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Research Article

Molecular Docking of Bioactive Compounds from *Piper* Plants Against Secreted Aspartyl Proteinase of *Candida albicans* Causing Oral Candidiasis

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ABSTRACT

Oral candidiasis is a common opportunistic fungal infection of the oral cavity caused by *Candidaalbicans*. A the most serious level, mortality rates of candidiasis are high. Resistance to antimicrobial agents among bacteria and fungi is a persistent problem complicating the management of critically ill patients. In recent years, drug resistance to human pathogenic fungus has been commonly reported from all over the world. Even though pharmaceuticalsectors have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. Hence, the present study was carried out to evaluate the effect of *Piper nigrum*and *Piper betle* against opportunistic pathogen *Candida albicans* causing oral candidiasis. Totally 55 and 25 compounds were identified from *P.nigrum* and *P.betle* respectively. The identified compounds were tested against oral candidiasis causing enzymes by *C. albicans* through molecular docking and observed Piperine has great potential. The selected compounds from these plants could be utilizedfor thedevelopment of novel drugs against oral candidiasis.

Keywords: Oral candidiasis, SAP, GC-MS, Hex, phytochemical compounds, Piperine, *P. nigrum*, *P. betle*

INTRODUCTION

Candida albicans is a commensal fungus that is often part of the oral microflora of healthy people. Loss of host immunity, HIV infection, corticosteroid use, or alteration of the oral microflora following antibiotic therapies permits a pathogenic transition of *C. albicans* to cause oral candidiasis[1]. *C. albicans* is normally present as nonpathogenic yeast form in the oral cavity of healthy individuals and under favorable conditions, has the ability to transform into a pathogenic hyphal form. The ability of *C. albicans* to adhere to buccal epithelial cells is critical in establishing oral colonization. After colonization, the organisms may persist for months or years in low numbers in the absence of inflammation. *C. albicans* expresses specific sets of virulence factors that promote hypha formation and adhesion and invasion of host tissues[2]. Secreted aspartyl proteinases (Saps) are recognized important virulence factors because they degrade host proteins to provide nitrogen for fungal cell metabolism, contribute to adherence, facilitate fungal epithelial and endothelial penetration, and are immunogenic during infection. *C. albicans* expresses a family of 10 *SAP* genes that are clustered into groups *SAP1* to *SAP3*, *SAP4* to *SAP6*, *SAP7*, *SAP8*, and *SAP9* and *SAP10* based upon their sequence homologies and pH activities[3,4]. In the past few decades, most famous and effective synthetic anticandidal drugs became ineffective due to the virulence of *C. albicans* especially in immunodeficient patients. These drugs have various drawbacks in terms of toxicity, efficacy, cost and their frequent use has led to the emergence of resistant strains. In particularthe effective treatment for the multidrug resistant *C. albicans* causing OPC emerged as a new challenge for fungal therapy. There is an emergence need for novel molecules, which is lower side effect and can serve as lead compound for the treatment of oral candidiasis. To overcome this problem plant based drugs is only solved this problem. In this context, many natural products and essential oils have shown to be alternative therapies for the treatment of candidiasis[5,6]. *P. nigurm* is used in treatment for asthma, cough, diabetes and heart problems. *Piper betle*was shown to contain compounds that have anti-diabetic and anti-allergic effects[7]. On the other hand, over the past year there have been some interesting and significant advances in computerbased ligand protein docking techniques and related rational drug-design. Docking is an estimated by energy values. As a result, the successful use of computational tools to help generate interesting new drugs for targeted receptors[8]. Hence, in the present study phytochemical compounds isolated from *P. nigrum* and *P. betle*were tested against SAP enzymes of *C.albicans* through molecular docking.

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MATERIALS AND METHODS

Collection of plant materials

Plant samples such as disease free fresh fruits of *P. nigrum*, and leaves of *P. betle*were collected from various area in Kollihills, Namakkal, Tamilnadu, India. The plant materials were authenticated and voucher specimens were deposited in herbarium (Rapinat Herbarium) of St. Joseph's college, Trichy, Tamil Nadu, India.

