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Molecular Docking and Zone Inhibition Analysis of Fractionated Ethanol Extract of *Zingiber officinale var. rubrum* Against *Candida albicans* as Oral Antifungal

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Abstract

Ginger is a herb that can be used as an alternative medicine because it has anti-bacterial, antioxidant, anti-inflammatory, analgesic and antifungal properties. This study aims to identify the potency of red ginger ethanol extract against *Candida albicans* protein by using *in silico* and *in vitro* approaches. Several ginger-derived compounds were obtained from PubChem, while the target protein 4J14 was obtained from the Protein Data Bank (PDB) database. Prediction of Activity Spectra for Substances (PASS) Online was used to predict each compound's biological function. Before molecular docking analysis, the 3D ligand site web server determined the binding site coordinates. PyRx was then used to perform molecular docking analysis on ginger compounds and the target protein 4J14, with fluconazole and posaconazole serving as controls. Additional analysis was used to identify amino acid residues in the complexes. The Chimera software was employed to describe protein compound complexes. The inhibitory zones of red ginger and fluconazole on fungal growth were determined *in vitro*. The molecular docking results showed that gamma-sitosterol had a more negative binding affinity (−9.6 kcal/mol) than fluconazole (−8.7 kcal/mol). Moreover, it affected the biological response of the target protein 4J14 or cytochrome 450, which is an essential protein in the fungal infection process. *In vitro* tests proved that a red ginger extract concentration of 15 mg/mL had antifungal potential. *In silico* and *in vitro* studies revealed that red ginger extract has the potential to be an antifungal agent.

Keywords: Antifungal, Molecular docking, Red ginger, Good health, Wellbeing

Introduction

Candida species (*Candida* spp.) is found in the oral cavity of 75 % of healthy people. When the amount of *Candida* spp. in saliva exceeds 400 colony-forming units per mL, an infection called oral candidiasis develops. Based on clinical examination, several phenotypes of *Candida* spp. include pseudomembranes, erythematous, hyperplastic, angular cheilitis, median rhomboid glossitis, denture stomatitis and linear gingival erythema. All of these disorders can cause a variety of symptoms, such as masticatory discomfort and food restriction [1-5].

Excessive or uncontrolled use of fluconazole can put infection-causing organisms under selection pressure, allowing them to develop drug-resistance mechanisms. Azole-resistant yeast isolates continue to proliferate due to the widespread use of azole drugs and prolonged antifungal therapy. For instance, oral isolates of *C. albicans* are resistant to azoles, including fluconazole, which is commonly used to treat denture-related stomatitis. Isolation of *C. albicans* capable of building biofilms is also a severe problem because it is one of the leading causes of antifungal drug failure [6,7].

Ginger (*Zingiber officinale Rosc.*; Zingiberaceae family) is rich in active ingredients such as phenolic compounds and terpenes. Its phenolic compounds are mainly gingerols, shogaols and paradols. In fresh ginger, gingerols, such as 6-gingerol, 8-gingerol and 10-gingerol are the main polyphenols. Red ginger, in particular, contains more phenolics and flavonoids than common ginger. Due to heating or extended storage, gingerols turn into shogaol. In the presence of hydrogenation, shogaol transforms into paradol. Ginger also contains phenolic compounds, such as quercetin, zingerone, gingerenone-A and 6-dehydrogingerdione. Moreover, some of ginger's terpene components, such as β-bisabolene, α-curcumene, zingiberene, α-farnesene and β-sesquiphellandrene, are the main constituents of ginger essential oil. In addition, polysaccharides, lipids, organic acids and raw fibre are also present in ginger [6,8].

It is critical to find more powerful antifungal drugs capable of treating such fungi. This property of herbal substances may lead to novel treatment options for infectious diseases. Accordingly, it is necessary to explore the content of fractionated red ginger ethanol extract to prove its antifungal potential. This study used the fractionation method to extract active compounds from red ginger that were free of essential oils, it was intended to determine their antifungal potential; this choice was made since many previous studies on the content of red ginger compounds were found as antifungal, but the content were mixed with essential oils. Therefore this research intended to identify the potency of red ginger ethanol extract against C.albicans protein by using *in silico* and *in vitro* approaches. This study offered an innovative method of using natural ingredients as antifungals with minimal side effects.

Materials and methods

Ethical approval

This study was approved by The Ethics Committee, Faculty of Dentistry, Airlangga University, Surabaya, Indonesia (No: 637/HRECC. FODM/XII/2021).

Compound preparation for *in silico* analysis

The simplified molecular input line entry system (SMILES) notation of the 22 compounds derived from red ginger essential oil, as obtained in a previous study, was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Each compound's ID number and 3D structure were obtained for analysis. In addition, the 3D structure was saved in structure data file (sdf) format.

Target protein sample preparation

The 3D structure of the cytochrome P450 protein (PDB ID 4J14) was collected from the Protein Data Bank (PDB) database (https://www.rcsb.org/). The Chimera software was utilised to remove water molecules and native ligands. Furthermore, polar hydrogen was added to strengthen the protein structure.

Predicted biological functions of selected herbal compounds

The prediction of activity spectra for *substances* (PASS) Online web server (http://way2drug.com/passonline/) was used to analyse the compounds' biological activity. It aimed to determine the potency of antifungal-related functions, such as Candida pepsin, CYP2C8 and CYP2C19 inhibitors. The biological function is indicated by the probability of activity (Pa score) and the probability of inactivity (Pi score). In brief, it was interpreted as follows: (i) only activity with $Pa > Pi$ is considered possible for a specific compound; (ii) if $Pa > 0.7$, the chance of finding experimental activity is high; (iii) if $Pa\ 0.5 < Pa < 0.7$, the chance of finding experimental activity is lower, but the compound may not be as similar to known pharmaceutical drugs; (iv) if $Pa < 0.5$, the chance of finding experimental activity is lower, but there is an opportunity to find a structurally new compound [9,10].

Predicted target protein binding site

The target protein's binding site position was determined using the 3DLigandSite web server (http://www.sbg.bio.ic.ac.uk/3dligandsite/). It aimed to predict the binding site's precise location within the protein for further analysis.

Molecular docking simulation of ligand-protein complex

The 22 compounds' interactions with the target protein were simulated through molecular docking using PyRx software using *Autodock Vina*. Specific docking was used to perform molecular docking. With fluconazole and posaconazole as controls, the binding affinity scores were compared.

Amino acid residue analysis within ligand-protein complex

The amino acid residues within the ligand-protein complex was analysed using the LigPlot software. Its goal was to discern how amino acid residues interact with potent compounds. The compound's position and pose within the binding site were also determined.

Visualisation of ligand-protein complex

The Chimera software was utilised to visualise the target protein and selected potent compounds. Each complex's 3D structure was depicted in different structures and colours. It intended to highlight the interaction between protein and ligand compounds.

Target protein prediction

Compounds derived from red ginger were analysed to predict the target protein for pathway analysis. It was conducted by using the NetInfer web server. The top 5 target protein candidates were selected based on their probability.

Analysis of protein-protein interaction network (PPIN)

Several proteins that were predicted as target proteins were analysed by using the Search Tool for the Retrievel of Interacting Genes (STRING) web server. It aimed to identify protein-protein interaction within the network. The False Discovery Rate (FDR) score was used as a data validity parameter.

Visualisation of protein-protein interaction network

The PPIN was depicted using different coloured nodes and edges.

Plant collection and preparation of red ginger fractionated ethanol extract

Zingiber officinale var. rubrum rhizomes were collected from the Herbal Laboratory of Materia Medica in Malang, East Java, Indonesia. The rhizomes were cleaned with tap water to remove any undesired materials; subsequently, they were cut off and rinsed with sterilised distilled water, then sun-dried for 3 days. The sample extraction process was modified based on Prastiyanto *et al.* [11]. The dried rhizome was ground into a fine powder and placed in steam distillation equipment for up to 6 h. Rhizome extract was processed using the maceration technique with ethanol solvent. A rotating evaporator at 50 °C concentrated the solution under reduced pressure.

