Chemical composition, anti-tyrosinase, and molecular docking studies of *Knema furfuracea* Warb. essential oil

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Knema furfuracea Warb., a tree species belonging to the family Myristicaceae and indigenous to Southeast Asia, has been traditionally used by the Dai people for the treatment of inflammation and other illnesses. This work investigates the chemical composition of the essential oil of *K. furfuracea*, antityrosinase activity, and molecular docking studies. The essential oil was obtained through hydrodistillation and characterised through gas chromatography (GC/FID) and gas chromatography mass spectrometry (GC/MS). The anti-tyrosinase activity was determined using mushroom tyrosinase enzyme. The major components of the essential oil were studied for their potential interactions with tyrosinase using Autodock vina. A total of thirty-one components (96.0%) were identified from the leaf oil and composed mainly of bicyclogermacrene (23.1%), δ-cadinene (17.2%), (*E*,*E*)-α-farnesene (9.1%), and β-caryophyllene (7.7%). The essential oil demonstrated moderate activity towards tyrosinase with an IC_{50} value of 80.3 µg/mL. Regarding the molecular docking study, β-caryophyllene indicated a strong inhibitory activity (–6.7 Kcal/mol). These findings suggest that *K. furfuracea* essential oil has the potential as a natural source of tyrosinase inhibitors.

Key words: β-caryophyllene, essential oil, *Knema furfuracea,* Myristicaceae, tyrosinase

1. INTRODUCTION

Essential oils are a type of secondary metabolite produced by plants. Essential oils are concentrated, natural combinations of bioactive compounds with a wide range of organic structures [1]. Essential oils are a valuable natural resource used in a wide variety of industries, including cosmetics, food and beverage, home and personal care, and perfume and chemical industries [2]. Since antiquity, it has been understood that the essential oils extracted from aromatic and therapeutic plants contain biological activities, including, most notably, antibacterial, antifungal, and antioxidant characteristics [3-6].

The genus *Knema* Lour. (Myristicaceae) is commonly found in tropical countries such as Asia, Africa, and Australia. Southeast Asia has about 60 species, locally known as '*pala hutan*' or '*penarahan'* [7]. The seeds and bark of several *Knema* species are utilized in traditional medicine to treat diseases, including cancer, skin disorders, and ulcers in the mouth and throat [8]. The phytochemical composition of the genus *Knema* has been a topic of interest in previous studies. Despite this, only a limited number of species have been investigated. Several compounds have been identified, such as alkyl and acyl resorcinol derivatives of phenylalkylphenol, flavonoids, anarcadic acid, lignans, and stilbenes [9-14]. Moreover, various species of *Knema* have demonstrated significant properties such as acetylcholinesterase, cytotoxicity, anti-inflammatory, nematicidal, and antibacterial activities [15,16]. Against NCI-H187 and Vero cells, for instance, the IC_{50} values for the hexane extract of *K. globularia* were 47.5 and 29.0 µg/mL, respectively [17]. Additionally, IC_{50}

values for knecorticosanone B and malabarocone, both isolated from *K. globularia*, ranged from 8.76 to 18.74 μM against Hep-G2, MCF-7, and SK-LU-1 cell lines [18]. Acetylcholinesterase was inhibited strongly by 2-hydroxy-6-(10′(Z)-heptadecenyl) benzoic acid, also found in *K. laurina* stem barks (IC_{50} value 0.57 μM) [19]. Knepachycarpanone A which was isolated from the fruit extract of *K. pachycarpa* showed considerable inhibitory action (IC_{50} value 26.92 μM) against the Hela cancer cell line [20]. The acetone extract of *K. furfuracea* included three compounds that actively inhibited nitric oxide (NO) generation in LPS-activated RAW264.7 macrophages (IC_{50} values of 3.79, 9.28, and 15.14 μM, respectively). These compounds were 7,4′ - dihdroxy-4′-methoxyflavanol, fisetinidol, and virolanol C [21].

Knema furfuracea Warb. is a species of tree in the family Myristicaceae. It is native to Southeast Asia and can be found in Thailand, Malaysia, Indonesia, and the Philippines. The tree can grow up to 25 meters of height. The twigs at the top are about 4-10 mm in diameter, with dense hairs that eventually fall off. The leaves are oblong to lanceolate, coriaceous, and about 10-50 cm long by 3-21 cm wide. The male and female flowers are small and have hairs about 0.5-1 mm long. The tree produces small, yellowish-green flowers that are grouped in clusters. The fruit is a brownish-red, pear-shaped nut that contains a single seed. The phytochemicals of *K. furfuracea* have been studied. It has been found to contain compounds such as phenolic acids, flavonoids, and lignans, some of which have shown promising biological activities [21,22].