Extraction of phytochemical compounds

Fruits of *P. nigrum*, and leaves of *P. betle* were washed with dechlorinated water, dried in shade and powdered with the help of an electric blender. The dried samples were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. 100 gm of crushed samples were continuously extracted with 95% methanol using soxhlet up to 48 hours. The extract was filtered and concentrated in rotatory evaporator at 35-40 ºC under reduced pressure to obtain a semisolid material, which was then lyophilized to get a powder (28.5%, w/v). The overall extraction procedure followed by method described in Harborne[9].

Qualitative analysis of phytochemical compounds

The methanolic leaves extract of two test samples weresubjected to following test for the identification of its various active constitutions by standard methods. Alkaloids were identified by Dragendroff's test, flavonoids and were identified by lead acetate test, carbohydrates were identified by Fehling's test, proteins were identified by Million's test, phenols were identified by Libermann's test and tannins were identified by Ferric chloride test. Saponins, Phytosterolterpenoids and Phlobatannins were identified by Harborne methods[9].

Gas Chromatography – Mass Spectroscopy (GC-MS) analysis

The powdered sample of two herbal products such as *P. nigrum*and*P. Betle* (20 g) were soaked and dissolved in 75 ml of methanol for 24 hours. Then the filtrates were collected by evaporated under liquid nitrogen. The GC-MS analysiswas carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatographequipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 x m dfcapillary column. Test methodology followed by protocol[10]. The instrument was set to an initial temperature of 110˚C, and maintainedat this temperature for 2 min. At the end of this period, the oven temperature was raised up to280˚C, at the rate of an increase of 5˚C/min, and maintained for 9 min. Injection porttemperature wasensured as 250˚C and Helium flow rate as 1 ml/min. The ionization voltagewas 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range wasset at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in thespectrometer database using National Institute of Standards and Technology Mass Spectraldatabase (NIST-MS). The percentage of each component was calculated from relative peakarea of each component in the chromatogram.

Molecular docking

The structure of SAPs 1-8 was retrieved from PDB[11]and the structure of bioactive compounds from drug bank[12]. Hex is an effective tool for molecular docking among the variety of computational methods. In the present study, bioactive compound act as ligand and SAP isoenzymes (1-8) from *C. albicans* act as receptor which made to dock with help of Hex software[13].

RESULTS

Qualitative analysis

The qualitative phytochemical analysis of methanolic extracts of *P. nigrum*fruitsand *P. betle*leavesrevealed the presence of alkaloids, carbohydrates, saponins, phenols, terpenoids, phytosterols, flavonoids, tannins, and phlobatannins. Tannins were absent in *P. betle* and present in *P. nigrum* (Table 1).

Gas Chromatography – Mass Spectroscopy (GC-MS) analysis

The phytochemical compounds present in *P. nigrum*and *P. betle*were identified by GC-MS analysis.Totally 55 compounds were identified from *P. nigrum*. The high peakcompounds in this sample were Piperine **(**26.6745 %), Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4Z,9S*)]- (4.8802 %), Eugenol (9.5204 %), o-Anisic acid,2-adamantyl ester (17.9390). The GC-MS chromatogram of *P. nigrum*is shown in Figure 1. The result of the GC-MS analysis of 25 compounds of *P.betle*samples was identified. High percentage of 2,5-Dimethoxybenzoic acid (65.8992 %), Phenol, 2-methoxy-3-(2-propenyl)- (6.1550 %), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (4.9169 %), Phytol (8.0181 %), Piperine (1.6883 %) compounds were identified (Fig.2).

Molecular docking

The structure of SAPs 1-8 was retrieved from PDB data bank and the structure of bioactive compounds from drug bank. All the ligand molecules were made to dock against the active sites of the target antigen using Hex software. The docking results were represented in the form of e-negative values (Tables.2& 3). In the docking studies, higher negative e-values represent high binding affinity between the receptor and ligand molecules, indicating the higher efficiency of the bioactive compounds. SAPs of *C. albicans*, on docking with bioactive compounds namely Eugenol, Bicyclo, Piperine (Fig.3) and O-Anisic acid of *P. nigrum* produced energy values such as - 149.68, -143.16, - 698.77 and -205.91respectively. Similarly, the bioactive compounds, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Phytol, Phenol and 2-methoxy-3-2-propenyl2,5-Dimethoxybenzoic acid of *P. betle* produced energy values such as-223.87, - 223.87- 166.50 and -159.45 respectively. The results showed that all the bioactive compounds with target antigens produced high negative e-value. Thus, it is clear that the bioactivecompounds were able to interact with any of the available binding sites of the SAP familyenzymes effectively. The above study clearly indicates that the bioactive compoundswere able to inhibit

