in both herbal extracts using gas chromatography technique (GC) (Chrompack-Packard 438A) equipped with an FID Detector at a temperature of 325ºC and a separation column type 30-SE with an inner diameter of 0.25 mm and a length of 30 m based on the method described in(20).

Molecular Docking studies

The key hypothesis and locking were to find the best fit between bioactive compounds and the studied factors of virulence. Biological active compounds extracted and characterized from *Camellia sinensis* , and *Mentha piperita* L leaf extracts were docked separately against exozyme-S from *Pseudomonas aeruginosa*, coagulase enzyme, extended- spectrum β-lactamase, and beta-lactamase binding protein 2 (PBP2) from *Staphylococcus aureus*. Each protein crystal structure has been recovered by RCSB as a PDB file and imported into molegro virtual Docker (MVD). Water molecules were not involved in the binding process of the ligand - receptor. In order to improve calculation and prevent potential distortion following a docking process, water molecules to prevent (21).

Biological active compounds

Fifteen different bioactive compounds detected by GC-MS were used in the present computerized study. These compounds were analyzed from aqueous extracts of *Camellia sinensis* and *Mentha piperita* L dried leaf. Downloaded from SDF-format ZINC15 and then imported into MVD workspace the chemical structures for all phyto-materials obtained from GC-MS and selected for drying. The ligands were then prepared for docking with the preparation of MVD molecules. The 2D structures were derived from the compound database of ZINC15 and the 3D ligand structures were drawn with the help of UCSF. The UCSF Chimera Structure Build module has been used to create ligands minimizes energy consumption, adds hydrogen atoms, and adds charges when needed). Each compound's prepared 3D structure was stored in pdb format and finally optimized to dock with UCSF Chimera tools (22).

Analysis of docking and post docking

The MVD docking wizard has been started and the structure of the proteins and all 15 ligands chosen has been selected as docking. However, the binding sites constraints were set to include the largest cavity detected on the protein structure and its dimensions were minimized in order to reduce simulation processing time and increase accuracy. All docking specifications were left as their original default settings. The maximum population was designed to a hundred based on the MVD recommendations (23), and fifty runs/ligands were established.

Docking results were imported and the best position of each ligand was loaded into the workplace on the basis of the re-rank score. The reception-liner-interaction diagrams for the best poses were visualized using BIOVIA Space Visualizer, while UCSF Chimera software used protein molecule and poses were visualized in MOL2 format (24).

Results and Discussion

GC-MS analysis was performed for the aqueous extract of *Camellia sinensis* and *Mentha piperita* L dried leaf to identify the biological active phytochemical constitutes. Ten Different compounds existed in the extract of *Mentha piperita* L and fourteen other biochemical compounds extracted from black tea (table 1). These phytochemical constitutes have a different bioactive group such as phenols, flavonoids, alcohol, and furan ring. They might interact with protein molecule inhibiting their enzymatic activity or denaturation of the proteins molecule.

The antioxidants and antibacterial properties of Tea Polyphenols have been fully documented; hydroxyl group on B-ring of each the 3 adjacent hydroxyl groups on the B-ring of GCG, EGCG,GC , and EGC are more strong scavenging free radical ability than the other free OH groups of ECG, CG, and EC leading to inhibit lipid peroxidation as well as chelate metal ions especially ferrous ions (25). Regarding phytochemical compounds, tea has antibacterial activity against various bacterial gram-positive and gram-negative species.

Myricetin is one of the most hydroxylated flavonols structure 3,3′,4′,5,5′,7-hexahydroxyflavone, it might have many antimicrobial mechanisms that involve in membrane disruption, inhibition of cell envelope synthesis, inhibition of nucleic acid synthesis, inhibition of bacterial virulence , and quorum sensing, which impairs their ability to form biofilms (26).