Tyrosinase, an enzyme that inhibits melanogenesis and contains copper, has been extensively studied for its potential as a cosmetic agent. While human and mammalian tyrosinases are glycosylated monomeric enzymes anchored to the melanosome membrane, mushroom tyrosinase is a soluble tetrameric enzyme found within the cytoplasm [23]. Due to its low price and commercial availability, mushroom tyrosinase has been widely used as the foundation for developing tyrosinase inhibitors of melanogenesis. However, despite some promising compounds, only some have been used in clinical settings due to a lack of efficacy or undesirable side effects such as potential carcinogenicity. Natural derivatives such as azelaic acid, hydroquinone, kojic acid, and arbutin have been utilised as skin lightening products for scientific and beauty purposes. However, concerns have been raised about their safety and potential toxicity to various systems [24]. Therefore, there is a need for new and effective tyrosinase inhibitors to address these side effects.

As we continue our investigation into bioactive volatile components from Malaysian species, this study focuses on the chemical composition of the essential oil of *K. furfuracea* and its potential anti-tyrosinase activity through molecular docking studies. This research is motivated by the fact that previous studies on the essential oils of the *Knema* genus are limited, and there is a need to further explore their chemical constitution and potential bioactivity.

2. MATERIAL AND METHODS

2.1 PLANT MATERIAL

The plant material used in the study was obtained by collecting leaves from *K. furfuracea* in Behrang, Perak, (3° 44’ 51.612” N 101° 27’ 19.9008” E) during October 2019. The identification of the plant was carried out by Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM) and voucher specimens (SK376/19) were placed in the UKMB Herbarium at UKM.

2.2. ISOLATION OF ESSENTIAL OIL

The 300 g of *K. furfuracea* leaves were cut up and hydrodistilled in a Clevenger apparatus for 4 hours. After collecting the essential oil, it was dried with anhydrous magnesium sulphate, filtered, and stored in brown glass vials at 0°C until further examination could be performed. The average moisture content was between 85% and 89%, and the oil yield was 0.21% based on the weight of the fresh leaves [25].

2.3 ANALYSIS OF ESSENTIAL OIL

Gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS) analyses were conducted using Agilent Technologies 7890B and 7890A GC systems, respectively. GC-FID and GC-MS were equipped with HP-5MS capillary columns of 30 m × 0.25 mm × 0.25 μm film thickness. Helium was used as the carrier gas with specific flow rates for each analysis. Injector and detector temperatures were set at 250 and 280°C, respectively. The oven temperature was programmed to increase from 50°C to 280°C at a rate of 5°C/min and held isothermally for 15 min. Diluted samples were manually injected with split ratios and volumes as mentioned. Peak area percentage was calculated using the GC HP Chemstation GC software. GC-MS detection was performed with electron ionisation at 70 eV, using a scan rate of 0.5 s (cycle time: 0.2 s) and covering a mass range of 40-400 amu. Identification of essential oil components was done using co-injections with selected standards, retention index, and mass spectra comparisons with libraries and literature [26-27]. Semi-quantification was performed by normalising peak areas using the same response factor for all volatile components. Standards used were obtained from Sigma-Aldrich.

2.4 ANTI-TYROSINASE ACTIVITY

A modified version of the previously reported method [29] was used to conduct the tyrosinase inhibition assay. Essential oils and kojic acid were dissolved in dimethyl sulfoxide (DMSO) at concentrations ranging from 20 to 100 μg/mL. The reaction was carried out