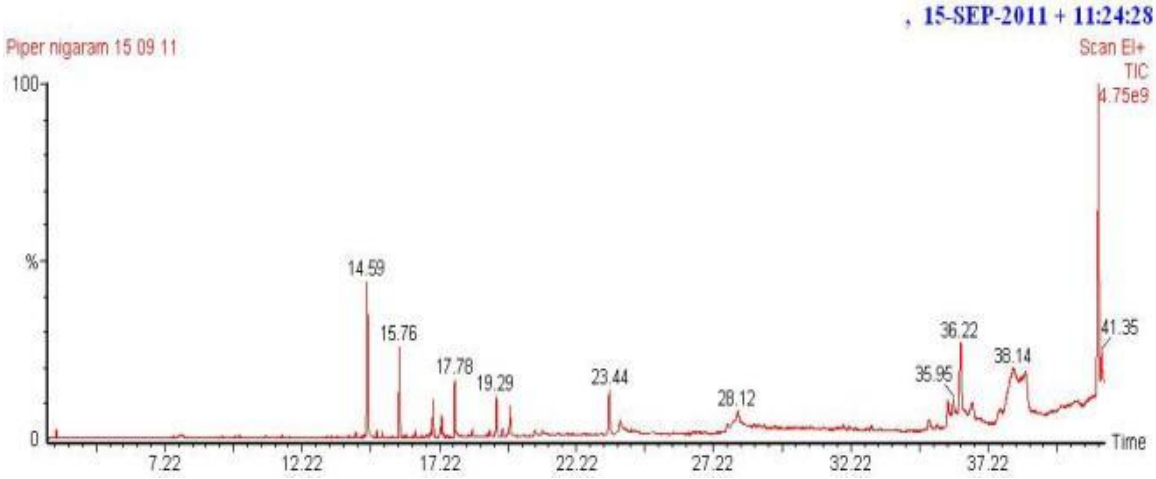

Figure 1: GC-MS chromatogram of *Piper nigrum*

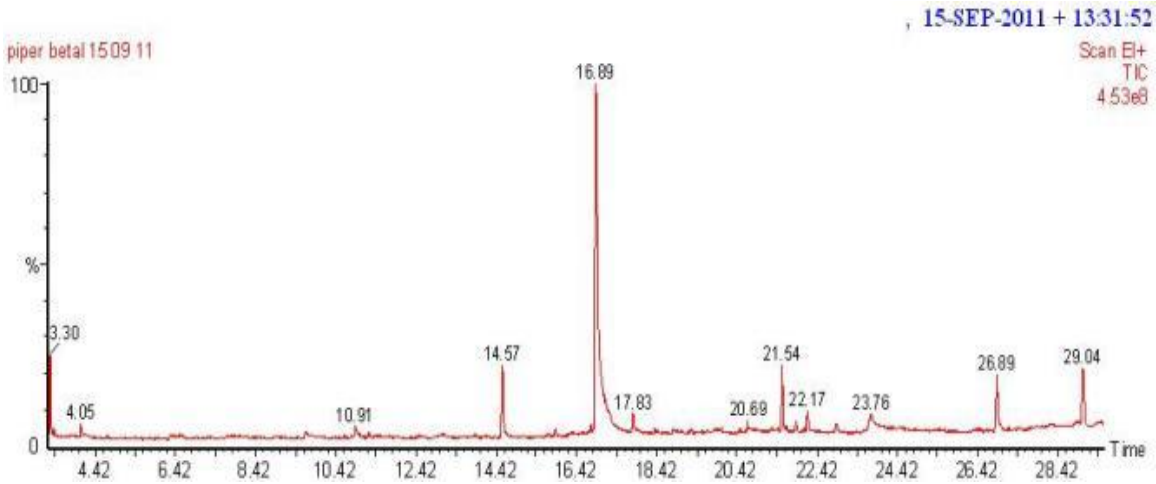

Figure 2: GC-MS chromatogram of *Piper betle*

Table 1: Qualitative analysis of *Piper nigrum*and*Piper betle*

Phytochemicals	*P. nigrum*	*P. betle*
Alkaloids	Present	Present
Flavonoids	Present	Present
Carbohydrates	Present	Present
Protein	Present	Present
Phenols	Present	Present
Saponins	Present	Present
Tannins	Present	Absent
Phytosterols	Present	Present
Terpenoids	Present	Present
Phlobatannins	Absent	Absent

