Phytochemical Analysis and Evaluation of Antifungal Properties of Eucalyptus Oil against *Candida* Species

Devika Nagappan
Department of Zoology, Arignar Anna Government Arts College, Cheyyar, Tamil Nadu, India

Madhavan Sivaraj
Department of Zoology, Arignar Anna Government Arts College, Cheyyar, Tamil Nadu, India

Sivamani Periyasamy
Microlabs, Institute of Research and Technology, Vellore, Tamil Nadu, India

Ashok Kamalanathan
Department of Microbiology and Biotechnology
Bharath Institute of Higher Education and Research (BIHER), Chennai, Tamil Nadu, India

Balasubramanian Gnanavel
Department of Zoology, Arignar Anna Government Arts College, Cheyyar, Tamil Nadu, India

Baskaran Chinnappan
Department of Pediatrics, UPMC Children's Hospital of Pittsburgh
University of Pittsburgh, Pittsburgh, Pennsylvania, United States of America
https://orcid.org//0009-0002-4887-7787

OPEN ACCESS

Manuscript ID:
ASH-2024-12017699

Volume: 12

Issue: 1

Month: July

Year: 2024

P-ISSN: 2321-788X

E-ISSN: 2582-0397

Received: 02.05.2024

Accepted: 25.06.2024

Published: 01.07.2024

Citation:
Nagappan, Devika, et al. "Phytochemical Analysis and Evaluation of Antifungal Properties of Eucalyptus Oil against *Candida* Species." *Shanlax International Journal of Arts, Science and Humanities*, vol. 12, no. 1, 2024, pp. 1-15.

DOI:
https://doi.org/10.34293/sijash.v12i1.7699

Abstract
This study aimed to investigate the phytochemical composition of eucalyptus oil and evaluate its antifungal properties against Candida species. Qualitative phytochemical analysis revealed the presence of various compounds, including carbohydrates, flavonoids, phenols, proteins, quinones, saponins, and tannins. Flavonoids were found to have the highest concentration, followed by tannins and polyphenols. GC-MS analysis identified several compounds in eucalyptus oil, including 2,3-Diamino-2-cyanosuccinonitrile as a major component. Antifungal assays demonstrated that eucalyptus oil inhibited the growth of Candida albigans, Candida tropicalis and Candida kefyr, with varying degrees of inhibition depending on the concentration used. The antifungal activity may be attributed to the presence of bioactive compounds such as 1-Butyne, 3-methyl-, 1-Hexen-4-ol, 1-chloro-3-methyl, N-(Methylphenylamino) methyl) benzamide. Further studies are required to explore the specific role and potential benefits of the identified compounds in eucalyptus oil and to evaluate the effectiveness of eucalyptus oil as an antifungal agent in clinical settings.
Keywords: Eucalyptus Oil, GC-MS, MIC, Phytocompounds, *Candida* Species

Introduction

Phytochemicals, inherent compounds found in plants, are renowned for their diverse biological activities, including antimicrobial effects (Lachumy et al.; Montazeri et al.; Soni et al.; Mokhber-Dezfuli et al.; Khan et al.: "Pharmacological Activities of Protocatechuic Acid"; Fitsiou et al.; Hussain et al.; Nieto; Pastorino et al.; Mullai Nila et al.). Among these, plant-derived essential oils have garnered notable interest for their therapeutic potential (Baptista-Silva et al.; Jin et al.; Oliyaei et al.; Koreti et al.). Eucalyptus oil, obtained from Eucalyptus tree leaves, boasts a rich phytochemical profile and a

history of medicinal use (Kesharwani et al.; Aljaafari et al.; Chandorkar et al.; El-Sakhawy et al.). Recently, there's been a surge in investigating eucalyptus oil's antimicrobial properties, especially against fungal pathogens.

Candida species, opportunistic fungal pathogens, pose significant health risks, particularly to immunocompromised individuals (Haynes; Yang; Silva et al.; Cadnum et al.; Kühbacher et al.; Cortegiani et al.; Pfaller et al.; Staniszewska; Aljaffary et al.). These pathogens are notorious for causing infections that vary from superficial mucosal infections to severe, life-threatening systemic infections. The increasing problem of antifungal resistance highlights the urgent need for alternative treatments. This situation has led to the investigation of natural compounds, such as essential oils, as potential sources of new antifungal agents. This study aims to perform a comprehensive phytochemical analysis of eucalyptus oil and evaluate its antifungal effectiveness against three Candida species: Candida albicans, Candida tropicalis, and Candida kefyr.

This study focuses on several key objectives related to eucalyptus oil and its antifungal properties. First, the phytochemical analysis aims to identify and quantify the major phytochemical constituents in eucalyptus oil. This will be achieved using techniques like gas chromatography-mass spectrometry (GC-MS) to determine its chemical composition. Next, the antifungal activity evaluation will assess the effectiveness of eucalyptus oil against Candida albicans, Candida tropicalis, and Candida kefyr by determining their minimum inhibitory concentration (MIC). The mechanism of action investigation will explore how eucalyptus oil exerts its antifungal effects against the tested Candida species. This includes studying its impact on cell membrane integrity, biofilm formation, and key cellular processes of the fungi. Furthermore, the comparison with standard antifungal agents will evaluate the efficacy of eucalyptus oil in comparison to commonly used antifungal drugs like fluconazole. This comparison aims to provide insights into the potential of eucalyptus oil as a natural alternative or adjunctive therapy for Candida infections.

By addressing these objectives, this study aims to enhance the understanding of the phytochemical composition of eucalyptus oil and its potential as an antifungal agent against Candida species. The findings may inform the development of new therapeutic approaches for Candida infections and inspire further investigations into the use of natural compounds in combating fungal pathogens.

Materials and Methods

All chemicals, solvents, and essential oils utilized in the investigations were sourced from Aromax Trading Pvt Ltd, Chennai, India, a reputable standard chemical supplier. For the qualitative phytochemical analysis of Eucalyptus oil, the methodology described by (Mishra et al.) was followed.

Test for Acids, Million's Test: In this test, 1.0 ml of oil was combined with five drops of Millon's reagent, then subjected to heating on a water bath for 5 minutes and subsequent cooling. Following this, 1% sodium nitrite solution was added. The presence of a red color indicates the presence of acids. Test for Alkaloids, Mayer's Test: For this test, 2.0 ml of oil was mixed with 2.0 ml of concentrated hydrochloric acid, followed by the addition of a few drops of Mayer's reagent. The appearance of a green color or white precipitate indicates the presence of alkaloids.