Table1: Chemical composition of peppermint and black tea aqueous extract using GC-mass analysis

Bioactive compounds in Mentha piperita(*Mentha piperita*) L. (peppermint) extract		
Compounds	**IUPAC name**	**dry weight%**
Carvone	**2-Methyl-5-(prop-1-en-2-yl)cyclohex-2-en-1-one**	**1**
Cineole	**1,3,3-Trimethyl-2-oxabicyclo(2,2,2)octane**	**7**
Isomenthone	**(2R,5R)-5-methyl-2-propan-2-ylcyclohexan-1-one**	**5**
Limonene	**1-Methyl-4-(1-methylethenyl)-cyclohexene**	**3**
Menthofuran	**3,6-Dimethyl-4,5,6,7-tetrahydro-1-benzofuran**	**4.5**
Menthol	**(1R,2S,5R)-2-Isopropyl-5-methylcyclohexanol**	**27**
Menthone	**(2S,5R)-2-Isopropyl-5-methylcyclohexanone**	**16**
Menthyl acetate	**Acetic acid ((1R,2S,5R)-2-isopropyl-5-methylcyclohexyl) ester**	**4.4**
Pulegone	**p-Menth-4(8)-en-3-one**	**4**
sopulegol	**5-methyl-2-prop-1-en-2-ylcyclohexan-1-ol**	**0.2**
Bioactive compounds in black tea extract		
Compounds		**dry weight%**
(-)-Epicatechin		**2.7**
(-)-Epicatechin-3-gallate		**7**
(-)-Epigallocatechin		**9.8**
(-)-Epigallocatechin-3-gallate		**10.9**
(+)-Catechin		**1.3**
(+)-Gallocatechin		**0.8**
Theaflavin		**1.8**
Theaflavin-3,3' -digallate		**1.8**
Theaflavin-3'-gallate		**1.7**
Theaflavin -3-gallate		**1.3**
Thearubigins		**59.2**
Kaempferol		**1.3**
Myricetin		**0.4**
Quercetin		**2**

Molecular docking is a powerful computer technology to decide the extent of interaction between ligand and target protein molecule of known three- dimension structure to predict new drug and figure (1) illustrated the virtual chemical design of the chosen bioactive compound for a chemical docking and the white line showed the hydrogen bonds in each component that might undergo chemical reaction with its receptor.

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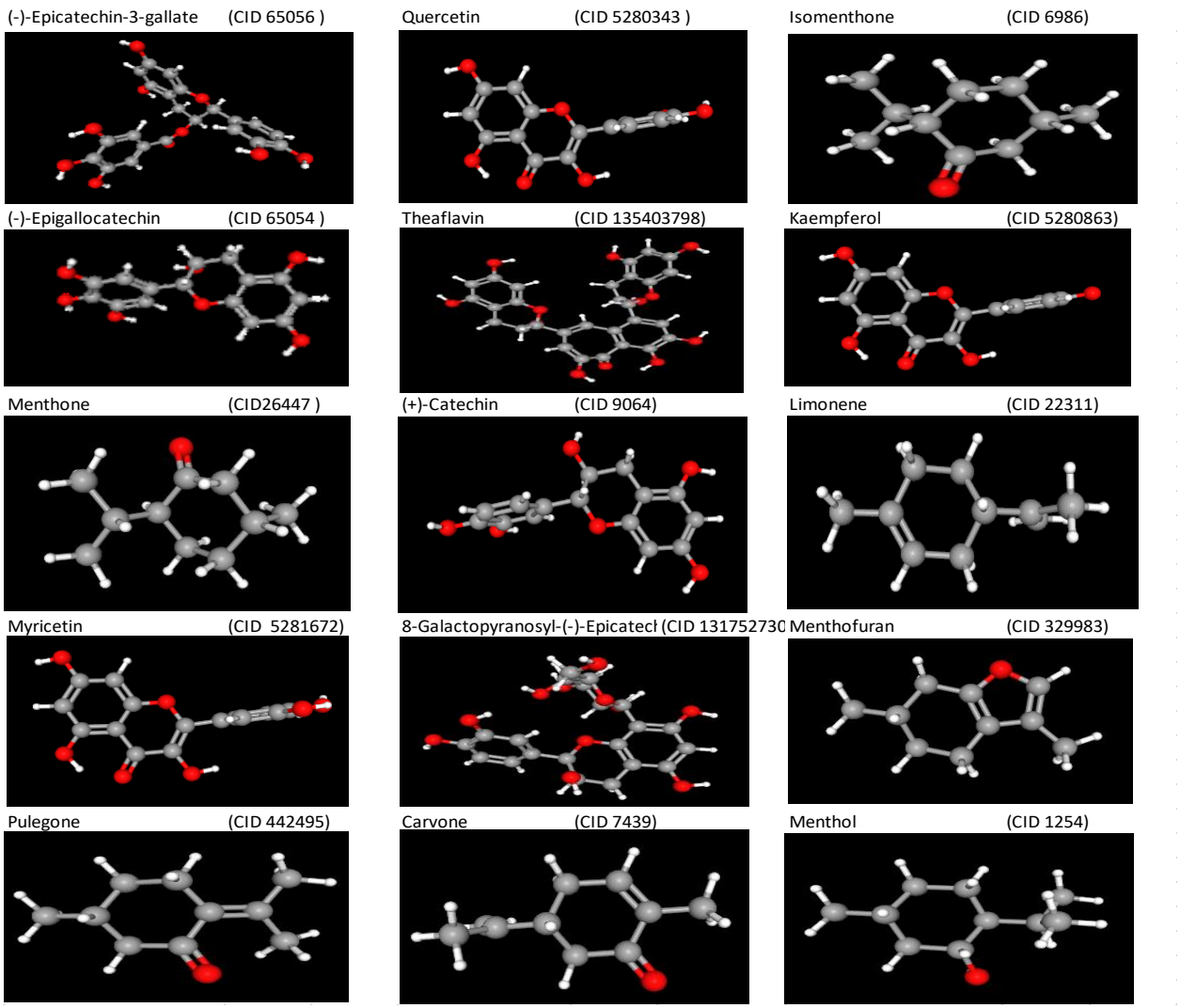