the activity of the SAPs 1-8 enzymes(Fig.4). Among the bioactive compounds studied, piperine from *P. nigrum* and *P. betle*s showed a higher negative energy value of -698.77(Fig.5) than that of other compoundsindicating as effective anticandidal activity over other compounds.

DISCUSSION

The quantitative GC/MSphytochemical analysis showed totally 55 compounds from *P. nigrum*and 25 compoundsfrom *P. betle*. These compounds belongto various groups like alkaloids, flavonoids, carbohydrates, saponins, tannins, phenol andterpenes. Flavonoids are reported to exhibit antioxidant activity[14] andare effective scavengers of superoxide anions[15]. Alkaloids havebeen identified to have antifungal activity; for examplepiperin a compound of the alkaloidclass isolated from *Piperspp*. The mechanism of action here is attributed to the alkaloidability to intercalate with DNA[16]. There is, therefore, reason to believethat the activity of piper could be attributed to the presence of alkaloids as revealed by thepreliminary qualitative phytochemical analysis. Terpenoids and alkaloids have been provedto have antifungal activity.

Presence of alkaloids and terpenoids in the leaf extracts of *P. nigrum* exhibit antifungal properties could serve as a basis for its traditional use as amedicinal plant. This agrees with what was reported by Ghoshal*et al*.[16] that alkaloids, terpenoids and lactones are responsible for antifungal activityand have beenfound to inhibit yeast growth[17].Emergence of multi-drug resistance in human and animal pathogenic fungi as well asundesirable side effects of certain antibiotics has triggered immense interest in the search fornew antimicrobial drugs of plant origin[18]. The antimicrobial activity ofphenolic compounds found in Piperaceae and Myrtaceaehave been studied mostly againstvarious fungal infections. The key roleof phenolic compounds as scavengers of free radicals is emphasised in several reports[19].Polyphenolic compounds have an