Test for Anthocyanin and Betacyanin: In the Sodium Hydroxide Test, 2.0 ml of oil is combined with 1.0 ml of 2N sodium hydroxide and heated for 5 minutes at 100°C. The formation of a bluish-green color indicates the presence of anthocyanin, while a yellow color indicates the presence of betacyanin. Test for Carbohydrates: Conducting Molisch's Test involves adding 1.0 ml of Molisch's reagent and a few drops of concentrated sulphuric acid to 2.0 ml of oil. The formation of a purple or reddish ring indicates the presence of carbohydrates. Test for Cardiac Glycosides: In the Ferric Chloride Test, 0.5 ml of oil is mixed with 2.0 ml of glacial acetic acid and a few drops of 5% ferric chloride. This mixture is layered with 1.0 ml of concentrated sodium hydroxide, and the formation of a brown ring at the interface indicates the presence of cardiac glycosides. Test for Coumarins: In the Sodium Hydroxide Test for coumarins, 1.0 ml of oil is mixed with 1.0 ml of 10% sodium hydroxide. The formation of a yellow color indicates the presence of coumarins. Test for Flavonoids: The

Sulphuric Acid Test involves treating 1.0 ml of oil with a few drops of concentrated sulphuric acid. The formation of an orange color indicates the presence of flavonoids. Test for Glycosides: In the Sulphuric Acid Test for glycosides, 2.0 ml of oil is mixed with 1.0 ml of glacial acetic acid, 5% ferric chloride, and a few drops of concentrated sulphuric acid. The presence of a greenish-blue color indicates the presence of glycosides.

Test for Phenols: Using the Ferric Chloride Test, 1.0 ml of oil was mixed with 2.0 ml of distilled water, followed by the addition of a few drops of 10% ferric chloride. The formation of a blue or green color indicates the presence of phenols. Test for Proteins: In the Ninhydrin Test, 2.0 ml of oil was treated with a few drops of 0.2% ninhydrin and heated for 5 minutes. The appearance of a blue color indicates the presence of proteins. Test for Quinones: Through the Sulphuric Acid Test, 1.0 ml of oil was combined with 1.0 ml of concentrated sodium hydroxide. The presence of a red color indicates the presence of quinones. Test for Saponins: The presence of saponins was determined using the Foam Test, where 1.0 ml of oil was mixed with 5.0 ml of distilled water and shaken well. Formation of a 1.0 cm layer of foam indicates the presence of saponins. Test for Starch: The Iodine Test involved adding a few drops of iodine solution to 2.0 ml of oil. The formation of a blue-purple color indicates the presence of starch.

Test for Steroids: Using the Salkowski Test, 5.0 ml of oil was mixed with 2.0 ml of chloroform and a few drops of concentrated sulphuric acid. The appearance of a red color indicates the presence of steroids. Test for Tannins: The Ferric Chloride Test was conducted by adding 2.0 ml of 5% ferric chloride to 1.0 ml of oil. The formation of a dark blue or greenish-black color indicates the presence of tannins. Test for Terpenoids: In the Sulphuric Acid Test, 0.5 ml of oil was mixed with 2.0 ml of chloroform, followed by the careful addition of concentrated sodium hydroxide. The formation of a red-brown color at the interface indicates the presence of terpenoids. Test for Triterpenoids: The Liebermann-Burchard's test involved adding Liebermann-Burchard's reagent (acetic anhydride and concentrated sodium hydroxide) to 1.5 ml of oil. The formation of a blue-green color indicates the presence of triterpenoids.

Quantitative Phytochemical Analysis

Primary metabolites are essential compounds synthesized in plants, directly contributing to normal growth, development, and reproduction, offering insights into the nutritional potential of plant parts. Tests for primary metabolites such as carbohydrates (Hedge and Hofreiter) and proteins (Pradeepa et al.) were conducted following standard procedures. Secondary metabolites, produced by plants for various purposes including defense and signaling, were evaluated for their properties and potential industrial applications. Secondary metabolites like flavonoids, polyphenols, quinones, saponins, and tannins were quantified. The total phenolic content was determined using the Folin-Ciocalteu method (McDonald et al.), while flavonoids were estimated through the Aluminum chloride colorimetric method (Chang et al.). Additionally, the estimation of tannins and saponins was conducted according to the method described by Schanderl (Schanderl).

In-vitro Antimicrobial Analysis of Selected Essential Oils

The species employed in the test consisted of all microbial stock cultures, which were procured from Microlab, Institute of Research and Technology, located in Vellore, Tamilnadu, India. The microorganisms were cultivated overnight at 37°C in Mueller-Hinton Broth with a pH of 7.4. The fungal species employed for the test were Candida albicans, Candida tropicalis, and Candida kefyr

Preparation of the Culture Media

The Sabouraud broth or Sabouraud dextrose (SDA) broth (Himedia, India) for fungus was prepared by dissolving 28 grams of nutrient agar and Sabouraud dextrose agar in 1000 milliliters of distilled water, following the method described by Mueller and Hinton. The pH of the medium was kept at 7.0, and it was stored at room temperature.

Inoculum Preparation and Antifungal Efficacy

The essential oil extracts were also evaluated for their antifungal efficacy, comparing them with the

standard antibiotic ketoconazole (at a concentration of 10 µg/mL) using an in-vitro well diffusion method. To prepare the agar plates, 20 ml of Sabouraud agar or Sabouraud dextrose agar (SDA) was poured onto Petri dishes and allowed to solidify. In test tubes, a loopful of each fungus was introduced into 10 ml of SDA broth, continuing this process until the turbidity matched the standard 0.5 McFarland. The overnight-cultured fungus strain was adjusted to an inoculum size of 106 CFU/ml for the subsequent inoculation of agar plates. Using cotton swabs, the inoculum was gently distributed across the medium's surface and allowed to air dry. A well with a 5 mm diameter was meticulously created using a sterile cork borer No. 4, and then the agar discs were carefully removed. The prepared wells were filled with different concentrations of each essential oil using a microtiter-pipette, and the corresponding antibiotics were used as positive controls. The plates were then allowed to sit at room temperature for a period of two hours for diffusion. Following this, the plates were incubated in an upright position at 37°C for a total of 24 hours. The dimensions of the growth inhibition zones were measured, and the outcomes were averaged, with the mean values subsequently documented in a table.

Analysis of Minimum Inhibitory Concentration (MIC)

To determine the Minimum Inhibitory Concentration (MIC), various concentrations of the selected essential oils were evaluated against the previously mentioned fungi, spanning from 0.05 to 2 mg/ml. The MIC is defined as the lowest concentration of the essential oils (highest dilution) that effectively inhibits the growth of the respective bacteria and fungi. The MIC (Minimum Inhibitory Concentration) was determined as the lowest dilution at which complete inhibition of visible growth was observed in the tubes. To confirm the MIC, samples from the MIC tubes were plated onto agar media to check for the presence or absence of growth. DMSO served as the solvent control, while Ketoconazole was used as the reference antifungal agent (Abdellatif et al.). All tests were conducted in triplicate to ensure accuracy and reliability of the results. By following these detailed methodologies and justifications, the study ensures that the determination of MIC for eucalyptus oil against Candida species is accurate, reliable, and scientifically valid.

GC-MS Spectral Analysis

GC-MS spectral analysis was conducted to identify and analyze the presence of aromatic compounds within the essential oil samples. The analysis utilized an Agilent 7890 gas chromatograph interfaced with a 240-mass selective detector and an ion trap. Interpretation of the GC-MS data was performed using the extensive database of the National Institute of Standards and Technology (NIST), containing over 62,000 patterns. During analysis, the spectrum of the unknown component was compared with the spectra of known components stored within the NIST library. This comparison allowed for the determination of the name, molecular weight, and structural characteristics of the components present in the test materials. Through this method, a comprehensive understanding of the chemical composition of the essential oil samples was achieved, aiding in the characterization and identification of specific aromatic compounds.