Figure (1): The modus operandi used in the study, from pharmacophore development to virtual screening to molecular docking (white dashes are hydrogen bond)

In drug discovery, molecular docking would reduce both cost and time when predicting ligand and receptor binding modes and affinities; also describing the way by two molecules fit together in three-dimension space (27).

The image of Docking results for phytochemicals with coagulase, Exoenzyme S, Extended-spectrum β-lactamase and PBP2 a protein presented in figure (2).

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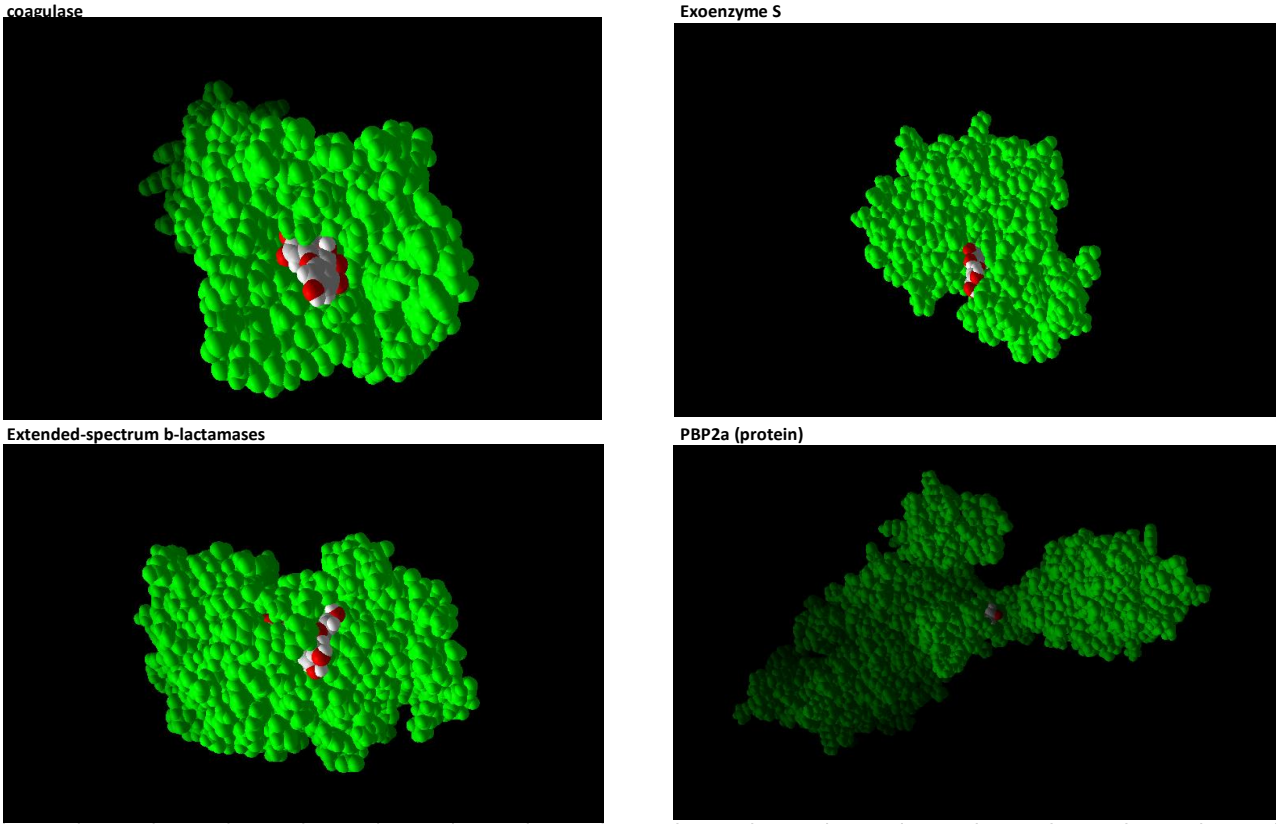