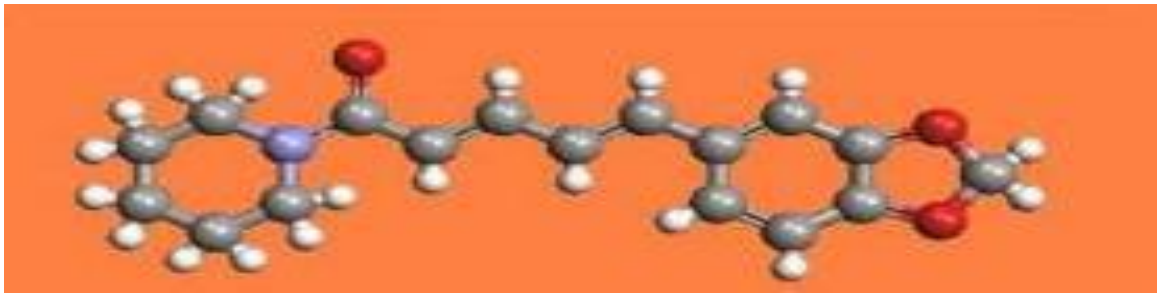

Figure 3: Three-dimensional structure of Piperine

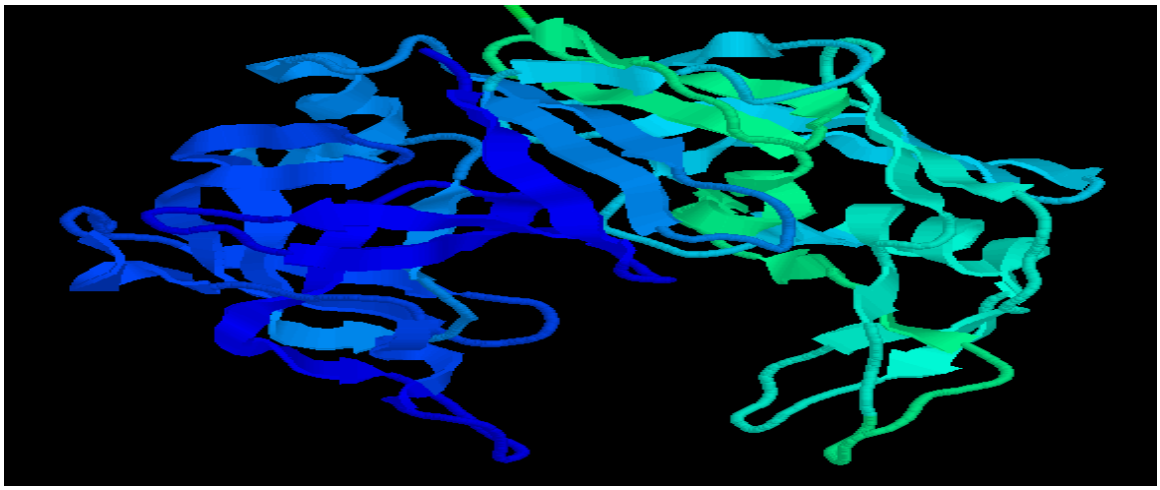

Figure 4: Molecular structure of SAP 1

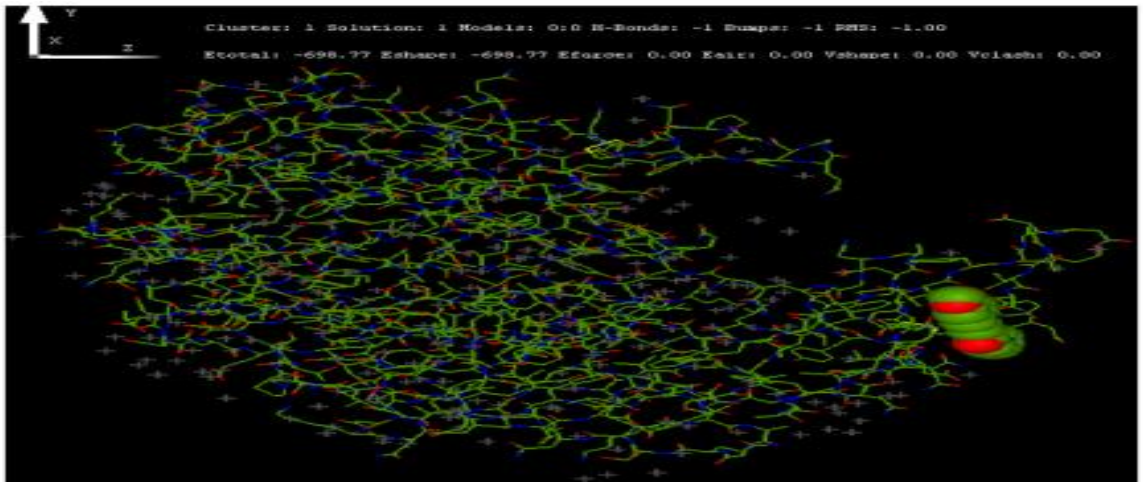

Figure 5: Molecular docking of Piperine with SAP1

important role in stabilizing lipid oxidation and areassociated with antioxidant activity[20]. The phenoliccompounds may contribute directly to anti-oxidative action. It is suggested that polyphenoliccompounds have inhibitory effects on mutagenesis and carcinogenesis in humans when up to10 g is ingested daily from adiet rich in fruits and vegetables[21].Thepowder of these two test plants can be used as natural healers against *C. albicans* because of their rich alkaloids and phenolic content. Saponins are secondary plant metabolites that occur in a wide range of plant species[22]. They are stored in plant cells as inactive precursors but arereadily converted into biologically active antibiotics by plant enzymes in response topathogen attack. The natural role of saponins in plants is thought to be protection againstattack by pathogens and pets[23]. These molecules also haveconsiderable commercial value and are processed as drugs and medicines, foaming agents, sweeteners, taste modifiers and cosmetics. In the present study, saponins of *P. nigrum*and *P. betle* havestudied for multidrug resistance *C. albicans* and allof these species are also rich in highly fungicidal saponins. It was concluded from this studythat the presence of these phytochemical in*P. nigrum* and *P. betle* have anticandidal activity. The result of this experiment indicates that thesemedicinal plants have potentiality to treat multiple resistant