Antifungal susceptibility test

The antifungal activity of red ginger rhizomes was evaluated using a diffusion test. In this method, 100 µL of each microorganism test is equivalent to a 0.5 McFarland standard inoculated on Sabouraud Dextrose Agar (SDA) medium. Bacteria were grown on the medium's surface using a sterile glass spreader. Moreover, a sterile paper disk was used for the sample treatment, employing as much as 0.01 mL of red ginger, with concentrations of 15, 30, 45 and 60 mg/mL; fluconazole with concentrations of 7, 8, 9 and 10 µg/mL was also used. The paper disk adhered to the medium's surface and then incubated aerobically for 48 h at a temperature of 37 °C. The diameters of the sample's inhibitory zones were observed and measured.

Statistical analysis

This study's data was collected using the Statistical Product and Service Solution (SPSS) software version 26. The normality of data distribution was analysed using the Kolmogorov-Smirnov test. In contrast, data homogeneity was assessed using Levene's Test; the results were then analysed using the 1-way ANOVA test. Moreover, to determine the difference between treatments, the Duncan test was employed. All analyses with $p < 0.05$ were considered statistically significant.

Results and discussion

Chemical compounds of red ginger fractionated ethanol extract

Several compounds, such as cyclohexane, cyclotetracosane, gamma-bisabolene, gamma-sitosterol, methylisonicotinic (isonicotinic acid), phenethanamine, propionate (ethyl 3-(4-hydroxy-3-methoxyphenyl) propionate), bisabolene (beta-bisabolene), curcumene, cyclooctacosane, methoxyphenol (4-ethyl-2-methoxyphenol), methylbenzyl (3-amino-4-methylbenzyl alcohol), methyl mandelate, methylenespiro (3r, 4s-2 ethyl), sesquiphellandrene, tolban, zingiberene, zingiberanol, nonacosene, tetradecylcyclohexane, shogaol (8-shogaol) and 6-gingerol, have been identified and were used for analysis. The SMILES notation of each compound was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) as sample

data. Moreover, the cytochrome P450 protein (PDB ID 4J14) from the PDB database was selected as a target protein.

Activity prediction of bioactive compounds contained in red ginger fractionated ethanol extract

The biological function of each compound as an antifungal was predicted using a Pa score between 0.5 - 0.7 ($0.5 < Pa < 0.7$). Results showed that gamma-sitosterol, bisabolene (beta-bisabolene), curcumene, sesquiphellandrene, zingiberene, zingiberanol, nonacosene, shogaol (8-shogaol) and 6-gingerol had significant antifungal properties, as evidenced by their high Pa scores. However, cyclohexane, cyclotetracosane, gamma-bisabolene, methylisonicotinic (isonicotinic acid), propionate (ethyl 3-(4-hydroxy-3-methoxyphenyl) propionate), cyclooctacosane, methoxyphenol (4-ethyl-2-methoxyphenol), methylbenzyl (3-amino-4-methylbenzyl alcohol), methyl mandelate and tetradecylcyclohexane scored less than 0.5 ($Pa < 5$) in antifungal function. Moreover, 3 compounds scored highly in other biological functions: (i) phenethanamine as a CYP2C8 and Candida pepsin inhibitor, (ii) methylnespiro (3r, 4s-2 ethyl) as an anti-infective and (iii) tolban as a CYP2C9 and Candida pepsin inhibitor (**Table 1**).

Table 1 Compounds of red ginger fractionated with ethanol extract.

Compounds	Pa	Pi
Cyclohexane	0.343	0.065
Cyclotetracosane	0.301	0.080
Gamma-bisabolene	0.361	0.060
Gamma-sitosterol	0.585	0.020
Methylisonicotinic	0.267	0.097
Phenethanamine	0	0
Propionate	0.296	0.082
Bisabolene	0.585	0.020
Curcumene	0.521	0.027
Cyclooctacosane	0.301	0.080
Methoxyphenol	0.332	0.069
Methylbenzyl	0.286	0.087
Methylmandelate	0.324	0.072
Methylnespiro	0	0
Sesquiphellandrene	0.638	0.015
Tolban	0	0
Zingiberene	0.607	0.018
Zingiberenol	0.680	0.011
Nonacosene	0.535	0.025
Tetradecylcyclohexane	0.413	0.047
Shogaol	0.534	0.025
6-gingerol	0.584	0.020
Fluconazole	0.726	0.008

Candida albicans is the most frequent cause of mucosal and systemic infections, accounting for 70 % of all fungal infections worldwide, and has been the leading cause of life-threatening invasive infections in recent years. The production of proteins crucial for adhesion and invasion is a factor in the pathogenic potential of *Candida albicans* [5,12,13]. Moreover, drug resistance has become a serious global health problem [4,12,14].

Computationally based drug discovery has become increasingly important in recent decades due to its advantages in terms of reduced risk, time, cost-effectiveness and resource utilisation when compared to traditional experimental approaches. This is possible because computing power has increased, and *in silico* methods have been developed. These computational methods are useful for limiting the use of animal models in pharmacological research, assisting in the rational design of novel and safe drug candidates and repositioning marketed drugs, thereby assisting medicinal chemists and pharmacologists throughout the drug discovery process. It supplements the experimental approach by narrowing the research scope and directing *in vivo* validation [15,16]. The results showed that each compound had different antifungal activity, as indicated by the activity probability score (Pa). Analysis of the biological function of each active compound in the red ginger fractionated ethanol extract revealed that most of the compounds have different Pa values according to their antifungal functions. A comparison of Pa and Pi values showed that the Pa value is greater than the Pi value, indicating that all red ginger compounds are active. As mentioned, if Pa > 0.7, the chance of finding experimental activity is high. Moreover, compounds with Pa values of 0.5 < Pa < 0.7 indicate a lower likelihood of finding experimental activity, but these compounds may not be the same as known pharmaceutical drugs. A compound with a Pa value of < 0.5 also has a lower probability of finding experimental activity [9,10].

Computational analysis of drugs target

Molecular docking analysis was employed to simulate the compound-target protein interaction. Fluconazole and posaconazole were selected as controls due to their commercial use as antifungal agents. The process was carried out using specific docking within a specific coordinates (Centre X: 0.1784; Y: −23.9700; Z: −26.7919 and Dimensions X: 25.8070; Y: 30.6638; Z: 39.5683). As controls, fluconazole and posaconazole were also simulated in the same coordinates (**Figure 1**).

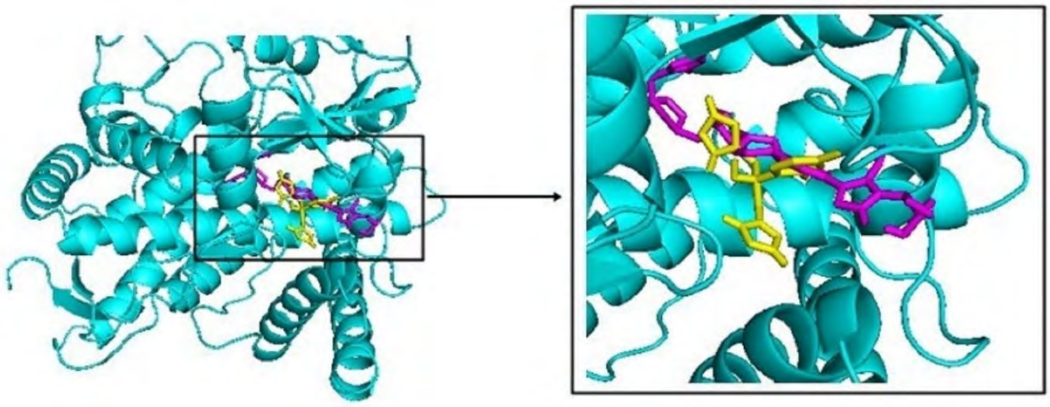

Figure 1 Complex of the target protein 4J14 (Cyan) with posaconazole (Magenta) and fluconazole (Yellow) ligand.

Moreover, the compounds were docked in the same binding site; the molecular docking simulation aimed to identify the interaction strength between the compound and the target protein. The bond's power was measured based on the binding affinity score (**Figure 2**).