Table I - Chemical components identified from *K. furfuracea* essential oil

No	Components	KI[a]	KI[b]	Percentage (%)	Identifications[c]
1.	α-Pinene	932	930	0.5	RI, MS
2.	Camphene	946	944	0.2	RI, MS
3.	Limonene	1033	1032	1.1	RI, MS, Std
4.	Linalool	1092	1090	0.2	RI, MS, Std
5.	Borneol	1165	1165	0.8	RI, MS
6.	Bornyl acetate	1283	1285	0.2	RI, MS
7.	α-Cubebene	1345	1345	2.4	RI, MS
8.	(*E*)-α-Damascone	1385	1383	1.9	RI, MS
9.	β-Cubebene	1388	1387	0.6	RI, MS
10.	β-Elemene	1390	1389	4.9	RI, MS
11.	Longifolene	1407	1407	1.4	RI, MS
12.	β-Caryophyllene	1415	1417	7.7	RI, MS, Std
13.	α-Humulene	1435	1436	1.8	RI, MS
14.	Aromadendrene	1439	1439	1.6	RI, MS
15.	(*E*)-β-Farnesene	1455	1454	0.7	RI, MS
16.	Germacrene B	1460	1559	1.4	RI, MS, Std
17.	γ-Muurolene	1476	1478	1.0	RI, MS
18.	β-Selinene	1486	1489	4.2	RI, MS
19.	α-Selinene	1495	1498	0.7	RI, MS
20.	Cadine-1,4-diene	1495	1495	1.6	RI, MS
21.	Bicyclogermacrene	1500	1500	23.1	RI, MS, Std
22.	α-Muurolene	1502	1501	2.1	RI, MS
23.	(*E*,*E*)-α-Farnesene	1504	1505	9.1	RI, MS
24.	γ-Cadinene	1516	1513	1.7	RI, MS
25.	δ-Cadinene	1520	1522	17.2	RI, MS, Std
26.	(Z)-Nerolidol	1530	1531	1.8	RI, MS
27.	α-Calacorene	1544	1545	1.1	RI, MS
28.	γ-Selinene	1598	1598	2.8	RI, MS
29.	1-*epi*-Cubenol	1625	1627	1.2	RI, MS
30.	τ-Muurolol	1645	1644	2.3	RI, MS, Std
31.	α-Cadinol	1650	1652	1.7	RI, MS
	Monoterpene hydrocarbons			**1.8**	
	Oxygenated monoterpenes			**1.2**	
	Sesquiterpene hydrocarbons			**89.0**	
	Oxygenated sesquiterpenes			**7.0**	
	Identified components			**99.0**	

[a] Linear retention index experimentally determined using homologous series of C_6-C_{30} alkanes
[b] Linear retention index taken from Adams and literatures
[c] Identification methods: Std, based on comparison with authentic compounds; RI, MS - based on comparison with Wiley, Adams, FFNSC2 and NIST08 MS databases

in a 96-well microplate, and the absorbance at 475 nm was measured using an ELISA microplate reader (VersaMax, Molecular Devices, USA). Each well contained 40 µL of essential oil dissolved in DMSO, 80 µL of phosphate buffer (pH 6.8), 40 µL of tyrosinase enzyme, and 40 µL of L-dopa. A control sample containing all components except L-dopa was included for each sample. Kojic acid was used as a reference inhibitor. The percentage of tyrosinase inhibition was calculated using the formula: $I\% = [A_{control} - A_{sample} / A_{control}] \times 100$,
where $A_{control}$ represents the absorbance of the control reaction and A_{sample} represents the absorbance of the essential oil/reference. The concentration of the sample causing 50% inhibition (IC_{50}) was determined by plotting the percentages of inhibition against the sample concentrations. The results were expressed as means ± standard deviation (SD) of triplicate analyses [29].

2.5. MOLECULAR DOCKING

The crystal structure of tyrosinase (PDB ID code 2Y9X) was obtained from the Protein Data Bank (PDB) website (http://www.rcsb.org). The retrieved protein structure was subjected to energy minimisation using the conjugate gradient algorithm and AMBER force field with UCSF Chimera 1.10.1. The major components, namely bicyclogermacrene, (E,E)-α-farnesene, δ-cadinene, and β-caryophyllene, were obtained from PubChem in sdf format. Each ligand was individually energy minimised using the OpenBabel tool embedded in PyRx, with default parameters including steepest descent steps of 100 with a step size of 0.02 Å, conjugate gradient steps of 100 with a step size of 0.02 Å, and an update interval fixed at 10 [30]. Molecular docking was performed using the PyRx virtual screening tool with the AutoDock VINA Wizard approach. The grid box centre values for X, Y, and Z were adjusted to -9.8702, -27.3402, and -40.6008, respectively, to ensure sufficient coverage

of the binding pocket and allow for ligand mobility in the search space. The exhaustiveness value was set to 8 to maximise binding conformational analysis. Docked compounds were evaluated based on the lowest binding energy (Kcal/mol) among all docked complexes. 2D and 3D visualisations of the docking complexes were generated using Discovery Studio 2021 [32]