Table 2: Molecular docking of effective bioactive compound from *P. nigrum*with SAP1-8

S. No	Name of the bioactive compound	Retention time	Peak area in percentage	Name of the receptor	E-value of docking
		EUGENOL			
1.	Name: Eugenol Formula: $C_{10}H_{12}O_2$ MW: 164	14.59	9.5204	SAP1	-147.36
2.	Name: Eugenol Formula: $C_{10}H_{12}O_2$ MW: 164	14.59	9.5204	SAP2	-48.43
3.	Name: Eugenol Formula: $C_{10}H_{12}O_2$ MW: 164	14.59	9.5204	SAP3	-80.68
4.	Name: Eugenol Formula: $C_{10}H_{12}O_2$ MW: 164	14.59	9.5204	SAP4	-10.89
5.	Name: Eugenol Formula: $C_{10}H_{12}O_2$ MW: 164	14.59	9.5204	SAP5	-57.76
6.	Name: Eugenol Formula: $C_{10}H_{12}O_2$ MW: 164	14.59	9.5204	SAP6	-149.68
7.	Name: Eugenol Formula: $C_{10}H_{12}O_2$ MW: 164	14.59	9.5204	SAP7	-16.59
8.	Name: Eugenol Formula: $C_{10}H_{12}O_2$ MW: 164	14.59	9.5204	SAP8	-19.06
		BICYCLO			
1.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene Formula: $C_{15}H_{24}$ MW: 204	15.76	4.8802	SAP1	-137.51
2.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene Formula: $C_{15}H_{24}$ MW: 204	15.76	4.8802	SAP2	-84.10
3.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene Formula: $C_{15}H_{24}$ MW: 204	15.76	4.8802	SAP3	-88.06
4.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene Formula: $C_{15}H_{24}$ MW: 204	15.76	4.8802	SAP4	-143.12
5.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, Formula: $C_{15}H_{24}$ MW: 204	15.76	4.8802	SAP5	-81.58
6.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene Formula: $C_{15}H_{24}$ MW: 204	15.76	4.8802	SAP6	-142.54
7.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene	15.76	4.8802	SAP7	-31.68

	Formula: $C_{15}H_{24}$ MW: 204				
8.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]- Formula: $C_{15}H_{24}$ MW: 204	15.76	4.8802	SAP8	-143.16
		PIPERINE			
1.	Piperine Formula: $C_{17}H_{19}NO_3$ MW: 285	41.24	26.6745	SAP1	-698.77
2.	Piperine Formula: $C_{17}H_{19}NO_3$ MW: 285	41.24	26.6745	SAP2	-31.60
3.	Piperine Formula: $C_{17}H_{19}NO_3$ MW: 285	41.24	26.6745	SAP3	-152.88
4.	Piperine Formula: $C_{17}H_{19}NO_3$ MW: 285	41.24	26.6745	SAP4	-594.16
5.	Piperine Formula: $C_{17}H_{19}NO_3$ MW: 285	41.24	26.6745	SAP5	-651.88
6.	Piperine Formula: $C_{17}H_{19}NO_3$ MW: 285	41.24	26.6745	SAP6	-92.87
7.	Piperine Formula: $C_{17}H_{19}NO_3$ MW: 285	41.24	26.6745	SAP7	-541.55
8.	Piperine Formula: $C_{17}H_{19}NO_3$ MW: 285	41.24	26.6745	SAP8	-187.94
		O-ANISIC ACID			
1.	Name: o-Anisic acid, 2-adamantyl ester Formula: $C_{18}H_{22}O_3$ MW: 286	36.22	17.9390	SAP1	-184.50
2.	Name: o-Anisic acid, 2-adamantyl ester Formula: $C_{18}H_{22}O_3$ MW: 286	36.22	17.9390	SAP2	-43.54
3.	Name: o-Anisic acid, 2-adamantyl ester Formula: $C_{18}H_{22}O_3$ MW: 286	36.22	17.9390	SAP3	-146.05
4.	Name: o-Anisic acid, 2-adamantyl ester Formula: $C_{18}H_{22}O_3$ MW: 286	36.22	17.9390	SAP4	-191.71
5.	Name: o-Anisic acid, 2-adamantyl ester Formula: $C_{18}H_{22}O_3$ MW: 286	36.22	17.9390	SAP5	-118.27
6.	Name: o-Anisic acid, 2-adamantyl ester Formula: $C_{18}H_{22}O_3$ MW: 286	36.22	17.9390	SAP6	-182.78
7.	Name: o-Anisic acid, 2-adamantyl ester Formula: $C_{18}H_{22}O_3$ MW: 286	36.22	17.9390	SAP7	-148.96
8.	Name: o-Anisic acid, 2-adamantyl ester Formula: $C_{18}H_{22}O_3$ MW: 286	36.22	17.9390	SAP8	-205.91