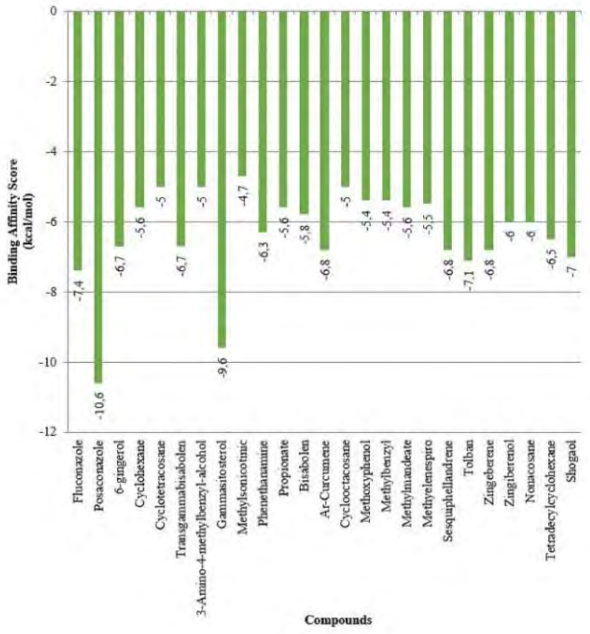

Figure 2 Binding affinity scores of compounds derived from red ginger fractionated ethanol extract towards the target protein.

The results showed that 4 compounds (i.e. gamma sitosterol, curcumene, tolban and shogaol) had binding affinity scores similar to the control (**Figure 3**). This suggests that the aforementioned compounds had strong interaction and biological effects on the target protein cytochrome 450. It is an essential protein associated with the infection process.

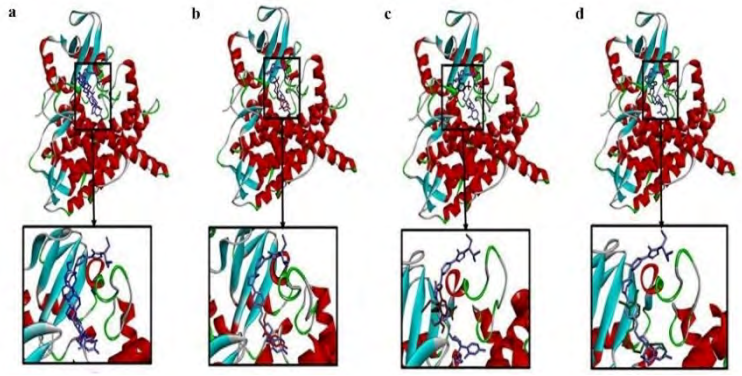

Figure 3 Amino acid residues within the complex of the target protein with (a) gamma sitosterol (blue), (b) curcumene (orange), (c) tolban (yellow) and (d) shogaol (green).

Furthermore, the findings revealed that the compounds interacted with several proteins involved in the immune response following the onset of infection. The results also showed that compounds associated with cellular homeostasis interacted with ESR1, EPHX2, OPRL1, DRD2, OPRM1 and SLC6A2 proteins. This was confirmed by the FDR score, which supports the analysis's validity. The FDR score had a significant validity value of 0.0083 ($p < 0.05$) (**Table 2**).

Table 2 Biological process within protein-protein interaction network (PPIN).

GO term	Biological process	Protein	False Discovery Rate (FDR) score
GO:0019725	Cellular homeostasis	ESR1, EPHX2, OPRL1, DRD2, OPRM1 and SLC6A2	0.0083

Further analysis focused on the compound's interaction with the target protein. The analysis was performed using molecular docking. The goal of molecular docking analysis was to identify compounds that interacted well with the target protein. All antifungal azoles work by inhibiting the fungal 4J14 or cytochrome 450 enzyme, which is required for the biosynthesis of ergosterol, the major sterol of fungal plasma membranes. Inhibiting fungal growth prevents ergosterol synthesis [17-19]. Additionally, changes in membrane sterol composition affect the fluidity and integrity of fungal membranes as well as the activity of several membrane-bound enzymes. Cell lysis and death are the end results [20].

The findings revealed that gamma-sitosterol had a higher binding energy (−9.6 kcal/mol) than fluconazole (−7.4 kcal/mol) but a lower binding affinity compared to posaconazole (−10.6 kcal/mol). Compounds such as tolban (−7.1 kcal/mol), shogaol (−7.0 kcal/mol), sesquiphellandrene (−6.8 kcal/mol), zingiberene (−6.8 kcal/mol) and curcumene (−6.8 kcal/mol) had lower bond affinity than fluconazole (−7.4 kcal/mol) and posaconazole (−10.6 kcal/mol). The results of molecular docking in the form of binding energy from several red ginger compounds indicate that these compounds may be able to affect the biological response of the target protein 4J14 or cytochrome 450, which is an important protein in the fungal infection process. Higher negative values suggest a strong interaction between the ligand and the target protein [21,22].

The effect of red ginger fractionated ethanol extract and fluconazole on *Candida albicans* growth

Red ginger fractionated ethanol extract and fluconazole were tested for antifungal activity against *Candida albicans*; the results are summarised in **Table 3**. The antifungal inhibitory activity of red ginger fractionated ethanol extract increased with concentration, as did fluconazole as an antifungal drug control. Statistical analysis revealed that the higher the concentration of fluconazole and red ginger fractionated ethanol extract, the greater their inhibitory power as antifungals.

Table 3 Inhibition of Fluconazole and red ginger fractionated ethanol extract against the growth of *Candida albicans*.

Groups	N	Diameter (mm) mean ± std. deviation	*p*-value
Fluconazole 7 μg/mL	4	6.00 ± 0.00^{a}	0.05
Fluconazole 8 μg/mL	4	8.20 ± 0.05^{c}	
Fluconazole 9 μg/mL	4	10.10 ± 0.15^{e}	
Fluconazole 10 μg/mL	4	12.53 ± 0.06^{g}	
Red ginger 15 mg/mL	4	7.00 ± 0.00^{b}	
Red ginger 30 mg/mL	4	8.23 ± 0.14^{c}	
Red ginger 45 mg/mL	4	9.31 ± 0.08^{d}	
Red ginger 60 mg/mL	4	11.60 ± 0.20^{f}	

This study found that a 15 mg/mL of red ginger fractionated ethanol extract had a higher inhibitory power than 7 μg/mL of fluconazole, while a 30 mg/mL of red ginger extract had an identical inhibitory power as 8 μg/mL of fluconazole. Moreover, red ginger concentrations of 45 and 60 mg/mL had lower inhibitory power than 9 and 10 μg/mL of fluconazole, respectively (**Figure 4**).

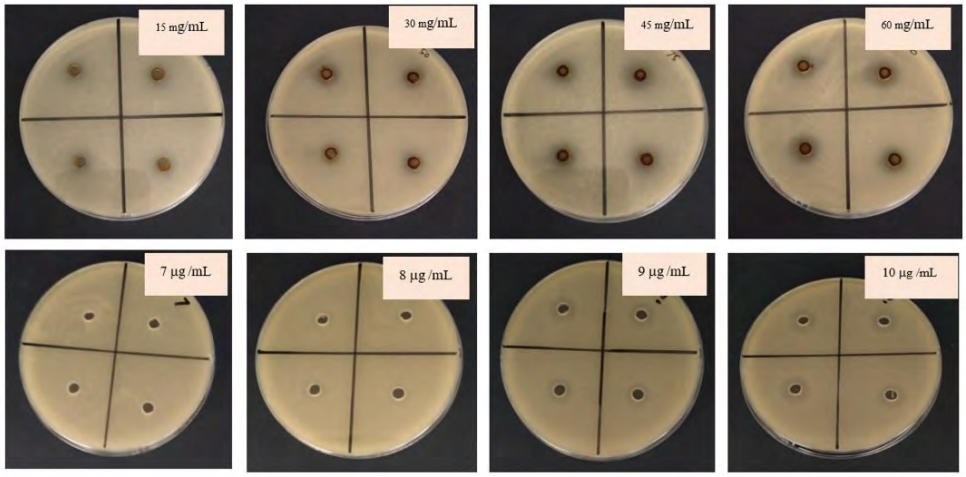

Figure 4 Average inhibitory effect of red ginger extract and Fluconazole on the growth of *Candida albicans*.

This study was conducted *in vitro* and used colonies of *Candida albicans* cultured and incubated at 37 °C for 48 h. This research also utilised red ginger fractionated ethanol extracts with different concentrations (15, 30, 45 and 60 mg/mL) as well as various fluconazole concentrates (7, 8, 9 and 10 µg/mL); the results revealed that inhibitory potency against fungal growth increased with higher concentrations. In this study, ginger extract was found to be effective in inhibiting *Candida albicans* growth. Furthermore, the inhibitory effect of ginger extract was comparable to that of fluconazole.