Figure(2): Docking results of phytochemicals with coagulase, Exoenzyme S, Extended-spectrum b-lactamases and PBP2a (protein)

The ligand molecules were analyzed based on their re-ranking score after they were finished. The present of phytochemicals extracted from the mint and black tea showed that: highest the re-rank score for coagulase enzyme was for Myricetin -67.152 followed by (-)-Epicatechin-3-gallate -63.374 from black tea and Menthofuran -61.042. While the appearance Re-rank scores against exozyme S was for myricetin -80.19 followed by Kaempferol from mint with re-rank score - 79.54. (-)-Epicatechin-3-gallate followed by Myricetin both extracted from black tea had promising results in extended beta- lactamase inhibition; with re-rank score value -81.82, -69.04, while results presented in table (2) indicated that lower interaction between compounds from mint with Extended- spectrum beta- lactamase. Phytochemical compounds that gave the highest re- rank against PBP2 protein were Kaempferol from mint -90.195 followed by Myricetin from black tea -80.906.

In response to the various conventional hydrogen bonds; extracted plant components Different alkyl and/or alkyl bindings were found that could contribute positively to the ligand-receptor hydrophobic interaction different alkyl and/or alkyl bindings were found that could contribute positively to the ligand-receptor hydrophobic interaction. The most hydrophobic interaction recognized with Theaflavin -11.77 followed by (-)-Epicatechin-3-gallate 11.1 for coagulase enzyme; Quercetin -15.61 then (-)-Epicatechin-3-gallate-14.29 with exozyme S. While the highest hydrophobic ligand recognized with (-)-Epicatechin-3-gallate -16.7 and -19.2 interacted with extended- spectrum β-lactamase and PBP2 protein respectively. β-lactam antibiotics have continued to be the most popular drug for treating most bacterial infections however Most commonly used β-lactam drugs as well as the third generation of β-lactam extensively subjected to hydrolyzed by a group of beta- lactamase enzyme; such enzyme secreted by penicillin-resistant *S. aureus* (28).

The extended- spectrum β-lactamase is the most class of beta lactamase and has the ability to degrade many of the third generation beta- lactam antibiotics conferring resistance to *S. aureus* and *P. aeruginosa* and inhibit by calvulanate (29,30). Results of molecular docking presented here were in accordance with previous research when found that aqueous extraction of both mint and black tea inhibited the growth of *S. aureus* (31) and the

inhibition would increase with the concentration of extract. Earlier studies recognized that gram-positive bacteria were more susceptible to black tea than gram -negative bacteria and found that (-)-Epicatechin-3-gallate inhibited the growth of *S. aureus* from tea (32).