Table 3: Molecular docking of effective bioactive compound from *P. betle*with SAP1-8

S. No	Name of the bioactive compound	Retention time	Peak area in percentage	Name of the receptor	E-value of docking
	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL				
1	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	21.54	4.9169	SAP1	-223.87
2	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	21.54	4.9169	SAP2	-196.42
3	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	21.54	4.9169	SAP3	-141.82
4	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	21.54	4.9169	SAP4	-202.49
5	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	21.54	4.9169	SAP5	-170.52
6	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	21.54	4.9169	SAP6	-209.39
7	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	21.54	4.9169	SAP7	-164.87
8	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	21.54	4.9169	SAP8	-214.95
	PHYTOL				
1	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	26.89	8.0181	SAP1	-223.87
2	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	26.89	8.0181	SAP2	-196.42
3	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	26.89	8.0181	SAP3	-141.82
4	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	26.89	8.0181	SAP4	-202.49
5	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	26.89	8.0181	SAP5	-176.81
6	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	26.89	8.0181	SAP6	-209.39
7	Name: Phytol	26.89	8.0181	SAP7	-164.87

	Formula: $C_{20}H_{40}O$ MW: 296				
8	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	26.89	8.0181	SAP8	-10.00
	PHENOL, 2-METHOXY-3-(2-PROPENYL)				
1	Name: Phenol, 2-methoxy-3-(2-propenyl)- Formula: $C_{10}H_{12}O_2$ MW: 164	14.57	6.1550	SAP1	-144.64
2	Name: Phenol, 2-methoxy-3-(2-propenyl)- Formula: $C_{10}H_{12}O_2$ MW: 164	14.57	6.1550	SAP2	-95.36
3	Name: Phenol, 2-methoxy-3-(2-propenyl)- Formula: $C_{10}H_{12}O_2$ MW: 164	14.57	6.1550	SAP3	-87.27
4	Name: Phenol, 2-methoxy-3-(2-propenyl)- Formula: $C_{10}H_{12}O_2$ MW: 164	14.57	6.1550	SAP4	-141.33
5	Name: Phenol, 2-methoxy-3-(2-propenyl)- Formula: $C_{10}H_{12}O_2$ MW: 164	14.57	6.1550	SAP5	-105.86
6	Name: Phenol, 2-methoxy-3-(2-propenyl)- Formula: $C_{10}H_{12}O_2$ MW: 164	14.57	6.1550	SAP6	-155.19
7	Name: Phenol, 2-methoxy-3-(2-propenyl)- Formula: $C_{10}H_{12}O_2$ MW: 164	14.57	6.1550	SAP7	-91.51
8	Name: Phenol, 2-methoxy-3-(2-propenyl)- Formula: $C_{10}H_{12}O_2$ MW: 164	14.57	6.1550	SAP8	-166.50
	2,5-DIMETHOXYBENZOIC ACID				
1	Name: 2,5-Dimethoxybenzoic acid Formula: $C_9H_{10}O_4$ MW: 182	14.57	65.8992	SAP1	-145.84
2	Name: 2,5Dimethoxybenzoic acid Formula: $C_9H_{10}O_4$ MW: 182	16.89	65.8992	SAP2	-101.48
3	Name: 2,5-Dimethoxybenzoic acid Formula: $C_9H_{10}O_4$ MW: 182	16.89	65.8992	SAP3	-92.96
4	Name: 2,5-Dimethoxybenzoic acid Formula: $C_9H_{10}O_4$ MW: 182	16.89	65.8992	SAP4	-149.51
5	Name: 2,5-Dimethoxybenzoic acid	16.89	65.8992	SAP5	-87.44