Fluconazole's application as a highly successful commercial azole antifungal agent has been limited due to the emergence of fluconazole-resistant Candida [23-25]. Hence, *Candida albicans* fungal pathogen was investigated in this study. Moreover, ginger has been found to have antifungal properties in several studies [11,26,27]. Ginger extract (*Zingiber officinale)* acts as a reducing, capping and antifungal agent [28,29].

Herbs have long been used in traditional herbal medicine. Herbal products may also be effective against drug-resistant *Candida* sp. Ginger, in particular, contains various antifungal and medicinal ingredients [27,30-32]. Herbal products and their active constituents may have a multi-directional mechanism of action. Herbs' possible mechanisms of action against drug-resistant *Candida* sp. include inhibition of budding yeast transformation into hyphae, inhibition of biofilm formation [33,34], inhibition of cell wall or cytoplasmic membrane biosynthesis [35], production of reactive oxygen species (ROS) and over-expression of membrane transporter [11,14].

Conclusions

This research showed that ginger root contains gamma sitosterol, curcumene, tolban and shogaol had binding affinity scores similar to fluconazole implying that it can be used as an antifungal medication. Soon, antifungal extracts with a strong fungicidal effect will be developed as an alternative to commercial synthetic fungicides.

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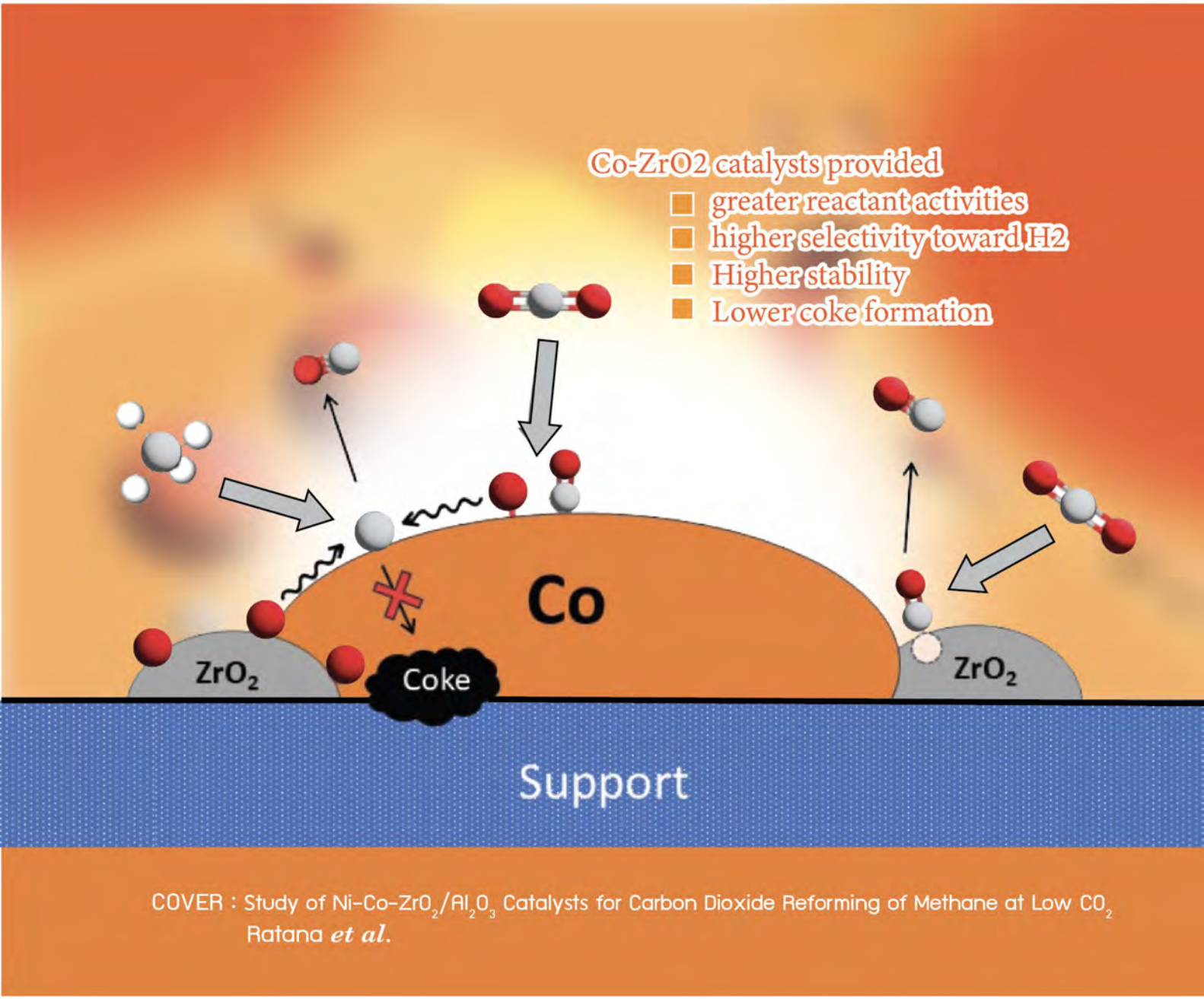

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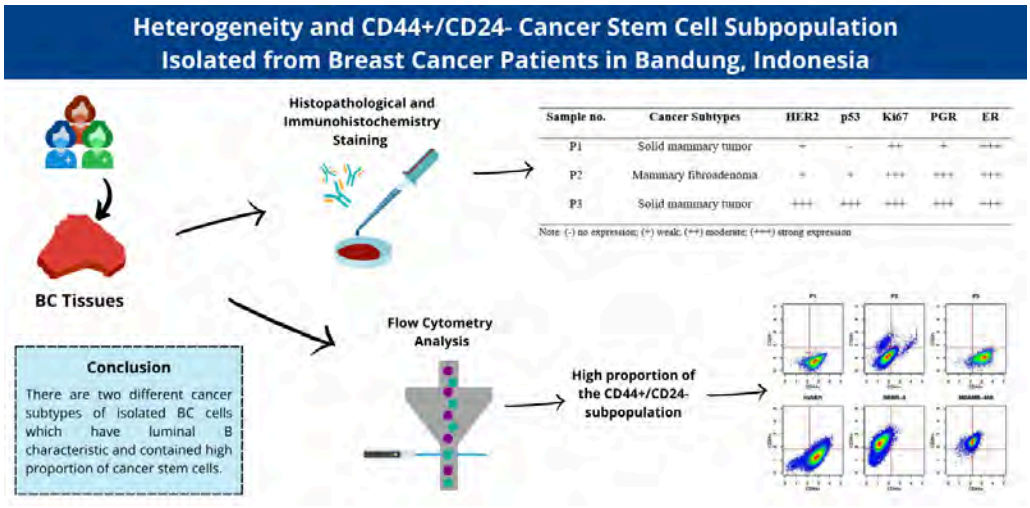

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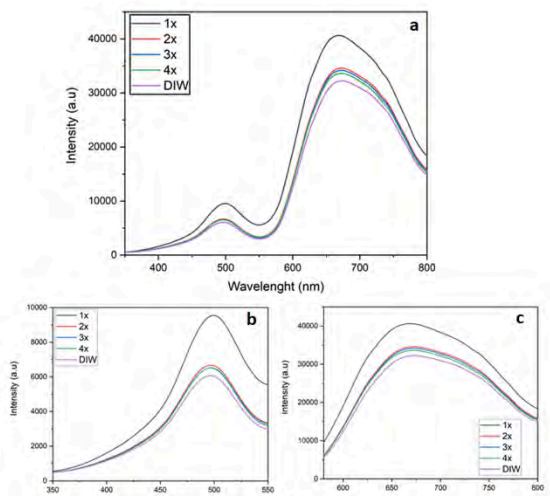

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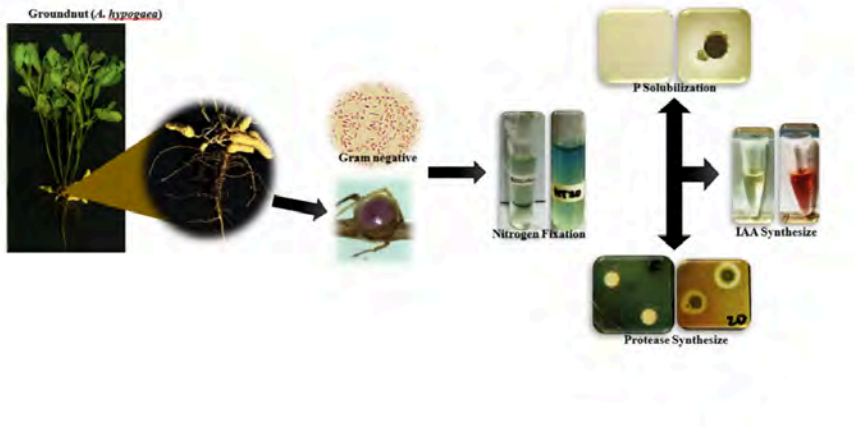

