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Molecular Docking of Phytocompounds from Mentha Piperita Leaf Extract as Promising Inhibitory Agents against Candida Albicans's Glucosamine-6-Phosphate Synthase

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Abstract

The Mentha piperita plant, also known as peppermint, is a perennial aromatic herb grown throughout most of the world and has long been utilized in traditional medicine. It is a member of the Lamiaceae family. This study aimed to assess and determine the active phytochemical components of Mentha piperita leaf extract that inhibit the glucosamine-6-phosphate synthase activity of Candida albicans. The plant materials were collected from Janyau, Gada Biyu area along Sokoto Road, Gusau, Zamfara state, Nigeria, and identified at the herbarium section of the Biological science Department, Federal University Gusau. The plant leaf was extracted using the soxhlet method and analyses the phytochemical constituents' presence. To identify the bioactive compounds in the extracts of Mentha piperita, thin layer chromatography (TLC) was used on glass slides coated with silica gel (0.2mm Kiesel-gel 60 F254, Merck). The resulting fraction was then analyzed using HPLC-DAD analysis and the Bioautography Agar Overlay Technique. Phytochemical analysis results showed the presence of flavonoids, glycosides, phenol, tannins, saponins, and alkaloids as secondary metabolites. Thin layer chromatography (TLC) separation of aqueous extracts provided one compound with an R_f value of 0.52, while methanol and n-hexane extract showed two compounds, each with R_f values of 0.79 and 0.74, 0.72 and 0.70. As bioautography shows, the most bioactive component among the five components is M1. In conclusion, the bioactive constituents identified by high-performance liquid chromatography are rutin, Chlorogenic acid, Neohesperidin, Rosmarinic acid, and Eriodictyol.

Keywords: Molecular Docking, Phytocompounds, Mentha piperita, Candida albicans, Chromatography, Retention Factor RF

INTRODUCTION

Scientists studying infectious diseases have always been very interested in biologically active substances derived from natural sources. (Prasathkumar *et al.*, 2021). With their ability to inhibit bacteria, fungi, and yeasts, higher and aromatic plants have long been employed in folk medicine and to prolong the shelf life of food. (Mutlu-Ingok *et al.*, 2020). Many species are in the genus Mentha, part of the Lamiaceae family. These species vary greatly in terms of their ploidy level and other traits. Mentha species are perennials that can proliferate through both vegetative and reproductive means. This family's members are very important both commercially and pharmacologically. *Mentha piperita* grows wild and in cultivation in many different parts of the world. According to published research, Mentha piperita is used externally as a rub or liniment and internally as a tea, tincture, oil, or extract. (Silva 2020).

Astringent, antiseptic, antipyretic, antispasmodic, anticatarrhal, antimicrobial, rubefacient, stimulant, emmenagogue, and anti-aging are some qualities that botanists believe it to possess. The essential oils of Mentha piperita, which comprise around 1% of the herb, are its main active ingredients. Monoterpenes predominate in the oils, primarily menthol and menthone and their derivatives. (e.g., iso menthone, neomenthol, acetyl menthol, pulegone) (Anwar *et al.*, 2019). The fungicidal properties of menthol and peppermint oil inhibit the growth of Candida albicans, Aspergillus

albus, and Dermatophytic fungus. (Abd Rashed *et al.*, 2021).

The fragrant perennial herb known as Mentha piperita, or peppermint, is grown worldwide and has long been utilized in traditional medicine. It grows to a height of 50 to 90 cm. Mint leaves are commonly used in herbal teas and culinary applications to enhance flavor and fragrance. The cyclic terpene alcohol known as menthol, which occurs naturally, gives Mentha species a unique flavor and aroma. For gastrointestinal issues, the common cold, and skeletal muscle pain, menthol is prescribed. (Fayed, 2019). Iron and magnesium, essential for human nutrition, are abundant in mint plants. (Farooq *et al.*, 2019).