3. RESULTS AND DISCUSSION

The fresh leaves of *K. furfuracea* were hydrodistillated to produce yellow oil with a yield of 0.21%. The volatile components have been successfully identified by GC-FID and GC-MS analysis, and their percentages are shown in Table 1 in relation to their Kovats index in the HP-5 column. The essential oil of *K. furfuracea* revealed the presence of thirty-one components with a percentage of 96.0%. The essential oil was characterised by the high concentration of sesquiterpene hydrocarbons (89.0%) and dominated by its richness in bicyclogermacrene (23.1%), δ-cadinene (17.2%), (*E*,*E*)-α-farnesene (9.1%), β-caryophyllene (7.7%), and β-elemene (4.9%). The other minor components detected in the essential oil in more than 2% were β-selinene (4.2%), γ-selinene (2.8%), α-cubebene (2.4%), τ-muurolol (2.3%), and α-muurolene (2.1%).
According to the available literature, only four studies have been conducted on the essential oils belonging to the genus *Knema* [33-36]. The chemical composition of Malaysian *K. kunstleri* and *K. hookeriana* and *K. intermedia* leaf oil has been recently described by us [33,35,36]. The essential oil of *K. kunstleri* leaf contained 77.3% of sesquiterpene hydrocarbons, with β-caryophyllene (23.2%) as the major component [33], and that of *K. hookeriana* consisted majorly

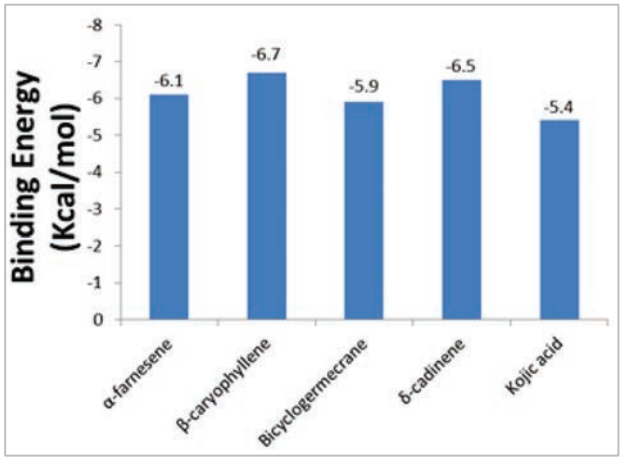

Figure 1 - Docking energies of the major components and control

of β-caryophyllene (26.2%), germacrene D (12.5%), δ-cadinene (9.2%), germacrene B (8.8%) and bicyclogermacrene (5.5%) [35], while *K.intermedia* contains mainly of τ-muurolol (20.1%), α-copaene (14.4%), δ-cadinene (13.9%), germacrene B (9.5%), and δ-selinene (7.0%) [36]. Meanwhile, another study on the leaf oil of *K. globularia* from Vietnam gave β-elemene (25.48%) as the major component [34]. The components' differences between *Knema* species may be influenced by the method of extraction, genetic factors, the season, stage of development, chemotype, distinct habitat in which the plant was collected, and the nutritional status of the plants, which influence plant biosynthetic pathways and consequently, the relative proportion of the main characteristic compounds [33].
Essential oil was tested for its anti-tyrosinase activi-

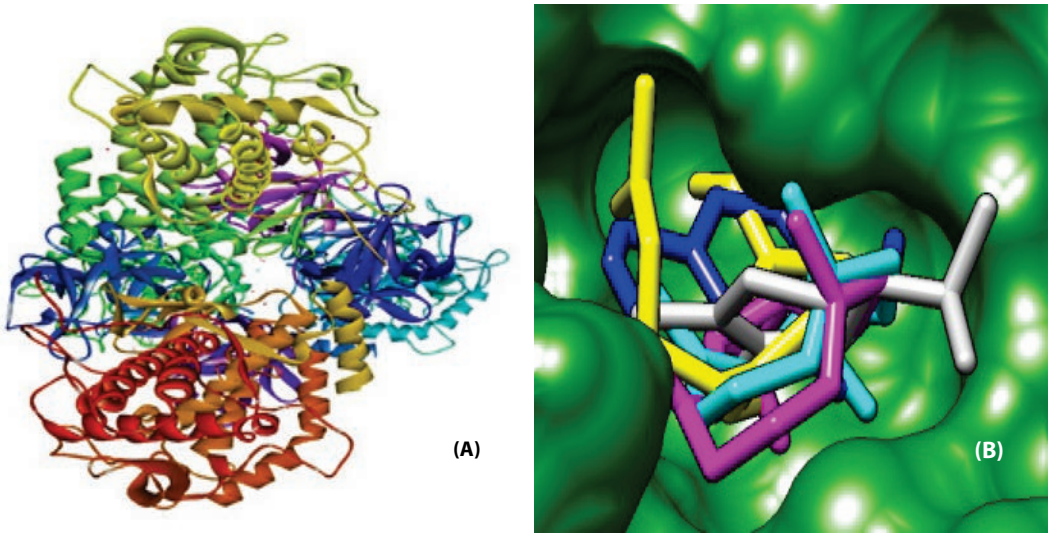

Figure 2 - (**A**) Structure of Tyrosinase; PDB ID: 2Y9X (**B**) The binding pocket of target protein in surface format is represented in dark green color with conformational position of ligands.