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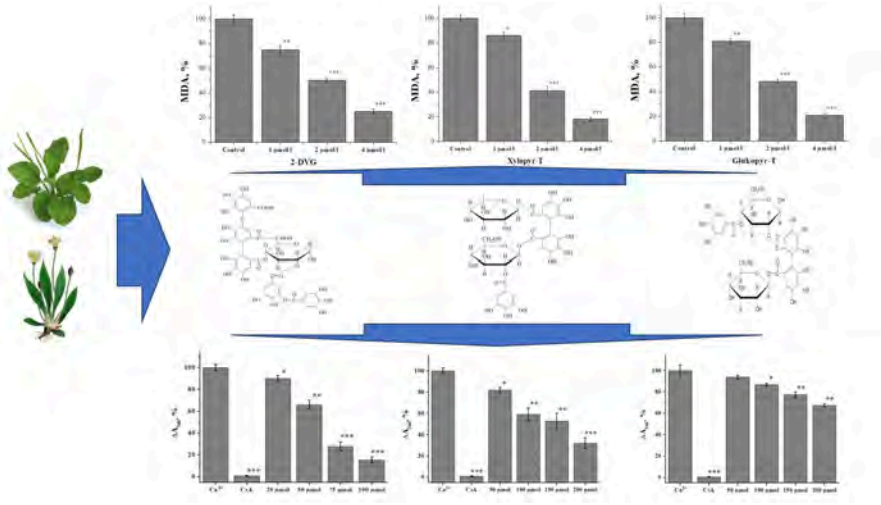

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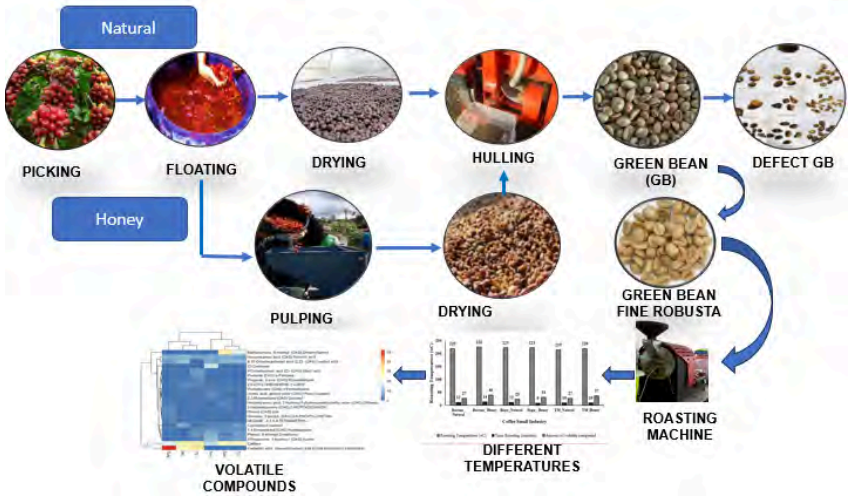

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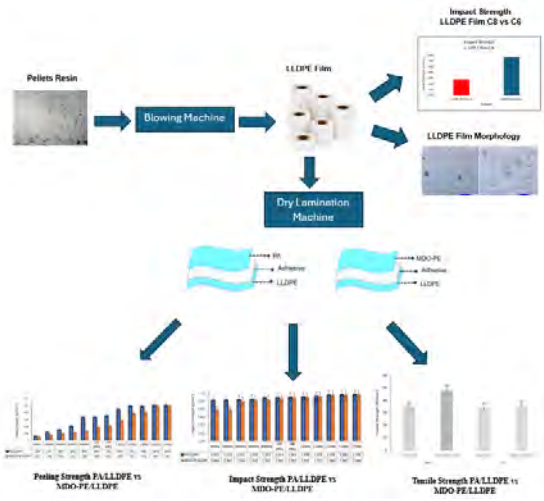

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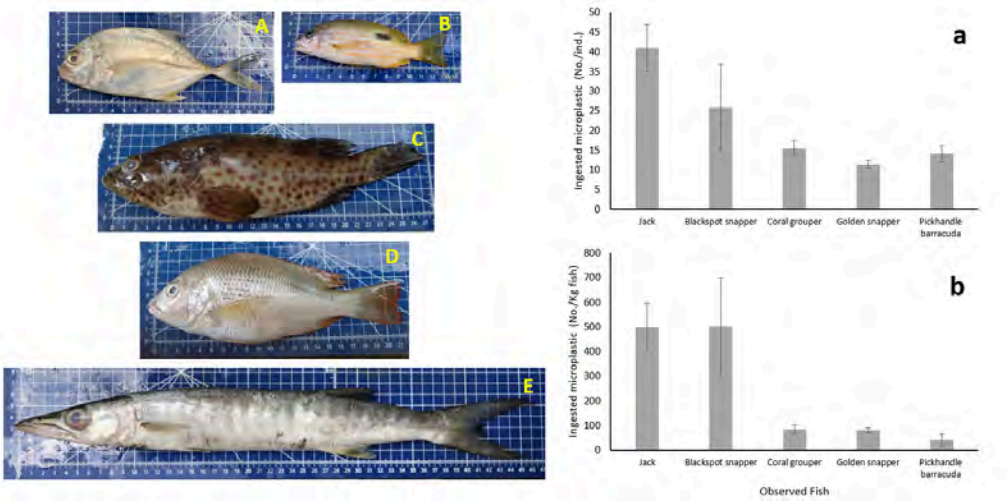

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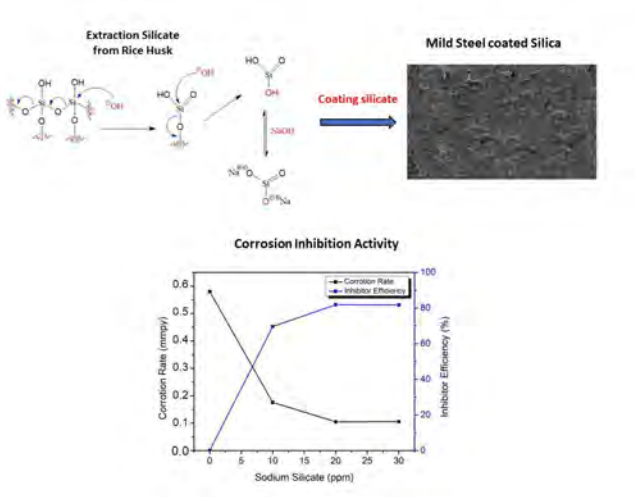

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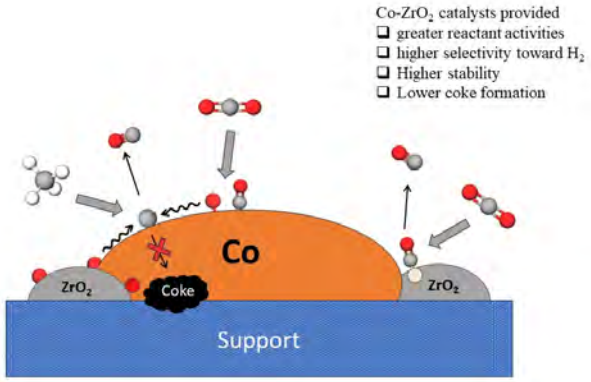