In 30 to 50% of healthy individuals, Candida species are common skin, mucosal, or gastrointestinal tract pathogens. However, they can also be opportunistic pathogens that cause serious infections in vital organs. Over the past few decades, there has been an increase in the number of patients who are susceptible to invasive candidiasis. These factors can be attributed to several factors, including more immunocompromised hosts, a greater use of invasive procedures and indwelling medical devices, longer hospital stays in intensive care units (ICUs), and improper use of broad-spectrum antibiotics. Furthermore, candidemia bloodstream infection linked to the species Candida—is one of the main reasons hospitalized patients develop severe illnesses, which have a notable mortality rate. (Raja, 2021)

MATERIAL AND METHODS

Study area

The study area is Gusau, the capital city of Zamfara State, Nigeria. It is located between Latitude 12°13'N to 12°18N and Longitude 6°29E to 6°45E with a total population of 383,162 people and an estimated 682,700 inhabitants in 2022 (NBS, 2022), from its annual growth rate of 2.7%. Gusau has a typical semiarid/arid climate, with two alternating rainy and dry seasons brought on by wet, northerly continental and dry, southerly air masses. (Kanoma and Abdulkadir 2022).

Collection, Identification, and Preparation of Plants Materials

The plant materials were collected from Janyau, Gada Biyu area along Sokoto road Gusau, Zamfara, Nigeria. The taxonomic identification of plant specimens was conducted at the herbarium section of the Biological Science Department, and the voucher number FUG/BIO/HEB/21/0076 was obtained; the plant material was transferred to the Microbiology laboratory, Federal University Gusau, for analyses. The specimens were rinsed with distilled water and air-dried at 40°C for seven (7) days before examination. A sterile electric blender was used to powder the dried leaves. (El Jurdi *et al.*, 2023).

Extraction of plant materials

Three distinct bottles containing the powdered leaves were each extracted separately using a 10:100 ratio of water, methanol, and n-hexane. The Soxhlet method was used for more than four hours. Using a rotary evaporator set to 40–50 ^{0}C, the extracts were concentrated until they were dry under low pressure. Mayorga *et al.* (2010) described gently heating the aqueous extract to 45^{0}C using a water bath. The dried extracts were placed in an airtight container to facilitate further analysis.

Phytochemical Screening

The analyses for the main group of natural constituents present in the plant extract were done using different specific reagents. Chemical tests were done to identify bioactive compounds of pharmacological importance through standard methods. Phytochemicals such as tannins, alkaloids, saponins, flavonoids, terpenoids, and phenols (Alqethami and Aldhebiani, 2021)

Antifungal Susceptibility Assay of Mentha piperita Leaf Extract against *Candida albicans*

Muller Hilton broth (100µl) was added to all 96 test wells; 50µl of the working concentrations of plant extract (400mg/ml) was added. Serial dilution was prepared for the plant extracts. The cultures were assessed and adjusted to 0.5 McFarland standards (i.e 1.5×10^{8}cell/ml), and 5µl of isolate suspension was added to each test well. The controls are microorganism control (50µl MH broth and 5µl microorganisms) was used as positive control and DMSO control (50µl of DMSO and 50µl of plant extract) as negative control. The plates were sealed with microplate sealing tape and incubated at 25^{0}c for 73 hours. After the incubation period, 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) was added to each well, and the plates were incubated for 30mins at 25°C until there was a change of colour. Viable cells reduce the yellow dye to a pink/purple colour, whereas no

colour change indicate inhibition of fungal growth (Mfengwana and Mashele, 2016)

Thin-Layer Chromatography

Glass slides coated with silica gel (0.2 mm Kiesel-gel 60 F254, Merck) were used for the thin layer chromatography (TLC) analysis of the *Mentha piperita* extracts. The plates were dried and activated in an oven for 30 minutes at 110^{0}C. After applying the crude leaf extracts to the TLC plates using micro-capillaries, the plates were dried and developed with ethanol and chloroform. UV light (365 and 254 nm) was used to visualize the separated compounds in a single plate. (Gurib-Fakim, 2006).