Statistical Analysis

The results were expressed in mean ± Standard Deviation. The statistical analysis was performed using Graph Pad Prism. All the assays were performed in triplicate.

Results and Discussion

Qualitative Phytochemical Analysis of Eucalyptus Oil

Qualitative phytochemical analysis is a method utilized to identify and ascertain the presence of various chemical compounds within plants (Hossain et al.). In the case of eucalyptus oil, this analysis unveiled the existence of several compounds, including carbohydrates, flavonoids, phenols, proteins, quinones, saponins, and tannins (Table 1). Carbohydrates are organic compounds crucial for providing energy in living organisms (Maod et al.), while in plants, they serve pivotal roles in offering structural support and storing energy (Deng et al.). Flavonoids, on the other hand, represent a group of plant pigments responsible for the vibrant hues observed in flowers and fruits (Giusti et al.). Renowned for their antioxidant

properties, flavonoids are also recognized for their potential health benefits (Wang et al.). Phenols constitute a diverse class of plant-derived compounds renowned for their antioxidant characteristics and antimicrobial properties (Saxena et al.).

Phenols contribute significantly to the characteristic aroma and taste of plants (Zeb), while proteins represent essential macromolecules crucial for various biological processes (Wyman and Gill). Acting as structural components, enzymes, and signaling molecules in plants (Ellis), proteins play a pivotal role in plant growth and development. Quinones, aromatic compounds present in plants (Mackiewicz et al.), participate in diverse biological processes, including electron transfer reactions and defense mechanisms against pathogens (Mackiewicz et al.). Saponins, glycosides responsible for the foaming properties often observed in plants (Góral and Wojciechowski), exhibit multiple biological activities, including antimicrobial and anti-inflammatory effects (Desai et al.). Moreover, saponins are known for their interaction with cell membranes (Gemede and Ratta). Tannins, polyphenolic compounds commonly found in plants (Nguyen et al.), possess astringent properties and contribute to plant defense against herbivores (Ashok and Upadhyaya). Additionally, tannins can interact with proteins, forming complexes that impact digestion and absorption (Crozier et al.).

The presence of these compounds in Eucalyptus oil can be attributed to the natural composition of the plant. Each compound serves various functions within the plant, such as protection against pathogens, pests, and UV radiation, in addition to playing roles in signaling and providing structural support. These diverse compounds likely contribute to the overall properties and potential benefits of Eucalyptus oil, including its aroma, antimicrobial activity, and potential therapeutic effects.

Table 1 Qualitative Phytochemical Analysis of Eucalyptus Oil

Phytocompounds	Eucalyptus oil
Acids	-
Alkaloids	-
Anthocyanins and Betacyanins	-
Carbohydrates	+
Cardiac Glycosides	-
Coumarins	-
Flavonoids	+
Glycosides	-
Phenols	+
Proteins	+
Quinones	+
Saponins	+
Starch	-
Steroids	-
Tannins	+
Terpenoids	-
Triterpenoids	-

whereas + denotes presence of phytocompounds and – denotes absence of phytocompounds

Quantitative Phytochemical Analysis of Eucalyptus oil

Upon comparing the concentrations of phytocompounds, it is evident that flavonoids exhibit the highest concentration, measured at 74±8.27 mg/ml (Table 2). This indicates a relatively abundant presence of flavonoids in the sample compared to other compounds. Following flavonoids, tannins present the next highest concentration at 17.75±3.12 mg/ml, albeit lower than that of flavonoids. Polyphenols, with a concentration of 57.75±3.12 mg/ml, rank slightly below flavonoids but still surpass the concentrations of carbohydrates, proteins, quinones, and saponins. Among these compounds, carbohydrates exhibit the lowest concentration at 2.24±0.05 mg/ml. Similarly, proteins, quinones, and saponins also display comparatively lower concentrations when juxtaposed with flavonoids, polyphenols, and tannins. To summarize the given phytochemical results, flavonoids emerge as the compound with the highest concentration, followed by tannins and polyphenols.

Table 2 Quantitative Phytochemical Analysis of Eucalyptus Pil

Phytochemicals	Eucalyptus oil
Carbohydrates	2.24±0.05 mg/ml
Flavonoids	74±8.27 mg/ml
Polyphenol	57.75±3.12 mg/ml
Protein	1.512±0.03 mg/ml

Quinones	0.04±005 mg/ml
Saponins	0.925±0.04 mg/ml
Tannins	17.75±3.12 mg/ml

Mean ± Standard Deviation

The quantitative phytochemical analysis of eucalyptus oil reveals the presence and concentration of various phytocompounds as follows:

Eucalyptus oil contains approximately 2.24±0.05 mg of carbohydrates, serving as a primary source of energy and nutrients. Within eucalyptus oil, these carbohydrates may contribute to its nutritional value or potential medicinal properties (Bello et al.). The oil also boasts about 74±8.27 mg of flavonoids, a diverse group of plant compounds celebrated for their antioxidant and anti-inflammatory properties. With links to various health benefits, including cardiovascular protection and immune system support, the substantial concentration of flavonoids in eucalyptus oil suggests noteworthy antioxidant and anti-inflammatory effects (Kang et al.; Rubió et al.; Khan et al. "Antioxidant and Anti-Inflammatory Effects of *Citrus* Flavonoid Hesperetin"; Rakha et al.). Furthermore, eucalyptus oil houses approximately 57.75±3.12 mg of polyphenols, renowned for their antioxidant prowess. Associated with reducing the risk of chronic diseases and shielding against cellular damage from oxidative stress, the presence of polyphenols underscores eucalyptus oil's potential as an antioxidant-rich substance (Frankel et al.; Amira et al.; Ahangarpour et al.; Oszmiański et al.). Additionally, the oil contains around 1.512±0.03 mg of protein, essential macronutrients crucial in numerous biological processes. Although the protein concentration in eucalyptus oil is relatively low compared to other compounds, its presence hints at a contribution to the oil's nutritional value (Chouhan et al.; Bhavaniramya et al.; Horst et al.).

Eucalyptus oil contains approximately 0.04±0.05 mg of quinones, a class of organic compounds known for their antimicrobial and antifungal properties (Macías et al.; Dulo et al.; Dahlem Junior et al.). Although their concentration in eucalyptus oil is relatively low, their presence may contribute to the oil's potential antimicrobial effects. Additionally, the oil contains about 0.925±0.04 mg of saponins, plant compounds renowned for their potential anti-inflammatory and immune-modulating properties (Ghosh et al.). With reported cholesterol-lowering effects and the ability to form a soapy lather when mixed with water, the presence of saponins in eucalyptus oil suggests it may possess these beneficial properties (Salehi et al.; Tiwari et al.). Furthermore, the oil harbors approximately 17.75±3.12 mg of tannins, a group of plant compounds acclaimed for their astringent properties and traditional use for antimicrobial and antioxidant effects. The relatively high concentration of tannins in eucalyptus oil indicates its potential to exhibit astringent, antimicrobial, and antioxidant activities (Aleksic and Knezevic; Nasr et al.). In summary, the quantitative phytochemical analysis of eucalyptus oil unveils the presence of various bioactive compounds, including flavonoids, polyphenols, proteins, quinones, saponins, and tannins. These compounds collectively contribute to the potential health benefits and medicinal properties associated with eucalyptus oil, such as antioxidant, anti-inflammatory, antimicrobial, and immune-modulating effects.