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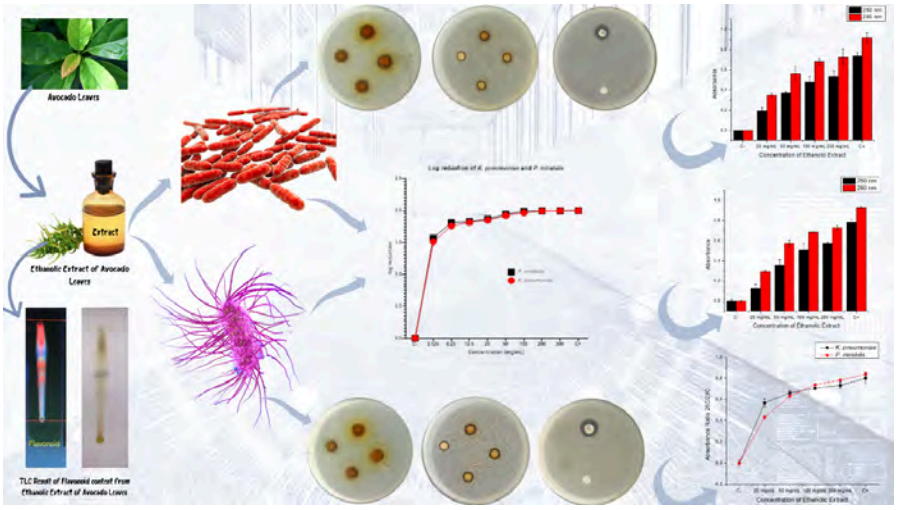

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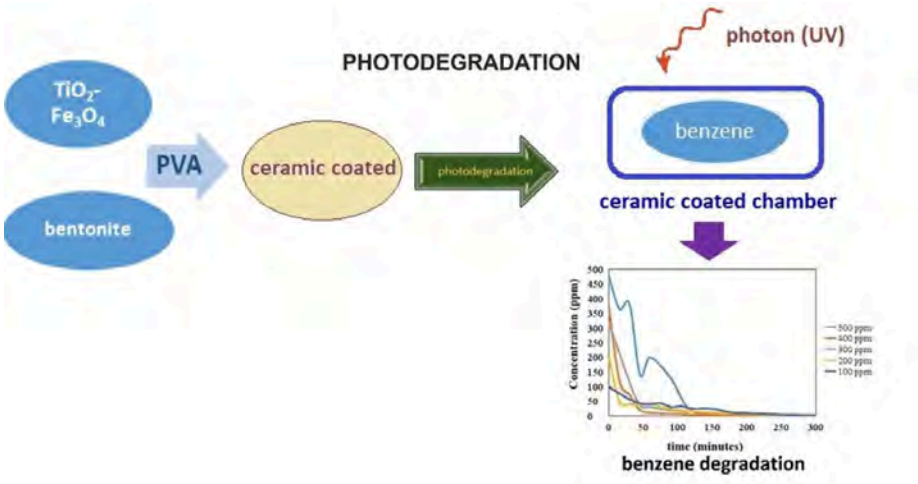

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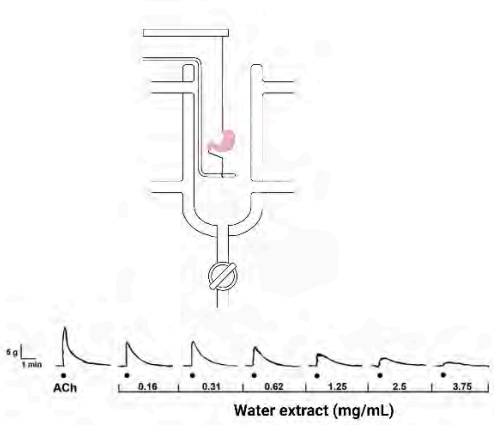

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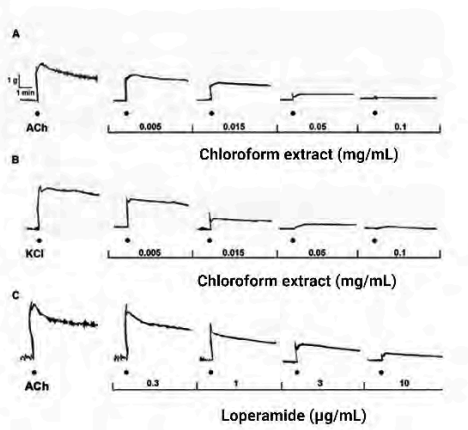

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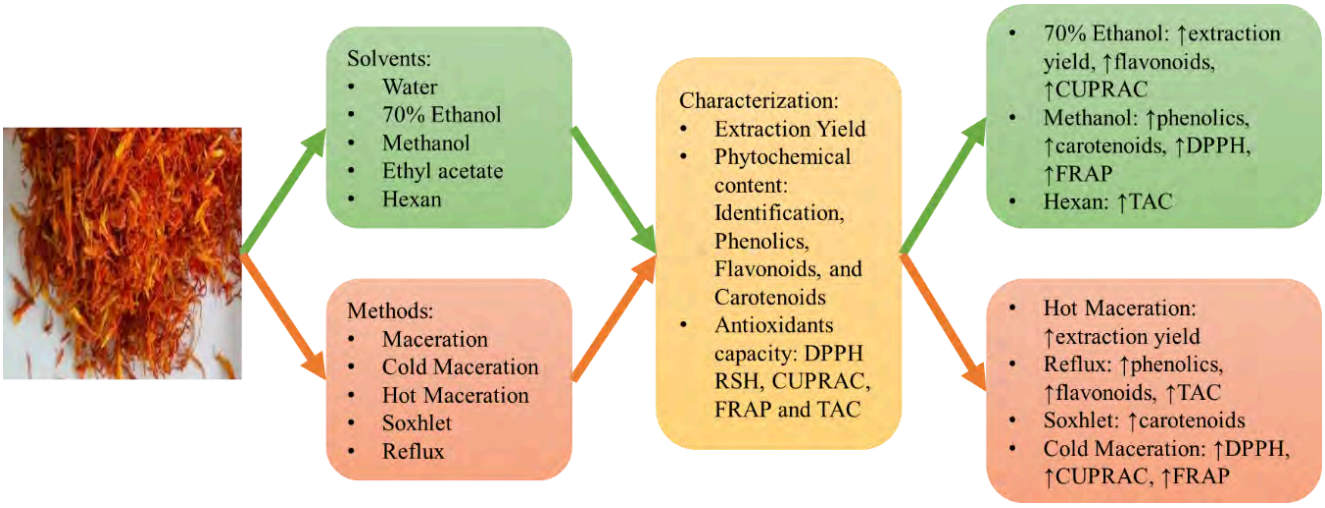

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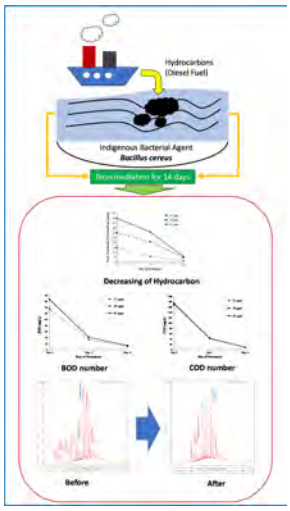

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Andi Kurniawan, Ainun Rahmadani Tri Wahyuni, Abd. Aziz Amin, Endang Yuli Herawati , Citra Satrya Utama Dewi, Wresti L. Anggayasti

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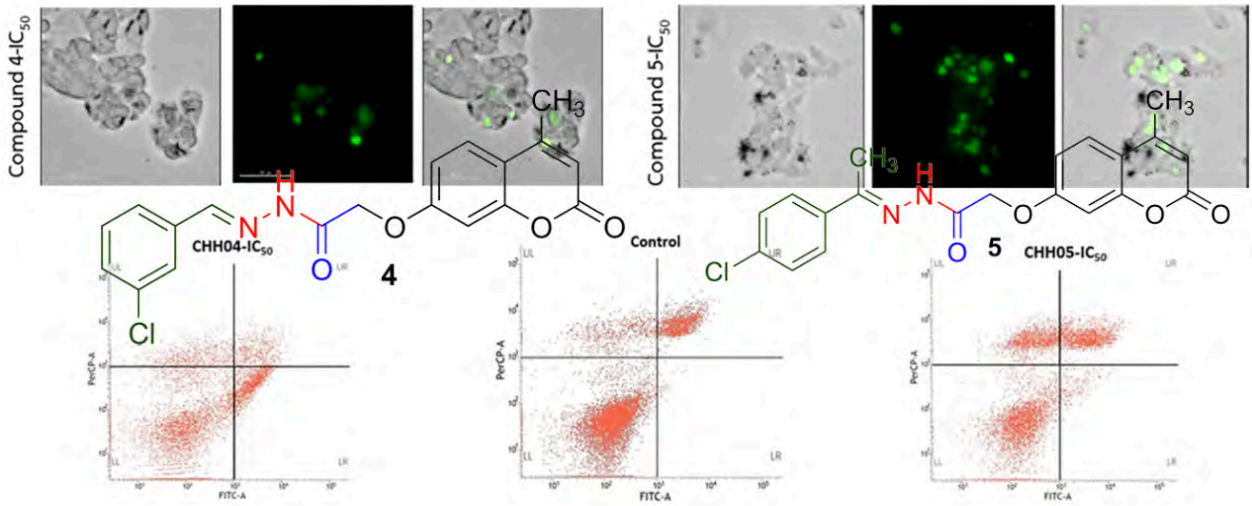