The following equation was used to calculate each extract's retention factor (Rf) measures through triplicate analyses.

$$Rf = \frac{\text{Distance Travelled by the Spot}}{\text{Distance Travelled by the Solvent}}$$

High-Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD) Analysis

The bioactive compounds in the natural extracts were identified using the HPLC-DAD analysis suggested by Kouri *et al.* (2007). An autosampler, a gradient pump, a column oven, and a diode array detector made up the Hitachi LaChrom Elite HPLC system. A 150 mm x 4.6 SVEA C18 column from Nanologica with a 5µm particle size and a 30˚C temperature maintained was used, with a flow rate of 0.5 mL min-1. Water, methanol, and acetonitrile, each containing 1% formic acid, made up the solvent system. The solvent gradient was carried out in this way: first 90% A, 6% B, 4% C 85% A, 9% B, 6% C, 0–5 min 30–60 minutes, 90% A, 6% B, 4% C; 5–30 minutes, 0% A, 85% B, 15% C 60–63 minutes. The analysis took 65 minutes in total. A volume of 20µl was used to inject the standards and sample. The chromatograms are represented at 225, 280, 355, and 370 nm, and the required spectra fall between 200 and 400 nm. Every analysis was performed three times. (Tsakni *et al.*, 2023).

Identification of Components of *Mentha piperita*

Using the National Institute Standard and Technique (NIST) database, which contains more than 62,000 patterns, the mass spectrum (MS) was interpreted. A comparison was made between the spectrum of the unknown and known components kept in the NIST library. With the help of MS analyses, the composition and relative percentages of the peppermint leaf were clarified. (Cajka *et al.*, 2017).

Protein Target Selection and Preparation

The 367 amino acid long and 1.8 Å resolution three-dimensional (3D) x-ray crystallographic structure of Candida albicans's glucosamine-6-phosphate synthase bound to glucosamine-6-phosphate was obtained from the protein data bank (PDB) under ID 2POC. Next, the protein was ready for docking and minimized using the appropriate tools in the Cresset Flare© software, version 4.0. The General Amber Force Field (GAFF), with a gradient cutoff of 0.200 kcal/mol/A and 2000 iterations set, served as the basis for the protein minimization process (Shi *et al.*, 2022).

Ligand selection and preparations

The compounds' three-dimensional (3D) structures were downloaded in simple document format (SDF) from the PubChem web server. They were optimized with Open Babel in Python Prescription (0.8), which used the Merck molecular force field (MMFF94) to energetically convert the ligands to the most stable structures. (Shi *et al.*, 2022)

***In silico* molecular docking**

The flexible docking protocol (Trott and Olson, 2010), previously employed by Ram *et al.*, (2023) was utilized to complete the molecular docking. To sum up, the Auto Dock Vina module in Python Prescription 0.8 suite was used to study the molecular docking of 17 compounds with Glucosamine-6-phosphate Synthase from Candida albicans. The grid box measuring 16.199 x 20.8158 x 23.6427 Å was used to determine the receptor's specific target site. The center was then modified in accordance with the glucosamine-6-phosphate binding site, which is made up of the amino acids Ser450, Ser452, Thr455, Gln451, Ser406, Glu591, Thr405, Lys588, Cys403, Gly404, Val501, Ala502, and Ser503. After the docking experiment, compounds with a docking score higher than the

control drug underwent molecular interaction analysis using LigPlot+ software (version 2.2.7) and PyMOL© Molecular Graphics (version 2.4, 2016, Shrodinger LLC). (Ram *et al.*,2023).

In vitro validation of molecular docking using Bioautography Agar Overlay Technique

After adjusting the Candida suspensions to the 0.5 MacFarland standards, one milliliter (1 ml) of the suspension was added to ten milliliters of melted Mueller Hinton agar supplemented with 5% glucose. TLC plates that had been developed were arranged in petri dishes, and the culture was added to the petri dish that held the TLC plate. The medium was allowed to solidify before being incubated for 24 hours at 25^{0}C. Methylthiazol tetrazolium was sprayed onto the TLC-bioautography plates in an aqueous solution. A distinct area of inhibition was discernible against a purple backdrop, and it was categorized as either positive (+) or negative (-) based on the chromatographical spot's length area. The TLC-identified compounds that demonstrated antifungal activity in the bioautographic method were selected and noted for additional investigation. (Demetrio *et al.*, 2016).