	Formula: $C_9H_{10}O_4$ MW: 182				
6	Name: 2,5-Dimethoxybenzoic acid Formula: $C_9H_{10}O_4$ MW: 182	16.89	65.8992	SAP6	-159.45
7	Name: 2,5-Dimethoxybenzoic acid Formula: $C_9H_{10}O_4$ MW: 182	16.89	65.8992	SAP7	-116.20
8	Name: 2,5-Dimethoxybenzoic acid Formula: $C_9H_{10}O_4$ MW: 182	16.89	65.8992	SAP8	-157.59

opportunistic *C. albicans.* Infections and it could be utilized to create a healthy environment. The treatment of patients with oral candidiasis involving only antifungal medicationsis difficult to achieve or it may not be possible in patients with oral candidiasis. Successfultreatment of candidiasis could be hampered when *C. albicans*get well established in the oralcavity. *C.albicans* in the oral cavity exhibits significantly higher tolerance to traditionalantifungal agents. Therefore, alternative strategies like elucidation of bioactivecompounds from medicinal plants to treat the oral candidiasis. This process involvesscreening of various bioactive compounds from medicinal plants through GC-MS analysis. The protein-ligands interaction plays a significant role in determining the suitable drugs for the treatment. A variety of computational methods to identify the suitable drugs are available. One such method is docking of drug molecules with receptors. The energy value obtained through docking is used as a criterion for the selection of drugs. Based on the energy values, lead molecules are identified. This infers that the lead molecules are one with maximum interaction having high negative e-value[24]. Thus the concept of protein-ligand docking helps in finding the suitable drugs for oral candidiasis. Among the variety of computational methods, Molecular docking using Hex is an effective method and it is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of Protein and DNA molecules. The docking results are ordered by negative energy values. The high negative energy value having compound is the seed member for drug designing. In molecular docking bioactive compound act as ligand and virulence factor of *C. albicans* act as receptor. *C. albicans*produces several virulence extra cellular hydrolyticenzymes such as phospholipases, lipases and secreted aspartyl proteinases (SAP). Among the virulent factorssaps are considered to bethe most important virulence factor in *C. albicans*and ithelpful to adhere and invade the host during the infection[25].At present ten aspartyl proteases have been identified in *C. albicans* (SAPs1-10). Monod *et al*.,[26] classified the SAPs according to their aminoacid sequences and Schaller*et al*.[27] classified the SAPs based on the role of infection. Naglik*et al*.[25]classifiedthe SAPs according to their active pH range. Among SAPs1-10, SAPs1-8 structure wasknown and SAPs9-10 was unknown. In the present study, SAPs 1-8 were taken for the study.In the docking studies, higher negative e-values represent high binding affinity between the receptor and ligandmolecules, indicating the higher efficiency of the bioactive compounds SAPs of *C. albicans*. The bioactive compounds were able to inhibit the activity of the SAPs1-8 enzymes. Among the bioactive compounds studied, piperine from *P. nigrum* and *P. betle* showed a higher negative energy value of -698.7than that of other compoundsindicating as effective anticandidal activity over other compounds.

CONCLUSION

GC-MS analysis of the present study revealed that occurrence of 55 bioactive compounds identified from *P. nigrum* and majority of the compounds are eugenol, bicyclo, piperine and o-anisic acid. In the case of *P. betle*25 compounds were identified, among 5 compounds such as3,7,11,15-Tetramethyl-2-hexadecen-1-ol, phytol, Phenol, 2-methoxy-3-(2-propenyl) and 2,5-Dimethoxybenzoic acid were detected to show anti-candida activity. From the molecular docking analysis, among the anti candidal bioactive compounds piperine alone was found to be more efficient (-698.77) against SAP enzymes. From the above results it is concluded that *P. nigrum* and *P. betle* have great potentialto designnew leadphy to medicine compounds showing higher inhibitory activities against opportunistic *C. albicans* infections.

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