Evaluation of Cytotoxicity and Apoptosis Induced by Coumarin Hydrazide-Hydrazone Derivatives in Human Hepatocellular Carcinoma Cell Line

Saisuree Prateeptongkum, Nongnaphat Duangdee, Wiratchanee Mahavorasirikul

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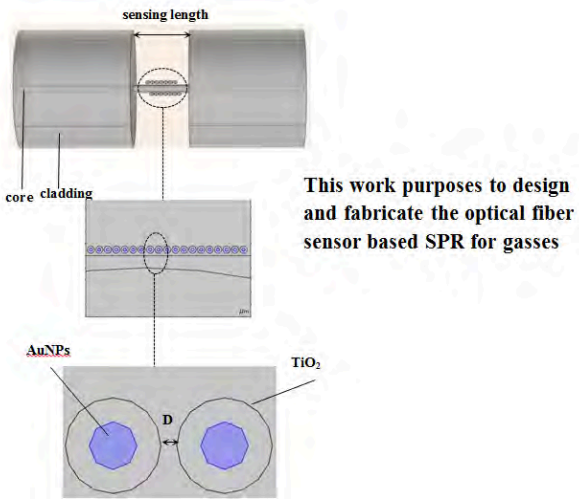

Structure Design and Sensitivity Optimization of Optical Fiber Biosensor Based Au Nano Column - TiO2 Heterostructure

Marwan Hafeedh Younus, Odai Falah Ameen, Hesham Anwar Salih

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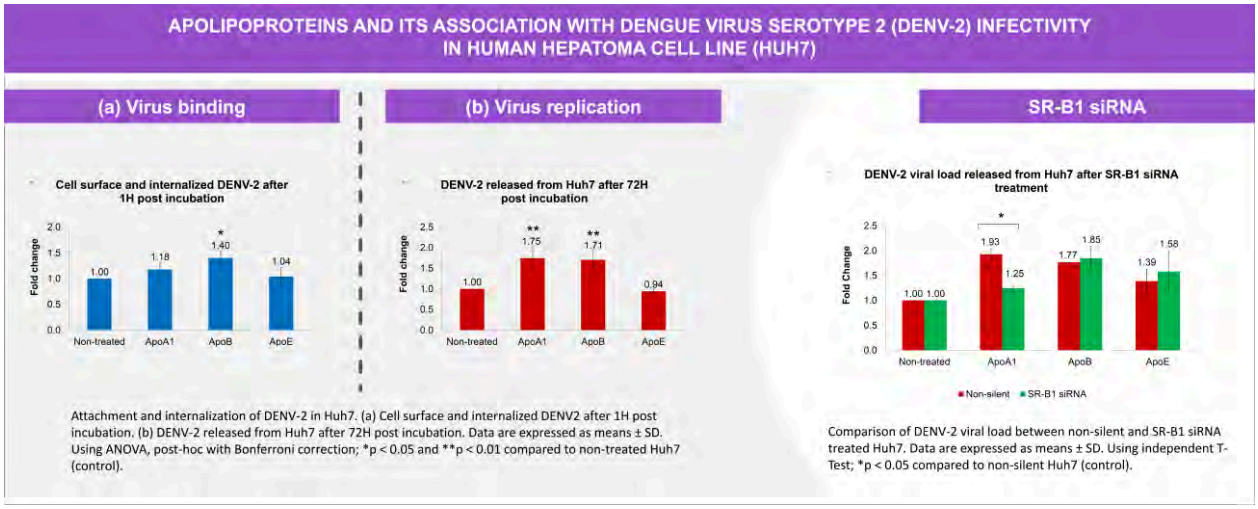

Apolipoproteins and Its Association with Dengue Virus Serotype 2 (DENV-2) Infectivity in Human Hepatoma Cell Line (Huh7)

Radzi Ikhsan Ahmad, Fadzilah Mohd Nor, Seok Mui Wang, Aletza Mohd. Ismail, Julina@Azimah Md Noor, Thuhairah Hasrah Abdul Rahman

7674

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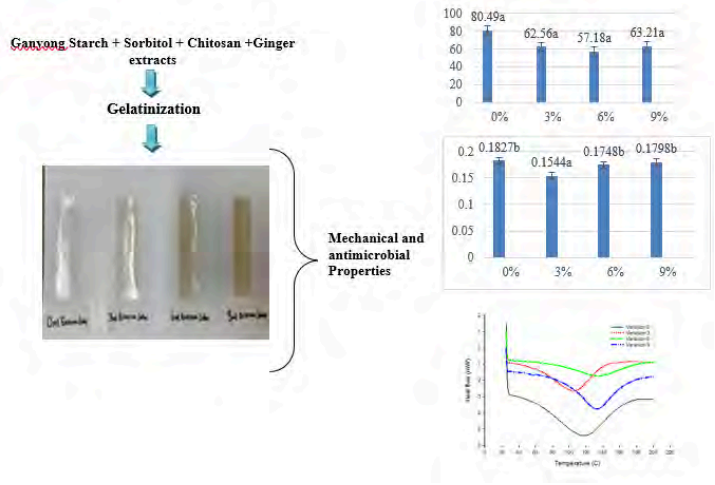

Effect of Ginger (Zingiber officinale) Extracts on Mechanical and Antimicrobial Properties of Ganyong Starch Edible Films as Primary Packaging of Crabstick

Deli Silvia, Zulkarnain, Rizki Ivan Fadhilah, Hanisah Kamilah, Nor Nadiah Abdul Karim Shah

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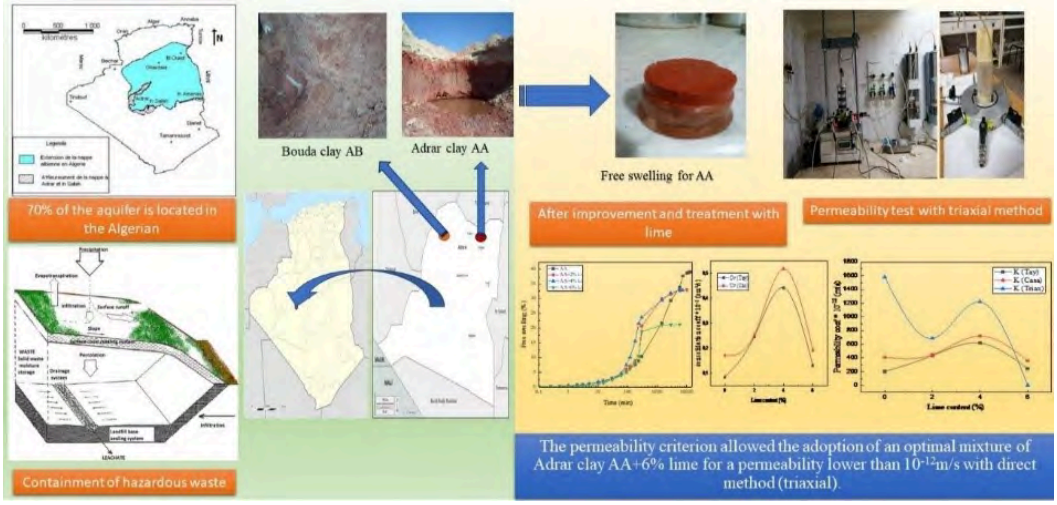

Evaluation of the Permeability of Clay-Lime Mixtures for Hazardous Waste Insulation Barriers, Case of Southern Algeria (Adrar)

Belaidi Khedidja, Zadjaoui Abdeldjalil, Mekerta Belkacem, Bassoud Abdelkader, Allal Mohamed-Amine