RESULTS

Table 1: Result of Phytochemical Constituents of *Mentha Piperita* Leaf Extract

Constituent	Aqueous	Methanolic	n-Hexane
Flavonoid	++	+	+
Tannins	+++	+++	+
Saponin	+	+++	-
Glycoside	+++	-	+
Alkaloid	+	+++	++
Phenol	++	+	+
Balsam	+++	+++	+
Cardial glycoside	++	++	+
Steroid	-	-	_
Saponin glycoside	-	+++	_-

Key: + =slightly positive, ++ = moderately positive, +++ = highly positive, - =negative

Table 2 shows the sensitivity test of Aqueous, Methanol, and n-hexane extract against *Candida albican*

	concentration (mg/ml)								
Extract	200	100	50	25	12.5	6.25	3.13	1.56	0.78
Aqueous	-	-	-	-	-	-	α	-	β
methanol	-	-	-	-	-	-	α	α/β	+
n-hexane	-	-	-	-	-	-	-	α	β

Key; β = MIC, α = MFC, + = Growth, - = No growth, 0= not determined

Table 3:TLC Profiling and Preparative Separation of Aqueous, Methanolic, And N- Hexane Extracts of *Mentha Piperita* Leaves

Extracts	Identity	Quantity (mg)	RF Value	Solubility
Aqueous	Aq1	0.03	0.52	Methanol and Distilled water
Methanol	M1	30	0.79	Methanol and Ethyl Acetate
	M2	40	0.74	Methanol and Ethyl Acetate
N-Hexane	H1	10	0.72	Hexane and Petroleum
	H2	40	0.7	Hexane and Petroleum

Key: RF= Retention factor

Table 4: Bioactive Compounds Identified in *Mentha Piperita* Leave Extracts using HPLC

Peak	Retention time (min)	Maxima Wavelengths	Bioactive Compound
A	45.1	205, 267, 258	Rutin
B	57.99	215,276,345	Chlorogenic acid
C	57.65	221, 235, 275	Naringenin
D	49.8	256,263,349	Luteolin
E	49.24	230,256,370	gallic acid
F	52.03	250,315	Diosmetin
G	52.21	267,338	Apigenin
H	43.2	206, 267 331	Rosmarinic acid
I	40.63	203, 258, 328	Neohesperidin
J	46.96	205, 230, 288	Eriodictyol
K	22.65	208, 265, 292	vanillic acid
L	44.5	206, 245, 330	Syringic acid
M	33.5	216, 308, 374	p-coumaric acid
N	36.77	210, 322	Ferulic acid
O	24.57	209, 240, 323	Caffeic acid
P	19.29	210, 260	Hydroxybenzoic acid
Q	42.23	273, 370	benzoic acid

Table 5: Result of Binding Energy between Phycompounds and *Candida albican's* Glucosamine-6- Phosphate Enzyme

S/No.	Compounds	PubChem ID	Binding Energy (kcal/mol)
1.	Diosmetin	5281612	-6.6
2.	Rutin	5280805	-7.5
3.	Chlorogenic acid	1794427	-7.0
4.	Naringenin	932	-6.7
5.	Gallic acid	370	-6.4
6.	Luteolin	5280445	-6.9
7.	Apigenin	5280443	-6.7
8.	Rosmarinic acid	5281792	-7.3
9.	Neohesperidin	442439	-7.4
10.	Eriodictyol	440735	-7.0
11.	Vanillic Acid	8468	-5.6
12.	Syringic acid	10742	-6.1
13.	p-coumaric acid	637542	-5.7
14.	Ferulic acid	445858	-6.3
15.	Caffeic Acid	689043	-6.1
16.	4-Hydroxybenzoic acid	135	-5.2
17.	Benzoic Acid	243	-5.0

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Table 6: Result of Bioautography of Chromatographic Spot of an Active Component