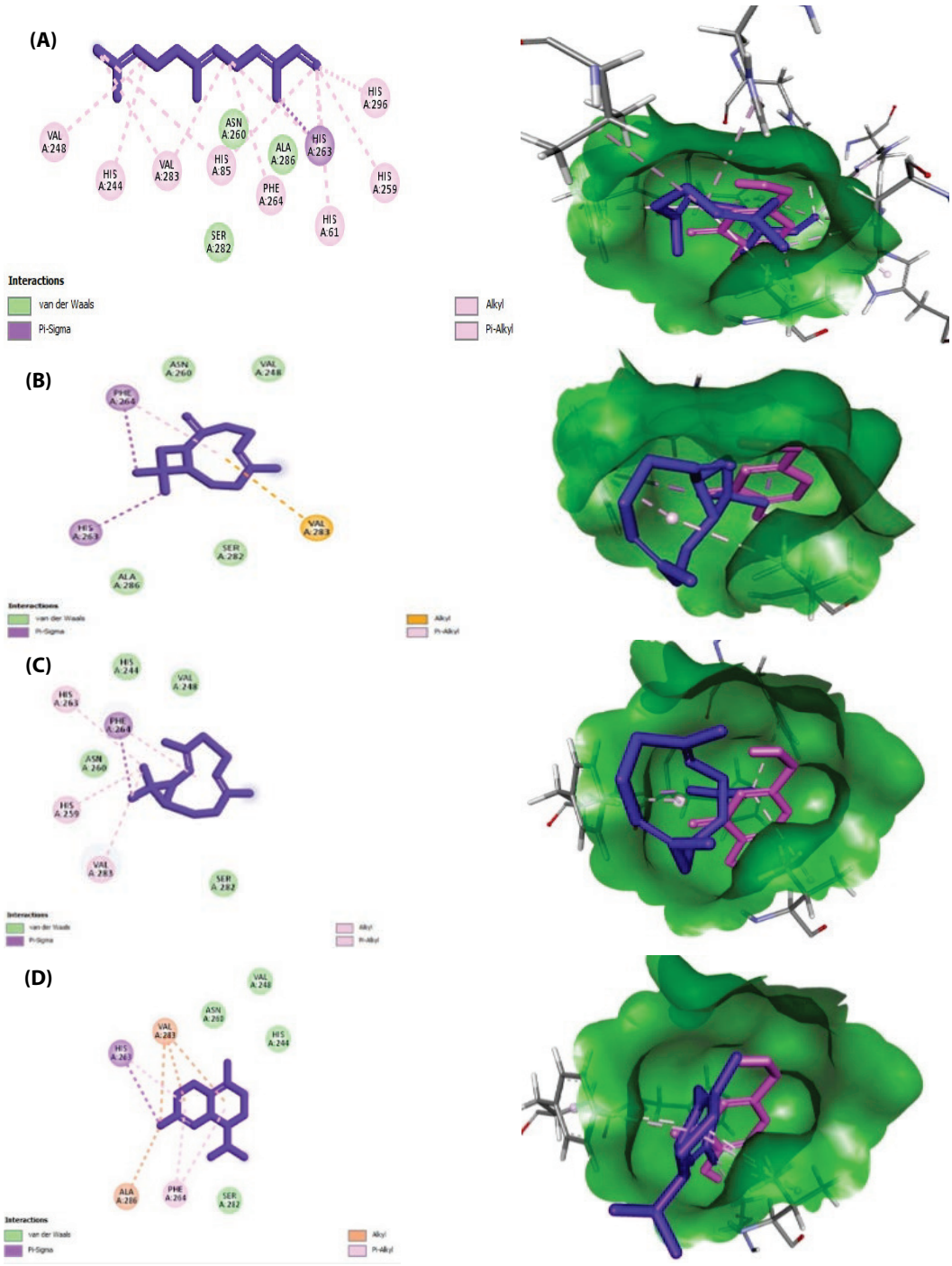

Figure 3 - 2D and 3D interactions view of ligands inside tyrosinase receptor; the representation of (**A**) α-farnesene (**B**) β-caryophyllene (**C**) bicyclogermacrane (**D**) δ-cadinene, superimposed unto the 2Y9X-kojic acid complex