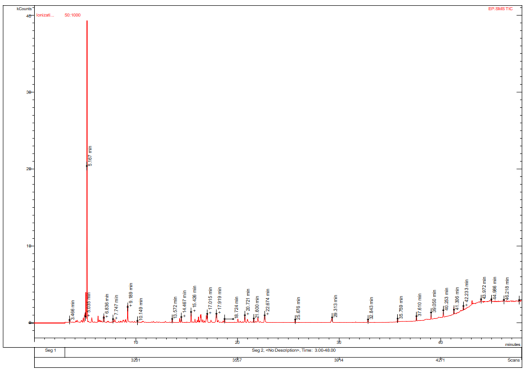

Figure 1 GC-MS chromatogram of Eucalyptus oil

Table 3 Phytochemical Compounds Identified through GC-MS Analysis of Eucalyptus Essential Oil

S. No	PubChem (CID)	Compound name	RT	Molecular formula	Molecular weight	Area	Amount
1	75549	1,4-Benzenediol, monobenzoate	3.466	$C_{13}H_{10}O_3$	214.22	1422	265
2	69019	1-Butyne, 3-methyl-	5.035	C_5H_8	68.12	13989	1837
3	543208	2,3-Diamino-2-cyanosuccinonitrile	5.167	$C_5H_5N_5$	135.13	97254	24446
4	70260	1,2,5-Trimethylpyrrole	6.836	$C_7H_{11}N$	109.17	3962	713
5	576892	Menthane, 3-chloro-8-phenyl	7.747	$C_{16}H_{23}Cl$	250.80	1652	298
6	5365328	1-Hexen-4-ol, 1-chloro-3-methyl	9.189	$C_7H_{13}ClO$	148.63	6355	1188
7	12752	Silane, phenyl	10.149	C_6H_8Si	108.21	947	147
8	10581	1,3-Cyclohexadiene, 1,5,5,6-tetramethyl	13.572	$C_{10}H_{16}$	136.23	1671	307
9	10686	Benzene, 1,2,3-trimethyl	14.467	C_9H_{12}	120.19	2384	436
10	10686	Benzene, 1,2,3-trimethyl	15.426	C_9H_{12}	120.19	4952	950
11	572209	N-((Methylphenylamino)methyl) benzamide	17.015	$C_{15}H_{16}N_2O$	240.30	8045	921
12	572209	N-(Methylphenylamino)methyl) benzamide	17.919	$C_{15}H_{16}N_2O$	240.30	7557	1048
13	570475	Heptane, 2,6-diphenyl-3-methyl	18.724	$C_{20}H_{26}$	266.4	2051	341
14	11170	Cyclooctasiloxane, hexadecamethyl	21.600	$C_{16}H_{48}O_8Si_8$	593.2	1808	350
15	12752	Silane, phenyl	22.674	C_6H_8Si	108.21	5628	801
16	11170	Cyclononasiloxane, octadecamethyl	25.676	$C_{16}H_{48}O_8Si_8$	593.2	1298	251
17	11169	Cyclotetrasiloxane, octamethyl	29.313	$C_8H_{24}O_4Si_4$	296.61	2775	494
18	11169	Cyclotetrasiloxane, octamethyl	32.843	$C_8H_{24}O_4Si_4$	296.61	1803	299
19	11169	Cyclotetrasiloxane, octamethyl	39.050	$C_8H_{24}O_4Si_4$	296.61	861	153
20	11169	Cyclotetrasiloxane, octamethyl	40.253	$C_8H_{24}O_4Si_4$	296.61	2352	442
21	11169	Cyclotetrasiloxane, octamethyl	41.306	$C_8H_{24}O_4Si_4$	296.61	2204	421
22	11169	Cyclotetrasiloxane, octamethyl	42.233	$C_8H_{24}O_4Si_4$	296.61	1662	339
23	91740709	2,5-Dihydroxyacetophenone, 2TMS derivati	43.972	$C_{14}H_{24}O_3Si_2$	296.51	2408	300
24	6329858	1,3,5,7-Tetraethyl-1-butoxycyclotetrasiloxane	44.986	$C_{12}H_{29}O_5Si_4$	365.70	1042	206
25	6329858	1,3,5,7-Tetraethyl-1-butoxycyclotetrasiloxane	46.218	$C_{12}H_{29}O_5Si_4$	365.70	1039	160

Eucalyptus oil showed 27 peaks (Figure 1 and table 3) with different retention times and a peak area was identified. The main compounds of essential oil were Benzoic acid is used as a preservative in food and beverages, as it inhibits the growth of mold, yeast, and some bacteria (Chipley). 1-Butyne, 3-methyl (5.035 min) used as a chemical intermediate in the production of pharmaceuticals. 2,3-Diamino-2-cyanosuccinonitrile (5.167 min) is a versatile building block used in the synthesis of a variety of compounds. It is used in the synthesis of pharmaceuticals, dyes, and other organic compounds. It is also used in the synthesis of polymers, such as polyurethanes, polyamides, and polyimides. It is also used in the synthesis of polymers for use in medical devices, such as catheters and stents. Additionally, it is used in the synthesis of polymers for use in the production of fuel cells (Mandoda). 2-p-Nitrobenzoyl-1,3,5-tribenzyl-.alpha(5.635 min) is a type of organic compound that can be used in a variety of applications. It can be used as a reagent in organic synthesis, as a catalyst in the production of polymers, and as a fluorescent dye in biological imaging. It can also be used as a ligand

in coordination chemistry, as a corrosion inhibitor, and as a stabilizer in the production of polymers. Additionally, it can be used as a fluorescent probe for the detection of metal ions and as a fluorescent marker for the detection of proteins (Abd El-Bar and Fawki).

1,3-Cyclohexadiene, 1,5,5,6-tetramethyl-8 (6.273 min) is a versatile organic compound that can be used in a variety of applications. It is used as a starting material in the synthesis of a variety of organic compounds, including pharmaceuticals, dyes, and fragrances. It is also used as a reagent in organic synthesis, as a ligand in coordination chemistry, and as a catalyst in polymerization reactions. Additionally, 1,3-cyclohexadiene can be used as a fuel additive to improve the combustion of gasoline and diesel fuels. Finally, 1,5,5,6-tetramethyl-8 is used as a precursor in the synthesis of a variety of organic compounds, including pharmaceuticals, dyes, and fragrances (Deb and Weber). Ethchlorvynol (6.408 min) is sometimes used to treat symptoms of vertigo, a condition characterized by a sensation of spinning or whirling and used to treat symptoms of motion sickness (Carr and Crampton). 2-Heptenoic acid, phenyl ester (8.757 min) used as anti-inflammatory drugs and antifungal agents. 1,3-Cyclohexadiene, 1,5,5,6-tetramethyl (13.572 min) can be used as a starting material for the synthesis of pharmaceuticals, such as anti-inflammatory drugs and antibiotics (Phillips et al.).