Chromatographic Spot	Activity
Q1	-
M1	+
M2	-
H1	-
H2	+

Key: Q= Aqueous extract, M= Methanolic extract, H= N- hexane extract

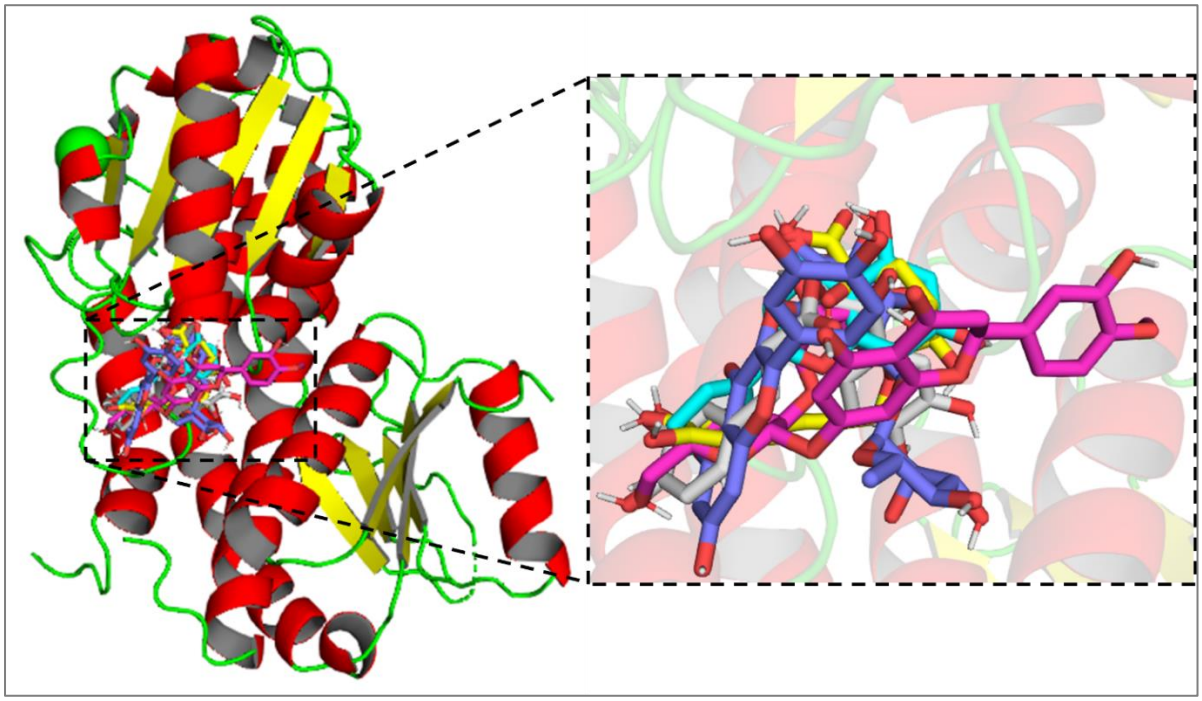

Figure 1: Molecular docking of selected polyphenols from *Mentha piperita* against Glucosamine-6-phosphate Synthase of *C. albicans*. 3D binding pose of polyphenols where they occupy a similar part of the binding site of the target receptor. Chlorogenic acid (yellow), eriodictyol (cyan), neohesperidin (purple), rosmarinic acid (grey), and rutin (light blue).

DISCUSSION

The result of preliminary phytochemical analysis of *Mentha piperita* leave extract showed that Methanol solvent is more effective in *Mentha piperita* soxhlet extraction than Aqueous solvent, while n-Hexane was observed to be least in *Mentha piperita* soxhlet extraction; this is due to the nature of their polarity and differences in their boiling point. This study is consistent with the finding of Abd Rashed et al., (2021) who also reported that Methanol is the better solvent for extraction than Aqueous and n-hexane in soxhlet extraction due to their difference in boiling point. The extraction of crude extracts from *Mentha piperita* leaves using three different solvents (aqueous, methanol, N-hexane) selected based on polarity was in agreement with the finding of Haris *et al.* (2021). The colour reaction identified the different phytochemical constituents present in the extracts with different reagents, which identified the presence of secondary metabolites in the plant extract, such as phenol, Tannins, flavonoids, steroids, alkaloid, Glycosides, and Saponins. The presence of these components in the plant extracts indicates that they may have some medicinal potential. This is consistent with the findings of Bipin Khana (2019).

The results of antifungal activities of *Mentha piperita* using micro broth dilution assay indicate that all the plant's extracts have shown good antifungal effects against the pathogens with a significant difference between the three extracts, the Methanolic extract exhibited higher antifungal activity with an average MIC of 7.59mg/ml followed by n-hexane with an average MIC of 23.24mg/ml and aqueous extract shows a less activity against the test organisms, There was a slight variation with the finding of

Haris *et al.*, (2021) who reported that Methanolic and aqueous extracts of the plant exhibited higher antifungal effects than n-hexane extract. When extracted with different solvents, the difference in the antifungal activity with the same source has proven that not all phytochemicals responsible for antifungal activities are soluble in a single solvent. Hence, solvent with different polarity employed in this study was in accordance with the report of Naiman and Khan, (2016). Two possibilities that account for this higher activity are the nature of biologically active components (alkaloids, flavonoids. tannins. phenols, etc.), which was enhanced in the presence of methanol and n-hexane solvents, and the method of extraction used (soxhlet) that have yielded a greater number of active constituents responsible for antifungal activity, this was in agreement with the report of Gosh *et al.*, (2008); Haris *et al.*, (2017). The activity of the extracts from the broth dilution assay at low concentrations corroborates with the findings of Pramila *et al.* (2012).