ty using L-DOPA as a substrate and kojic acid as a positive control. The t-test was used for statistical analysis, and a significance level of $p<0.05$ was used. In this experiment, the essential oil's enzyme-inhibiting ability was measured by looking at the doses at which 50% inhibition (IC_{50}) occurred. This investigation found modest success with the essential oil, with an IC_{50} value of 80.3 µg/mL compared to the standard's IC_{50} value of µ10.5 µg/mL for kojic acid. Previous studies have revealed that the essential oil's phenolic components may have served as tyrosinase examples, resulting in conformational or steric alterations. However, because of its low proportion of oxygenated components that may chelate or link with the copper metal [37,38], the enzyme displays less enzymatic behaviour.

Molecular docking is an excellent method for studying the binding configuration of ligands at the active site of target proteins. The binding affinity and active amino acid residues of the selected components in the target protein were investigated using molecular docking. The major components (bicyclogermacrene,

(*E*,*E*)-α-farnesene, δ-cadinene, and β-caryophyllene, since they constitute the highest percentage) were docked into the binding site of the mushroom tyrosinase, and their affinity was tested. Figure 1 shows the binding affinity values of the components and kojic acid (control). The lowest binding energy values (kcal/mol) and the interaction pattern were used to examine the docked complexes of the components with tyrosinase receptor. All ligands had comparable docking energies with conventional kojic acid (–5.4 kcal/mol) and interacted with the active site residues of the tyrosinase receptor.

Analysing the binding pocket, all ligands were found in the target protein's active region. Superimposing the docked complexes allowed us to verify the binding conformation of each ligand in the active domain of the target protein. Furthermore, the ligands bound in the binding pocket showed a comparable conformational pattern. All ligands are attached to the target protein with minor rotational deflection. The fact that most of the ligands were bound in the same area validated our docking results, as shown in Figure 2. The significant components had a binding energy range from –5.9 to –6.7 Kcal/mol, with β-caryophyllene having the highest (–6.7 Kcal/mol). All components showed better docking scores than kojic acid (–5.4 kcal/mol). The low activity observed might be due to many components' presence since the essential oil was tested and not the individual components.

Binding analysis showed that β-caryophyllene forms a two hydrophobic π-σ bond with the imidazole side chain of His263 at a distance of 3.89 Å and the benzyl side chain of Phe264 at a distance of 3.93 Å. With a binding distance of 4.81 Å, β-caryophyllene rings also form a π -alkyl bond with the aromatic portion of Phe264 and an alkyl bond with Val283 with a binding distance of 5.38 Å. Additionally, it displayed van der Waals interactions with Ala286, Ser282, Asn260, and Val248, as shown in Figure 3. According to molecular docking, compared with the reference drug kojic acid, the prepared ligand with the lowest binding energy might be a suitable inhibitor of tyrosinase receptors. The theoretical investigation suggested excellent outcomes when compared to the experimental study.

4. CONCLUSIONS

In conclusion, this article presents a study on the chemical composition and biological activities of the essential oil extracted from *K. furfuracea*, a plant species found in Southeast Asia. The chemical composition of the essential oil was analysed using GC/FID and GC/MS, which identified 31 compounds, with bicyclogermacrene, (*E*,*E*)-α-farnesene, δ-cadinene, and β-caryophyllene being the major components. The essential oil was also evaluated for its anti-tyrosinase activity, which is vital for its potential use in skin whitening products. Results showed that the essential oil exhibited a moderate anti-tyrosinase activity, indicating its potential as a natural skin whitening agent. Furthermore, molecular docking studies were performed to predict the interactions between the essential oil compounds and the tyrosinase enzyme. In addition, the major components obtained promising scores for docking in the active sites of the examined target enzymes. The results also provide insight into the molecular interactions between the essential oil major components and the tyrosinase enzyme. β-caryophyllene had the highest binding affinity with the tyrosinase enzyme, suggesting its potential as an active ingredient in anti-tyrosinase products. Overall, this study highlights the potential of *K. furfuracea* as a natural source of anti-tyrosinase agents, this could open ways for further research on the use of *K. furfuracea* essential oil in the development of tyrosinase inhibitors for commercial and medicinal applications.

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