Kaempferol extracted from black tea understudy gave the highest Re-rank score against PBP2 protein and exozyme S which predicted to the mint extract as a co- antibiotic to reduce bacterial resistance against beta- lactam drugs. As well as its interaction with exoenzyme S from *P. aeruginosa* which has multiple functions towards virulence upon burns infection; it acts as an antiphagocytic factor that enables the bacterial community invanding the immune system. Also acts as an ADP-ribosylating enzyme with multiple substrates including Ras, RalA, certain Rab proteins, Rac1, and Cdc42 low molecular-weight G (LMWG) proteins. ExoS protein has the activity of a GTPase-activating protein (GAP) which affects the cytoskeletal structure of eukaryotic burned tissue (33). Its function could extend to cause complex effects including inhibition of DNA synthesis of injury cells, alteration in cellular adherence microvillus effacement, and cell morphology leading to invade the skeletal tissue (34). From reviewing researches contribute to the bioactivity of phytochemical constitutes; it was presumed that very slow development of pathogenic bacterial resistance against drugs based on plant material. According to this perspective, it is novel to screen a variety of plants having antibacterial activity to manage the drug resistance among human pathogens as well as to reduce and inhibit the virulence factors activity. Moreover, Secondary metabolites of these spices are commonly recognized as a food safety material with insignificant adverse events. The majority of these spices are antimicrobial agents (35). Spices could therefore be candidates for the discovery and development of new antimicrobial agents for bacterial burn injury infections.

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Table (2): MolDock score, Rerank score and H-Bond score results generated from MVD docking using (MolDock score, Plant score) functions and (SE, Simplex, Optimizer) algorithms

Name	Molecular Formula	PubChem CID	Coagulase			Exoenzyme S			Extended-spectrum β-lactamase			PBP2a (protein)		
			MolDock Score	Rerank Score	HBond	MolDock Score	Rerank Score	HBond	MolDock Score	Rerank Score	HBond	MolDock Score	Rerank Score	HBond
(-)-Epicatechin-3-gallate	C22H18O10	65056	-107.81	-63.374	-11.109	-103.78	-18.21	-14.29	-121.29	-81.82	-16.7	-94.09	-61.117	-19.2
(-)-Epigallocatechin	C22H18O11	65064	-72.856	-45.598	-4.1633	-70.97	-54.26	-10.84	-63.236	-18.84	-11.3	-72.22	-46.825	-10.9
Menthone	C10H18O	26447	-59.171	-52.613	-0.7031	-55.45	-33.59	0	-46.324	-34.96	-2.1	-61.73	-53.956	0
Myricetin	C15H10O8	5281672	-82.011	-67.152	-9.4431	-87.669	-80.19	-7.743	-83.305	-69.04	-10.7	-89.09	-80.906	-16.5
Pulegone	C10H16O	442495	-71.641	-60.032	0	-62.538	-52.67	0	-54.849	-46.95	-0.95	-67.6	-55.567	0
Quercetin	C15H10O7	5280343	-88.621	-49.198	-6.7477	-101.87	-48.33	-15.61	-76.291	-43.72	-11	-96.96	-53.252	-10.6
Theaflavin	C29H24O12	135403798	-88.856	55.147	-11.777	-89.768	-54.93	-8.671	-47.247	-20.49	-7.36	-81.18	-62.531	-11.3
(+)-Catechin	C15H14O6	9064	-75.187	-58.189	-4.979	-70.673	-39.32	-9.376	-79.511	-45.14	-8.9	-82.66	-51.676	-5.97
8-Galactopyranosyl-(-)-Epicatechin	C21H24O11	13175230	953.438	-9.1592	-5.2704	940.61	-7.031	-13.7	953.925	-2.567	-7.87	948.05	-10.439	-9.96
Carvone	C10H14O	7439	-65.328	-54.643	0	-65.292	-52.63	-3.434	-49.359	-39.67	0	-68.49	-57.614	-2.5
Isomenthone	C10H18O	6986	-55.991	-42.695	-1.2916	-51.512	-35.85	-0.58	-43.13	-31.1	0	-64.27	-48.584	-2.19
Kaempferol	C15H10O6	5280863	-89.542	-40.772	-7.4262	-90.684	-79.54	-5.709	-75.62	-63.81	-3.23	-102.8	-90.195	-5.99
Limonene	C10H16	22311	-60.412	-40.436	0	-58.802	-36.99	0	-47.513	-29.71	0	-60.87	-50.746	0
Menthofuran	C10H14O	329983	-75.629	-61.042	0	-69.521	-53.28	0	-63.263	-45.71	-1.72	-79.27	-63.784	0
Menthol	C10H20O	1254	-65.428	-54.556	-2.5	-60.14	-46.05	-3.211	-51.629	-42.63	-3.86	-63.22	-49.235	-2.5