The result of thin layer chromatography (TLC) profiling and preparative separation of aqueous extract in Table 3 indicated five (5) chromatographic spots. This includes one (1) spot from Aqueous extract, and two (2) spots each from Methanol and n-hexane. The solvent system used is methanol: ethyl acetate (8:2), ethyl acetate: chloroform: methanol (8:1:1), ethyl acetate: chloroform (8:2), and butanol: acetic acid: water (6:2:2) as trials. The butanol: acetic acid: water gives a better separation, and the isolated compound was labeled aq1. For the methanol extracts, the solvent system used as trials are ethyl acetate: chloroform: methanol (8:1:1), ethyl acetate: chloroform (8:2) ethyl acetate: methanol (8: 2), and ethyl acetate: n-hexane (8:2), the ethyl acetate: chloroform gives a better separation and is used for separation. Two compounds were isolated and labelled as M1 and M2. For the N-Hexane, the solvent system (mobile phase) along with the precoated silver gel phase used as trials are hexane: ethyl acetate (8:2), hexane: ethyl acetate: (9:1), and 100% hexane. The hexane: ethyl acetate (8: 2) gave better separation and was used for preparative separations, two (2) were isolated and labelled as H1 and H2. This finding disagrees with Taura *et al.*, (2021) who reported that a higher chromatographic spot was observed in Aqueous extract than in Methanolic extract. The difference may be due to the extraction method involving heat.

In this study, Seventeen (17) bioactive compounds were identified by HPLC, as shown in Table 4. The majority of the components show maximum absorption and have a successful separation at a specific wavelength. The bioactive compounds detected in *Mentha piperita* fractions using HPLC were reported by several authors to have antimicrobial effects (Malika *et al.*, 2017; Tsakni *et al.*, 2021). The most abundant phenolic acids identified in the mint extract were caffeic acid (116.89±0.28 ppm) and benzoic acid (41.92±0.61ppm), and the main flavonoid is eriodictyol (32.23±0.75 ppm). Apigenin was found in traces in the samples. The peaks of the chromatogram can confirm these results. This finding agrees with the finding of Tsakni *et al.* (2021).

In silico molecular docking results shown in Table 5 revealed that, out of seventeen bioactive compounds identified by HPLC, only five have inhibitory potential against *Candida albicans's* Glucosamine-6-phosphate Synthase. These compounds include rutin (-7.5 kcal/mol), chlorogenic acid (-7.0 kcal/mol), rosmarinic acid (-7.3 kcal/mol), neohesperidin (-7.4 kcal/mol), and eriodictyol (-7.0 kcal/mol) these finding is in agreement to the finding of Zengin *et al.*, (2019).

The result of in silico molecular docking was validated by bioautography, which showed that two spots (M1 and H2) out of the five chromatographic spots separated by TLC have shown antimicrobial activity against the test isolate, while Q1 and H1 have no activity. This assay was applied to all extracts and the inhibitory activities are equal to chromatographic spot. This study agrees with the findings of Haris *et al.* (2017).

CONCLUSIONS

In conclusion, the research showed that Methanolic extract has a higher extraction potential than aqueous extract, while n-hexane has the least extraction potential due to its polarity.

In silico molecular docking revealed that out of seventeen bioactive constituents of *Mentha piperita* identified by HPLC, only five can inhibit the growth of *candida albicans* which include Rutin with a binding energy of (-7.5 kcal/mol), Chlorogenic acid (-7.0 kcal/mol), Rosmarinic acid (-7.3 kcal/mol), Neohesperidin (-7.4 kcal/mol), and Eriodictyol (-7.0 kcal/mol).

The in-vitro validation test confirmed that chromatographic spot 01 obtained from Methanolic extract (M1) and chromatographic spot 02 obtained from n-Hexane (H2) are the most active against test organism, while Q1 has no activity.