N-((Methylphenylamino) methyl) benzamide (15.811 min) is a chemical compound used in the synthesis of pharmaceuticals, such as anti-inflammatory drugs, anticonvulsants, and anti-depressants. It is also used as a reagent in organic synthesis (Liu et al.). 1,3-Oxathiolane-2-thione, 4-hydroxy-4,5-24 (16.039 min) can be used as an intermediate in the synthesis of various pharmaceuticals, such as anti-inflammatory drugs, antifungal agents, and anti-cancer agents (Kihara et al.). Benzenepropanesulfonamide,.beta.-hydrox (16.380 min) is used in the synthesis of pharmaceuticals, such as anti-inflammatory drugs, anticonvulsants, and anti-cancer agents. It is also used as a reagent in organic synthesis, as a catalyst in the production of polymers, and as a surfactant in detergents (Scozzafava et al.). [3,3-Dimethyl-2-(3-methylbuta-1,3-dienyl (16.750 min) can be used as an intermediate in the synthesis of pharmaceuticals such as anti-inflammatory drugs, antifungal agents, and anti-cancer drugs (Okoye et al.). (4S,4aR,6R)-4,4a-Dimethyl-6-(prop-1-en-2 (20.035 min) can be used as an active pharmaceutical ingredient in the development of new drugs (Chouni et al.).

Propenamide, 2-benzoyl amino-3-(2-fluoro (20.721 min) used in the synthesis of various pharmaceuticals, such as anti-inflammatory drugs, anticonvulsants, and anti-cancer drugs (Dixit and Bharatam). (S)-9-[(S)-2-(Hydroxymethyl) pyrrolidine-1 (43.080 min) can be used as an active pharmaceutical ingredient in the development of new drugs for the treatment of various diseases (Sato et al.) and 2,5-Dihydroxyacetophenone (43.972 min) used as a starting material for the synthesis of pharmaceuticals, such as anti-inflammatory drugs and antibiotics (Han et al.).

One noteworthy peak is Peak 6, which corresponds to 2,3-Diamino-2-cyanosuccinonitrile (CAS Number: 167320-30-1) with a retention time of 5.167 minutes. This compound exhibits a high peak area with a value greater than or equal to 100. The significant peak area suggests that 2,3-Diamino-2-cyanosuccinonitrile is present in relatively high abundance in the eucalyptus oil sample (Table 3). The presence of 2,3-Diamino-2-cyanosuccinonitrile in eucalyptus oil could have several implications. This compound may contribute to the overall aroma or flavor of the oil, as it is known that different chemical components can influence the sensory characteristics of essential oils. Further investigation is required to determine the specific role and potential benefits of 2,3-Diamino-2-cyanosuccinonitrile in eucalyptus oil. It is important to note that some peaks do not have associated CAS numbers, indicating that the exact chemical identity of those compounds is not readily available or requires further analysis for accurate identification. Overall, the GC-MS spectral analysis provides valuable information about the chemical composition of eucalyptus oil, highlighting the presence of various compounds and emphasizing the potential significance of 2,3-Diamino-2-cyanosuccinonitrile in the composition. Further studies can focus on exploring the biological activities of eucalyptus oil.

Minimum Inhibitory Concentration (MIC) Assay

Table 4 MIC of Tested Fungal Species Against Eucalyptus Oil at Different Concentrations

	Concentrations (µl)							
Fungal species	**1000**	**500**	**250**	**125**	**62.5**	**31.25**	**15.6**	**Ketoconazole (31.25)**
C. albicans	+	+	+	+	+	+	+	-
C. tropicalis	+	+	+	+	+	+	+	-
C. kefyr	+	+	+	+	-	-	-	-

Whereas, - denotes no growth and + denotes growth of fungal species Candida albigans, Candida tropicalis fungal growth observed at 1000 µl, 500 µl, 250 µl, 125 µl, 62.5 µl, 31.25 µl, and 15.6 µl of eucalyptus oil whereas Candida kefyr fungal growth observed at 1000 µl, 500 µl, 250 µl, 125 µl, no fungal growth at 62.5 µl, 31.25 µl, and 15.6 µl of eucalyptus oil concenrations. Whereas, ketoconazole, a known antifungal medication, showed no fungal growth at 31.25 µl concentration (Table 4 and Figure 2 a,b,c). Based on these results, we can infer that eucalyptus oil has antifungal properties against the tested Candida species. However, the growth inhibition differs depending on the concentration used. The mechanism of action for eucalyptus oil's antifungal activity can be attributed to its chemical constituents. Eucalyptus oil contains several bioactive compounds such as 1,8-cineole, alpha-terpineol, and terpinen-4-ol, which have demonstrated antifungal properties. 1,8-cineole, also known as eucalyptol, is a major component of eucalyptus oil and has been shown to possess antifungal effects. It can disrupt the fungal cell membrane, interfere with cellular processes, and inhibit fungal growth. Alpha-terpineol and terpinen-4-ol are monoterpenoids present in eucalyptus oil. These compounds have been reported to exhibit antifungal activity by disrupting fungal membrane integrity, altering membrane permeability, and interfering with cellular functions. The observed MIC values indicate the concentration at which eucalyptus oil is effective in inhibiting fungal growth. Higher concentrations (e.g., 1000 µl) may still allow fungal growth, while lower concentrations (e.g., 31.25 µl) inhibit growth for some Candida species, but not others. It's worth noting that these results provide valuable information about the susceptibility of Candida species to eucalyptus oil and ketoconazole in vitro. However, further studies are necessary to evaluate their effectiveness in clinical settings and to understand the exact mechanisms by which eucalyptus oil exerts its antifungal effects on Candida species.

a. C. albigans

b. C. tropicalis

c. C. kefyr

Figure 2 (a,b,c) MIC of Candida Species Tested against Different Concentrations of Eucalyptus Oil

Conclusion

In conclusion, the qualitative phytochemical analysis of eucalyptus oil has provided valuable insights into its chemical composition, revealing

the presence of various bioactive compounds. Among these, flavonoids were found to be present in the highest concentration, indicating their potential significance in the oil's overall properties. Additionally, the GC-MS analysis identified 2,3-Diamino-2-cyanosuccinonitrile as a major component of the oil, shedding light on its molecular composition. Furthermore, the antifungal assays conducted in this study demonstrated the inhibitory effects of eucalyptus oil against Candida species, underscoring its potential as an antifungal agent. This observed antifungal activity suggests that bioactive compounds present in the oil, such as 1-Butyne, 3-methyl-, 1-Hexen-4-ol, 1-chloro-3-methyl, N-(Methylphenylamino)methyl) benzamide, may play a crucial role in its effectiveness against fungal pathogens.

However, it is important to note that further studies are warranted to elucidate the precise mechanisms underlying the antifungal activity of eucalyptus oil. Additionally, clinical trials are needed to evaluate the efficacy and safety of eucalyptus oil in real-world settings. Future research should focus on elucidating the mechanisms of action and conducting clinical trials to validate the effectiveness of eucalyptus oil in clinical applications. Overall, the findings from this research provide a promising foundation for future investigations into the therapeutic potential of eucalyptus oil in combating fungal infections.