Conclusion

Molecular docking revealed that the aqueous extract of both black tea and mint have high re-rank as inhibitory substances against different virulence factors of S.aureus and P.aeruginosa.

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إنتاج الأعشاب الطبية لتثبيط والقضاء على الجراثيم التي تلوث الحروق: دراسة عبر محاكاة الكمبيوتر

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الملخص

يعاني المصابين من المدنيين بالحروق الشديدة من تداعيات عديدة أهمها هو انخفاض الفعالية المناعية الخلوية نتيجة الإصابات الجرثومية المصاحبة للحروق والتي من أهمها البكتريا الانتهازية للنوع *Staphylococcus aureus* والنوع *Pseudomonas aeruginosa* حيث يكونان مسؤولين عن أغلب إصابات المشافي بعد الرقود لفترات طويلة في المشفى. يفرز النوعان العديد من عوامل الضراوة التي تسهل من إصابة الحروق ، منها ما يساعد على الالتصاق والتموضع والتغلغل بالنسيج المصاب إضافة لمقاومة العديد من الصادات االحيوية المستخدمة في العلاج.

اعتمد نظام الالتحام الجزيئي في تحديد مدى ارتباط الرابط مع المستقبل (عامل الضراوة)، وقد صمم هذا البحث لمتابعة مدى تالف او تداخل بين المواد الفعالة المستخلصة من الشاي الاسود والنعنع مع عوامل ضراوة شملت كل من أنزيم تخثر الدم ، ايكسوزايم س، بيتا لاكتاميز واسع الطيف وبروتين مرتبط مع البيتا لاكتام وباستعمال نظام الالتحام الجزيئي.

حللت المستخلصات المائية لكل من الشاي الاسود والنعنع باستخدام جهاز GC- mass وحددت المواد الفعالة بيولوجيا لتتابع مدى تداخلها وبصورة منفردة مع عوامل الضراوة المذكورة أنفا باستخدام الالتحام الجزيئي. استخدم نتيجة عامل إعادة الترتيب في متابعة التداخلات الأفضل والأقوى بين الرابط والمستقبل.

وقد أوضحت النتائج الحالية أن المواد الفعالة من الشاي الأسود ذات تداخلات أقوى من المواد الفعالة لمستخلص النعنع. أثبتت المادة الفعالة الميرسيتين أنها أفضل حيث احتاجت إلى طاقة تفاعل قدرت 80.906 - و 80.19 - و 67.06 - لكل من بروتين المرتبط بالبيتا لاكتام و ايكسوأنزيم س و بيتا لاكتاميز واسع الطيف وأنزيم الكواكيوليز على التتابع. كما لوحظ ان الميرستين أكثر كفاءة بالارتباط مع عوامل ضراوة *P. aeruginosa* من تلك العائدة لبكتريا *S. aureus* . لوحظ أن الكامبفور ياتي بالمرتبة الثانية في كفاءة التداخل وقد سجلت نتيجة 90.195- و79.54- مع بروتين الارتباط بالبيتا لاكتام و الاكسوزايم س من بكتريا *P.aeruginosa*.

اوضحت تقنية المحاكاة ان المستخلص المائي لكل من الشاي الاسود والنعنع احتوى على عدد من المواد التي اظهرت اعادة ارتباط عالي لتثبيط عدد من عوامل الضراوة لبكتريا *S. aureus* وبكتريا *P.aeruginosa* مما يؤهلها لان تستخدم في علاج اصابات الحروق .

الكلمات المفتاحية : الالتحام الجزيئي ، درجة إعادة الترتيب، اكسوزايم س، أنزيم تخثر الدم.