REFERENCES

Abd Rashed, A., Rathi, D. N. G., Ahmad Nasir, N. A. H., & Abd Rahman, A. Z. (2021). Antifungal properties of essential oils and their compounds for application in skin fungal infections: Conventional and nonconventional approaches. *Molecules*, **26**(4), 1093. **[Crossref]**

Alqethami, A., and Aldhebiani, A. Y. (2021). Medicinal plants used in Jeddah, Saudi Arabia: phytochemical screening. *Saudi Journal of Biological Sciences*, **28**(1), 805-812. **[Crossref]**

Anwar, F., Abbas, A., Mehmood, T., Gilani, A. H., & Rehman, N. U. (2019). Mentha: A genus rich in vital nutra-pharmaceuticals—A review. *Phytotherapy Research*, **33**(10), 2548-2570. **[Crossref]**

Bipin K., and Kathmandu (2019) Phytochemical and Antibacterial Analysis of Mentha piperita (Peppermint)

Cajka, T., Smilowitz, J. T., & Fiehn, O. (2017). Validating quantitative untargeted lipidomics across nine liquid chromatography–high-resolution mass spectrometry platforms. *Analytical chemistry*, **89**(22), 12360-12368. **[Crossref]**

Cresset Flare© software, version 4.0 cresset-group.com

Demetrio, L., Valle j.r., Juliana janet, m., puzon, Esperanza, c., Cabrera and windell L. (2016). thin layer chromatography-bioautography and gas chromatography-mass spectrometry of antimicrobial leaf Extracts from Philippine piper betle L. against multidrug-resistant Bacteria. *Evidence based complementary and alternative medicine*. **[Crossref]**

El Jurdi, K., Al Souky, N., Melquist, S. J., Al-Hadeethi, D., Salyers, W. J., & El Zein, M. (2023). S682 Efficacy of Peppermint Oil in Irritable Bowel Syndrome: A Systematic Review and Meta-Analysis. *Official journal of the American College of Gastroenterology| ACG*, *118*(10S), S498-S499. **[Crossref]**

Farooq Anwar, F. A., Ali Abbas, A. A., Tahir Mehmood, T. M., Gilani, A. H., and Najeeb-ur Rehman, N. U. R. (2019). Mentha: a genus rich in vital nutra-pharmaceuticals-a review. **[Crossref]**

Fayed, M. A. (2019). Mentha Piperita L.-A promising dental care herb mainly against cariogenic bacteria. *Universal Journal of Pharmaceutical Research*, **4**(3), 33-38. **[Crossref]**

Gosh, A., Dashi, B.K., Roy, A., Mandala, B. and Chand, G. (2008). Antibacterial activity of some medicinal plant extracts. *Journal of Natural Medicine*; **62**: 259-262.

Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular aspects of Medicine*, *27*(1), 1-93. **[Crossref]**

Haris N. G., Abubakar A. I., Aisha S. B. and Sa'adu U. (2021) Phytochemical Constituent and Antifungal Potentials of Mentha piperita (Pepper Mint) Leaf Extract on Urophathogenic Candida albicans. *Dutse Journal of Pure and Applied Sciences;* **7**(1): 1-13

Haris, N.G., Taura, D.W., Abubakar, A.I., Yahaya, S.M., Ahmed, I., Aisha, S.B. (2017). Phytochemical screening and antibacterial activity of Menthapiperita (pepper mint) leaves extracts on urophatogenic *Escherichia coli*. *Journal of Microbial Research*; **3**(2): 51 – 58

Kanoma, M. S., & Abdulkadir, M. (2022). The impact of future climate change on water availability in Gusau, Zamfara State, Nigeria. Dutse J. Pure Appl. Sci, 7, 144-154. **[Crossref]**

Kouri, G., Tsimogiannis, D., B ardouki, H., &Oreopoullou, V. (2007).Extraction and analysis of antioxidant components from Origanum dictamnus. Innovative Food Science and Emerging Technologies, 8 (2), 155-162. 2007. **[Crossref]**

Malika, Y. L., Thilinika, D., Janendra, M. D., Saman, E., Herath, S. & Wijayasiri, W. (2017).Physiological traits determining tolerance to intermittent drought in Capsicum annum complex.Scientia Horticulture, 246 (2019) 21-33. **[Crossref]**