Recommendation

Based on the findings of this study, it is recommended to conduct further research to elucidate the specific mechanisms underlying the antifungal effects of eucalyptus oil and its bioactive compounds against Candida species. In-depth investigations are warranted to determine the optimal concentrations and formulations of eucalyptus oil, considering its potential as a natural alternative or adjunct to existing antifungal medications. Clinical trials are essential to evaluate the efficacy and safety of eucalyptus oil in treating fungal infections caused by Candida species, with a comparison to standard antifungal treatments. Additionally, exploring the potential synergistic effects of eucalyptus oil with other antifungal agents is crucial to enhance overall antifungal activity and mitigate the development of resistance.

Future Aspects

The findings of this study offer valuable insights into the phytochemical composition and antifungal properties of eucalyptus oil. However, there are several avenues for future research to explore. Firstly, investigating the bioactivity of other compounds identified in eucalyptus oil, particularly 2,3-Diamino-2-cyanosuccinonitrile, can provide a deeper understanding of their potential therapeutic applications and mechanisms of action. Secondly, exploring the potential antimicrobial effects of eucalyptus oil against other pathogenic microorganisms can expand its applications in the field of infectious diseases. Thirdly, evaluating the safety and tolerability of eucalyptus oil in human subjects, including potential adverse effects and interactions with other medications, is crucial for its clinical use. Lastly, the development of standardized extraction and purification methods is essential to ensure consistent quality and efficacy of eucalyptus oil for therapeutic purposes. These areas of research will further enhance our understanding and utilization of eucalyptus oil as a natural remedy.

Acknowledgements

The authors would like to thank the Director of Microlabs at the Institute of Research and Technology in Vellore, Tamil Nadu, India, for generously providing the necessary facilities for conducting this research. Additionally, their assistance in evaluating antimicrobial activity is greatly appreciated.

Declarations

Ethics approval Not applicable.

Funding No funding and worked with online and freely available software's.

Conflict of interests The authors declare that they have no conflict of interests.

Availability of data and materials All the data generated or analyzed during this study are included in this article.

References

Abd El-Bar, M., & S. Fawki. "Fumigant Activity and Chemical Composition of Three Essential Oils Used in Gelatin Capsules for the Control of *Acanthoscelides obtectus* (Say)

(Coleoptera: Chrysomelidae) in Egypt." *African Entomology*, vol. 29, no. 2, 2021, pp. 534-46.

Abdellatif, Fahima, et al. "Chemical Composition and Antimicrobial Activity of the Essential Oil from Leaves of Algerian Melissa Officinalis L." *EXCLI Journal*, vol. 13, 2014, pp. 772-81.

Ahangarpour, Akram, et al. "The Antidiabetic and Antioxidant Properties of some Phenolic Phytochemicals: A Review Study." *Diabetes & Metabolic Syndrome*, vol. 13, no. 1, 2019, pp. 854-57.

Aleksic, Verica, and Petar Knezevic. "Antimicrobial and Antioxidative Activity of Extracts and Essential Oils of *Myrtus communis* L." *Microbiological Research*, vol. 169, no. 4, 2014, pp. 240-54.

Aljaafari, Mariam Nasser, et al. "An Overview of the Potential Therapeutic Applications of Essential Oils." *Molecules*, vol. 26, no. 3, 2021.

Aljaffary, Mohammed, et al. "Effects of Nystatin oral rinse on oral *Candida species* and *Streptococcus mutans* among healthy adults. *Clinical Oral Investigations*, vol. 27, 2023.

Amira, El Arem, et al. "Effects of the Ripening Stage on Phenolic Profile, Phytochemical Composition and Antioxidant Activity of Date Palm Fruit." *Journal of Agricultural and Food Chemistry*, vol. 60, 2012.

Ashok, P. K., and K. Upadhyaya. "Tannins are astringent." *Journal of Pharmacognosy and Phytochemistry*, vol. 1, no. 3, 2012, pp. 45-50.

Baptista-Silva, Sara, et al. "The Progress of Essential Oils as Potential Therapeutic Agents: A Review." *Journal of Essential Oil Research*, vol. 32, no. 4, 2020, pp. 279-95.

Bello, Morenikejimi, et al. "Antioxidant Property of Eucalyptus globulus Labill. Extracts and Inhibitory Activities on Carbohydrate Metabolizing Enzymes related to type-2 Diabetes." *Biocatalysis and Agricultural Biotechnology*, vol. 36, 2021.

Bhavaniramya, Sundaresan, et al. "Role of Essential Oils in Food Safety: Antimicrobial and Antioxidant Applications." *Grain & Oil Science and Technology*, vol. 2, no. 2, 2019.

Cadnum, Jennifer L., et al. "Effectiveness of Disinfectants against *Candida auris* and other *Candida* Species." *Infection Control & Hospital Epidemiology*, vol. 38, no. 10, 2017.

Carr, D. J., and R. F. Crampton. "Ethchlorvynol." *British Medical Journal*, 1963.

Chandorkar, Nikhil, et al. "A Systematic and Comprehensive Review on Current Understanding of the Pharmacological Actions, Molecular Mechanisms, and Clinical Implications of the genus *Eucalyptus*." *Phytomedicine Plus*, vol. 1, no. 4, 2021.

Chang, C. C., et al. "Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods." *Journal of Food and Drug Analysis*, vol. 10, no. 3, 2002.

Chipley, John R. "Sodium Benzoate and Benzoic Acid." *Antimicrobials in Food,* CRC Press, 2020, pp. 41-88.

Chouhan, Sonaam, et al. "Antimicrobial Activity of some Essential Oils - Present Status and Future Perspectives." *Medicines*, vol. 4, no. 3, 2017.

Chouni, Anirban, et al. "GC-MS Analysis and Screening of Anti-Proliferative Potential of Methanolic Extract of *Garcinia cowa* on Different Cancer Cell Lines." *Pharmacognosy Journal*, vol. 13, no. 2, 2021.

Cortegiani, Andrea, et al. "What's New on Emerging Resistant *Candida* Species." *Intensive Care Medicine*, vol. 45, 2018, pp. 512-15.

Crozier, Alan, et al. "Phenols, Polyphenols and Tannins: An Overview." *Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Diet*, Blackwell Publishing, 2006, pp. 1-25.

Dahlem Junior, Marcos Aurelio, et al. "Quinones as an Efficient Molecular Scaffold in the Antibacterial/Antifungal or Antitumoral Arsenal." *International Journal of Molecular Sciences*, vol. 23, no. 22, 2022.

Deb, Sanghamitra, and Peter M. Weber. "The Ultrafast Pathway of Photon-Induced Electrocyclic Ring-opening Reactions: The Case of 1,3-Cyclohexadiene." *Annual Review of Physical Chemistry*, vol. 62, 2011, pp. 19-39.

Deng, Jiang, et al. "Biomass-derived Carbon: Synthesis and Applications in Energy Storage and Conversion." *Green Chemistry*, no. 18, 2016.

Desai, Sapna D., et al. "Saponins and their Biological Activities." *Pharma Times*, vol. 41, no. 3, 2009, pp. 13-16.

Dixit, Vaibhav A., and Prasad V. Bharatam. "SAR and Computer-Aided Drug Design Approaches in the Discovery of Peroxisome Proliferator-Activated Receptor γ (PPARγ) Activators: A perspective." *Journal of Computational Medicine*, 2013.