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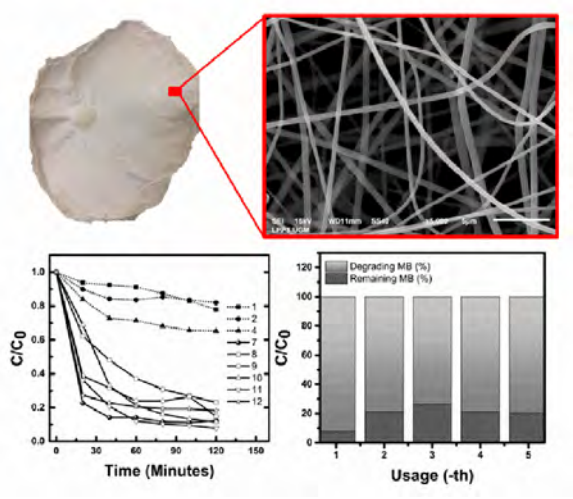

Photocatalysis-Assisted Process of Reusable PVA/TiO2 Nanofibers

Ahmad Kusumaatmaja, Ari Dwi Nugraheni, Diki Purnawati, Ani Rohmatillah , Dian Nur Mahardika

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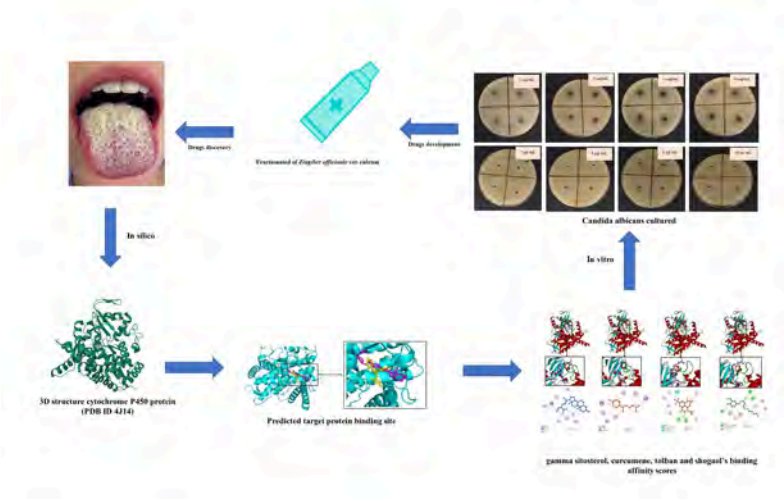

Molecular Docking and Zone Inhibition Analysis of Fractionated Ethanol Extract of Zingiber officinale var. rubrum Against Candida albicans as Oral Antifungal

Prawati Nuraini, Hendrik Setia Budi, Soegeng Wahluyo, Dimas Prasetianto Wicaksono, Akbar Aji Wiguno, Yulanda Antonius, Kittipong Laosuwan

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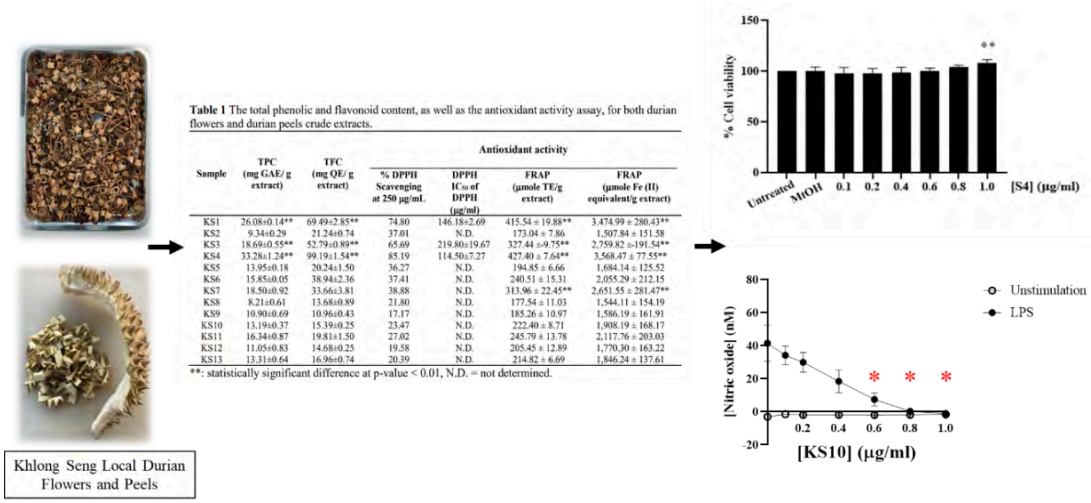

Anti-Inflammatory and Antioxidant Properties of Crude Methanol Extracts from the Flowers and Peels of Khlong Seng Local Durian (Durio Zibethinus Murr.)

Surachai Sang-Ngam, Worrawat Promden, Naunpun Sangphech, Arnon Chukaew, Siriluk Sintupachee

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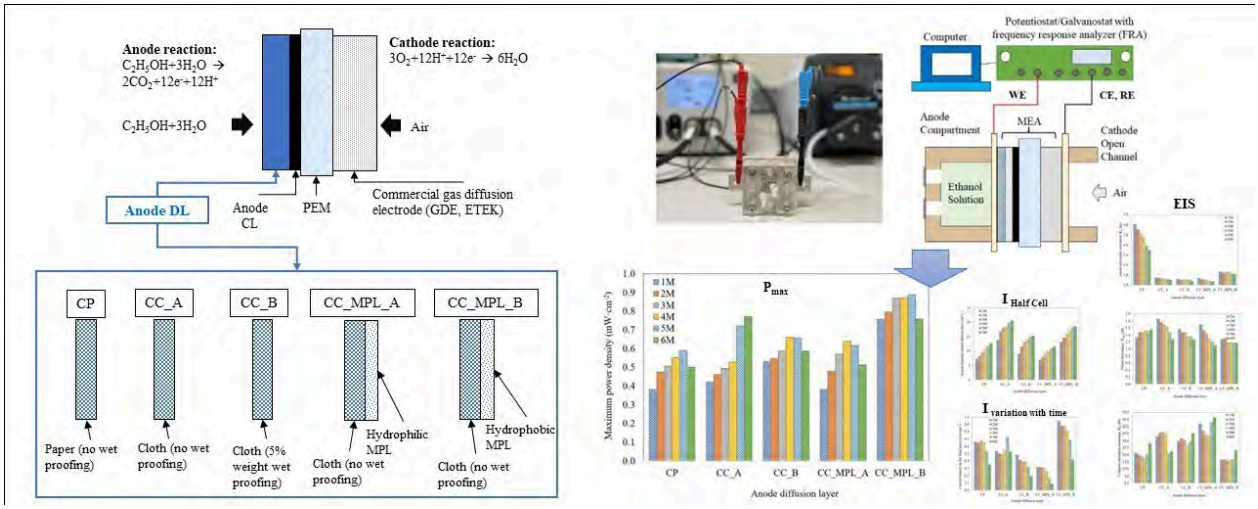

Influence of Anode Diffusion Layer on the Performance of a Passive Direct Ethanol Fuel Cell

Panuwat Ekdharmasuit

7867

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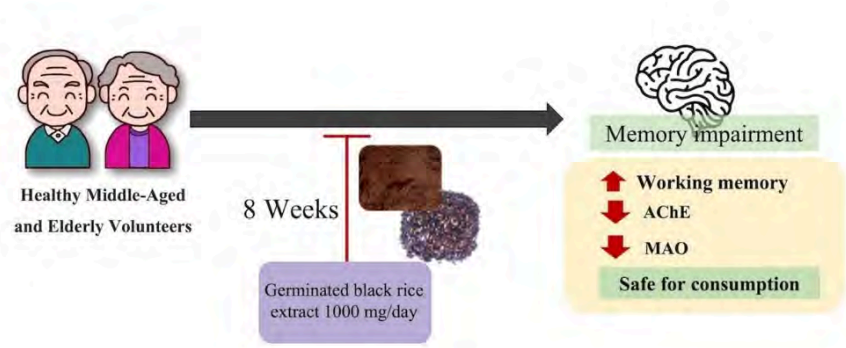

The Effect of an 8-Week Germinated Black Rice Extract Consumption on Cognitive Functions in Healthy Middle-Aged and Elderly Volunteers: A Randomized Controlled Trial

Panicha Pongnaratorn, Thanthika Kaewsoongnern, Chatchanok Nukulkit, Naruwat Pakdee, Yollada Sriset , Warin Ohnon

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