Mayorga, P., Pérez, K.R., Cruz, S.M., Caceres, A. (2010). Comparison of bioassays using the anostracan crustaceans Artemia salina and Thamnocephalus platyurus for plant extract toxicity screening. *Brasil Journal of Pharmacy*; **20**: 897-903. **[Crossref]**

Mfengwana. P.M.A and Mashele.S.S. (2016). Antimicrobial activity screening of

philenoptera violacea (Klotzsch) schrire and xanthocercis zambesiaca (baker) dumaz-Le-Grand. *Journal of pharmaceutical sciences and research*; **8**(10): 1132-1135

Mutlu-Ingok, A., Devecioglu, D., Dikmetas, D. N., Karbancioglu-Guler, F., & Capanoglu, E. (2020). Antibacterial, antifungal, antimycotoxigenic, and antioxidant activities of essential oils: An updated review. *Molecules*, **25**(20), 4711. **[Crossref]**

Naiman, A. K., Dominic, M. P., Mark, R. S. Lauren, B. R., Lauren, B. S. Dale, W. E. Arthur, F. K. & Charles, H. M. (2016). Moderate-to-vigorous activity, indices of cognitive control, and academic achievement in preadolescents.The Journal of Pediatrics, 1 (173), 136-142. **[Crossref]**

Pramila D.M., Xavier R., Marimuthu K., Kathiresan S., Khoo M.L., Senthil M., Sathya K. and Sreeramanan S. (2012). "Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (Mentha piperita: Lamiaceae)". *Journal of Medicinal Plants Research*; **6**(2), 331-335 **[Crossref]**

Prasathkumar, M., Anisha, S., Dhrisya, C., Becky, R., & Sadhasivam, S. (2021). Therapeutic and pharmacological efficacy of selective Indian medicinal plants–a review. *Phytomedicine Plus*, *1*(2), 100029.. **[Crossref]**

Raja, N. S. (2021). Epidemiology, risk factors, treatment and outcome of Candida bloodstream infections because of Candida albicans and Candida non-albicans in two district general hospitals in the United Kingdom. *International Journal of Clinical Practice*, **75**(1), e13655. **[Crossref]**

Ram, S., More-Adate, P., Tagalpallewar, A. A., Pawar, A. T., Nagar, S., and Baheti, A. M. (2023). An in-silico investigation and network pharmacology based approach to explore the anti-breast-cancer potential of Tecteria coadunata (Wall.) C. Chr. *Journal of Biomolecular Structure and Dynamics*, 1-12. **[Crossref]**

Shi, L., Wen, Z., Song, Y., Wang, J., & Yu, D. (2022). Computational investigation of potent inhibitors against SARS-CoV-2 2′-O-methyltransferase (nsp16): structure-based pharmacophore modeling, molecular docking, molecular dynamics simulations and binding free energy calculations. *Journal of Molecular Graphics and Modelling*, *117*, 108306. **[Crossref]**

Silva, H. (2020). A descriptive overview of the medical uses given to Mentha aromatic herbs throughout history. *Biology*, ***9***(12), 484. **[Crossref]**

Taura, M. G., Muhammed, A. A. & Alqahtani, D. O. (2021). Prevalence of congenital heart disease among patients with down syndrome in Southwestern Saudi Arabia. Annal of African Medicine, 20 (4), 265-269. 2012. **[Crossref]**

Tsakni, A., Chatzilazarou, A., Tsakali, E., Tsantes, A. G., Van Impe, J., & Houhoula, D. (2023). Identification of Bioactive Compounds in Plant Extracts of Greek Flora and Their Antimicrobial and Antioxidant Activity. *Separations*, **10**(7), 373. **[Crossref]**

Zengin, G., Stefanucci, A., Rodrigues, M. J., Mollica, A., Custodio, L., Aumeeruddy, M. Z., & Mahomoodally, M. F. (2019). Scrophularia lucida L. as a valuable source of bioactive compounds for pharmaceutical applications: In vitro antioxidant, anti-inflammatory, enzyme inhibitory properties, in silico studies, and HPLC profiles. *Journal of Pharmaceutical and Biomedical Analysis*, *162*, 225-233. **[Crossref]**