Dulo, Benson, et al. "Natural Quinone Dyes: A Review on Structure, Extraction Techniques, Analysis and Application Potential." *Waste and Biomass Valorization*, vol. 12, 2021.

El-Sakhawy, M. A., et al. "Anticandidal effect of Eucalyptus oil and three isolated compounds on cutaneous wound healing in rats." *European Review for Medical and Pharmacological Sciences*, vol. 27, no. 1, 2023, pp. 26-37.

Ellis, R. John. "Macromolecular Crowding: Obvious but underappreciated." *Trends in Biochemical Sciences*, vol. 26, no. 10, 2001, pp. 597-604.

Fitsiou, E., et al. "Phytochemical Profile and Evaluation of the Biological Activities of Essential Oils Derived from the Greek Aromatic Plant Species *Ocimum basilicum*, *Mentha spicata*, *Pimpinella anisum* and *Fortunella margarita*." *Molecules*, vol. 21, no. 8, 2016.

Frankel, Edwin N., et al. "Principal Phenolic Phytochemicals in Selected California Wines and their Antioxidant Activity in Inhibiting Oxidation of Human Low-Density Lipoproteins." *Journal of Agricultural and Food Chemistry*, vol. 43, 1995, pp. 890-94.

Gemede, Habtamu Fekadu, and Negussie Ratta. "Antinutritional Factors in Plant Foods: Potential Health Benefits and Adverse Effects." *International Journal of Nutrition and Food Sciences*, vol. 3, no. 4, 2014, pp. 284-89.

Ghosh, P., et al. "Morphological, Ethno biological and Phytopharmacological Attributes of *Tridax procumbens* Linn. (Asteraceae): A Review." *International Journal of Scientific Research in Biological Sciences*, vol. 6, no. 2, 2019, pp. 182-91.

Giusti, M. Monica, et al. "Flavonoids as Natural Pigments." *Handbook of Natural Colorants*, John Wiley, 2023, pp. 371-90.

Góral, I., and K. Wojciechowski. "Surface Activity and Foaming Properties of Saponin-rich Plants Extracts." *Advances in Colloid and Interface Science*, vol. 279, 2020.

Han, Yunkyung, et al. "2, 5-dihydroxyacetophenone Isolated from Rehmanniae Radix Preparata Inhibits Inflammatory Responses in Lipopolysaccharide-Stimulated RAW264.7 macrophages." *Journal of Medicinal Food*, vol. 15, no. 6, 2012, pp. 505-10.

Haynes, Ken. "Virulence in *Candida* Species." *Trends in Microbiology*, vol. 9, no. 12, 2001, pp. 591-96.

Hedge, J. E., and B. T. Hofreiter. *Carbohydrate Chemistry*. Academic Press, 1962.

Horst, Egon Henrique, et al. "Seasonal and Species Variations in the Nutritive Value of Eucalyptus Foliage as a Potential Feed Resource for Ruminants in Silvopastoral Systems." *Agroforestry Systems*, vol. 96, 2022.

Hossain, Farah, et al. "Evidence for Synergistic Activity of Plant-Derived Essential Oils against Fungal Pathogens of Food." *Food Microbiology*, vol. 53, 2016, pp. 24-30.

Hussain, Fahad, et al. "Phytopharmacological Potential of Different Species of *Morus alba* and their Bioactive Phytochemicals: A Review." *Asian Pacific Journal of Tropical Biomedicine*, vol. 7, no. 10, 2017, pp. 950-56.

Jin, L., et al. "Gut Microbes in Cardiovascular Diseases and their Potential Therapeutic Applications." *Protein & Cell*, vol. 12, no. 5, 2021, pp. 346-59.

Kang, J., et al. "Flavonoids from acai (*Euterpe oleracea* Mart.) Pulp and their Antioxidant and Anti-Inflammatory Activities." *Food Chemistry,* vol. 128, no. 1, 2011, pp. 152-57.

Kesharwani, V., et al. "A review on therapeutics application of eucalyptus oil." *International Journal of Herbal Medicine*, vol. 6, no. 6, 2018, pp. 110-15.

Khan, Abida Kalsoom, et al. "Pharmacological Activities of Protocatechuic Acid." *Acta Poloniae Pharmaceutica*, vol. 72, no. 4, 2015, pp. 643-50.

Khan, Amjad, et al. "Antioxidant and Anti-Inflammatory Effects of *Citrus* Flavonoid Hesperetin: Special Focus on Neurological Disorders." *Antioxidants*, vol. 9, no. 7, 2020.

Kihara, Nobuhiro, et al. "Preparation of 1,3-Oxathiolane-2-Thiones by the Reaction of Oxirane and Carbon Disulfide." *The Journal of Organic Chemistry*, vol. 60, no. 2, 1995, pp. 473-75.

Koreti, Deepali, et al. "Application of Essential Oils in Alternative Medicine." *Essential Oils: Sources, Production and Applications*, Walter de Gruyter, 2023, pp. 237-52.

Kühbacher, Andreas, et al. "Interaction of *Candida* species with the skin." *Microorganisms*, vol. 5, no. 2, 2017.

Lachumy, Subramanion, et al. "Pharmacological Activity, Phytochemical Analysis and Toxicity of Methanol Extract of *Etlingera elatior* (torch ginger) Flowers." *Asian Pacific Journal of Tropical Medicine*, vol. 3, no. 10, 2010, pp. 769-74.

Liu, Chunjian, et al. "Discovery of 4-(5-(Cyclopropylcarbamoyl)-2-methylphenylamino)-5-Methyl-*N*-propylpyrrolo [1, 2-*f*][1, 2, 4]triazine-6-carboxamide (BMS-582949), a Clinical p38α MAP Kinase Inhibitor for the Treatment of Inflammatory Diseases." *Journal of Medicinal Chemistry*, vol. 53, no. 18, 2010.

Mackiewicz, M., et al. "Macromolecule Biosynthesis: A Key Function of Sleep." *Physiological Genomics*, vol. 31, no. 3, 2007, pp. 441-57.

Macías, Francisco A., et al. "Recent Advances in Allelopathy for Weed Control: From Knowledge to Applications." *Pest Management Science*, vol. 75, no. 9, 2019.

Mandoda, Purvi. *The Exploration of Pyrolysis-GC/MS alongside UPLC to Identify Phenolic Compounds in a Variety of Legumes and Grains*. University of Helsinki, 2022.

Maod, Fitriana, et al. "Identification of Organic Compounds as Carbohydrates and Chemicals Contained in them." *Journal of Health, Technology and Science*, vol. 3, no. 1, 2022, pp. 10-18.

McDonald, Suzy, et al. "Phenolic content and antioxidant activity of olive extracts." *Food Chemistry*, vol. 73, no. 1, 2001, pp. 73-84.

Mishra, Priyanka, et al. "Phytochemical Analysis and Assessment of in *vitro* Antibacterial Activity of *Tinospora cordifolia*." *International Journal of Current Microbiology and Applied Sciences*, vol. 3, no. 3, 2014, pp. 224-34.

Mokhber-Dezfuli, Najmeh, et al. (2014). Phytochemistry and Pharmacology of *berberis species*. *Pharmacognosy Reviews*, vol. 8, no. 15, 2014, pp. 8-15.

Montazeri, Naser, et al. "Phytochemical Contents and Biological Activities of *Rosa canina* fruit from Iran." *Journal of Medicinal Plants Research*, vol. 5, no. 18, 2011.

Mullai Nila, K., et al. "Phytochemical Profiling and Antioxidant Activity of Red Spinach (*Amaranthus dubius*)." *International Journal of Zoological Investigations*, vol. 9, no. 1, 2023, pp. 178-83.

Nasr, Alyaa, et al. "Phenolic Compounds and Antioxidants from *Eucalyptus camaldulensis* as Affected by some Extraction Conditions, a Preparative Optimization for GC-MS analysis." *Preparative Biochemistry & Biotechnology*, vol. 49, no. 5, 2019, pp. 464-76.

Nguyen, Linh T., et al. "An Overview of Saponins – A Bioactive Group." *Bulletin UASVM Food Science and Technology*, vol. 77, no. 1, 2020, pp. 25-36.

Nieto, G. "Biological Activities of Three Essential Oils of the Lamiaceae Family." *Medicines*, vol. 4, no. 3, 2017.

Okoye, T. C., et al. "Antibacterial and Anticancer Activity of Kaurenoic Acid from Root Bark Extract of Annona Senegalensis." *Planta Medica*, vol. 77, 2011.

Oliyaei, Najmeh, et al. "Application of Essential Oils in the Treatment of Inflammatry Bowel Disease." *Essential Oils - Advances in Extractions and Biological Applications*, Intech Open, 2022, pp. 139-52.

Oszmiański, Jan, et al. "Phytochemical Analysis by Liquid Chromatography of Ten Old Apple Varieties Grown in Austria and their Antioxidative Activity." *European Food Research and Technology*, vol. 246, no. 3, 2020, pp. 437-48.

Pastorino, Giulia, et al. "Liquorice (*Glycyrrhiza glabra*): A phytochemical and pharmacological review." *Phytotherapy Research*, vol. 32, no. 12, 2018.

Pfaller, Michael A., et al. "Twenty Years of the SENTRY Antifungal Surveillance Program: Results for *Candida* Species from 1997-2016." *Open Forum Infectious Diseases*, vol. 6, 2019, pp. 79-94.

Phillips, Thomas W., et al. "Identification of Sex Pheromones from cowpea weevil, *Callosobruchus maculatus*, and related studies with C. analis (Coleoptera: Bruchidae)." *Journal of Chemical Ecology*, vol. 22, 1996.

Pradeepa, M., et al. "Qualitative and quantitative phytochemical analysis and bactericidal activity of *Pelargonium graveolens* L'Her." *International Journal of Applied Pharmaceutics*, vol. 8, no. 3, 2016, pp. 7-11.

Rakha, Allah, et al. "Anti-Inflammatory and Anti-Allergic Potential of Dietary Flavonoids: A Review." *Biomedicine & Pharmacotherapy*, vol. 156, 2022.

Rubió, Laura, et al. "Recent Advances in Biologically Active Compounds in Herbs and Spices: A Review of the Most Effective Antioxidant and Anti-Inflammatory Active Principles." *Critical Reviews in Food Science and Nutrition*, vol. 53, no. 9, 2013, pp. 943-53.

Salehi, Bahare, et al. "Dioscorea Plants: A Genus Rich in Vital Nutra-Pharmaceuticals-A Review." *Iranian Journal of Pharmaceutical Research*, vol. 18, 2019, pp. 68-89.

Sato, Toshio, et al. "Enantioface-Differentiating (Asymmetric) Addition of Dialkylmagnesium to Aldehydes by using the Lithium Salt of (2S, 2′S)-2-Hydroxymethyl-1-[(1-Methylpyrpolidin-2-Yl) Methyl] Pyrrolidine as aChiral Ligand." *Chemistry Letters*, vol. 7, no. 6, 1978, pp. 601-04.

Saxena, Mamta, et al. "Phytochemistry of Medicinal Plants." *Journal of Pharmacognosy and Phytochemistry*, vol. 1, 2013, pp. 168-82.

Schanderl, S. H. *Methods in Food Analysis*. Academic Press, 1970.

Scozzafava, Andrea, et al. "Anticancer and Antiviral Sulfonamides." *Current Medicinal Chemistry*, vol. 10, no. 11, 2003, pp. 925-53.

Silva, Sonia, et al. "Adherence and Biofilm Formation of Non-Candida Albicans Candida Species." *Trends in Microbiology*, vol. 19, no. 5, 2011, pp. 241-47.

Soni, Priyanka, et al. "Pharmacological Properties of *Datura stramonium* L. as a Potential Medicinal Tree: An Overview." *Asian Pacific Journal of Tropical Biomedicine*, vol. 2, no. 12, 2012.

Staniszewska, Monika. "Virulence Factors in *Candida* species." *Current Protein and Peptide Science*, vol. 21, no. 3, 2020, pp. 313-23.

Tiwari, Ritesh, and Ajay Kumar Shukla. "Plant Metabolites and their Role in Health Benefits: A Brief Review." *Advance Pharmaceutical Journal*, vol. 5, no. 2, 2020, pp. 47-53.

Wang, Juan, et al. "The Colour Variations of Flowers in wild *Paeonia delavayi* Plants are Determined by Four Classes of Plant Pigments." *New Zealand Journal of Crop and Horticultural Science*, vol. 50, no. 1, 2022, pp. 69-84.

Wyman, Jeffries, and Stanley J. Gill. *Binding and linkage: Functional Chemistry of Biological Macromolecules*. University Science Books, 1990.

Yang, Yun-Liang. "Virulence factors of Candida species." *Journal of Microbiology, Immunology and Infection*, vol. 36, no. 4, 2003, pp. 223-28.

Zeb, Alam. "Concept, Mechanism, and Applications of Phenolic Antioxidants in Foods." *Journal of Food Biochemistry*, vol. 44, no. 9, 2020.

Author Details

Devika Nagappan, *Assistant Professor, Department of Zoology, Arignar Anna Government Arts College, Cheyyar, Tamil Nadu, India,* ***Email ID:*** *devikajvnr@gmail.com*

Madhavan Sivaraj, *Assistant Professor, Department of Zoology, Arignar Anna Government Arts College, Cheyyar, Tamil Nadu, India,* ***Email ID:*** *madhavanmd1989@gmail.com*

Sivamani Periyasamy, *Director, Microlabs, Institute of Research and Technology, Vellore, Tamil Nadu, India,* ***Email ID:*** *sivamnee@gmail.com*

Ashok Kamalanathan, *Associate Professor, Department of Microbiology and Biotechnology, Bharath Institute of Higher Education and Research (BIHER), Chennai, Tamil Nadu, India,* ***Email ID:*** *ashok1984biotech@gmail.com*

Balasubramanian Gnanavel, *Associate Professor, Department of Zoology, Arignar Anna Government Arts College, Cheyyar, Tamil Nadu, India,* ***Email ID:*** *aaabala@gmail.com*

Baskaran Chinnappan, *Research Associate Fellow, Department of Pediatrics, UPMC Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, Pennsylvania, United States of America,* ***Email ID:*** *basphd@gmail.com*