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Formulation and Evaluation of Nano-Hydrogel Containing Essential Oil of *Mentha Piperita* for Antifungal Activity

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ABSTRACT:

Mentha piperita essential oil was incorporated into a nano-hydrogel formulation to enhance its antifungal activity. The selection of biocompatible polymers was crucial for creating a hydrogel with the ability to absorb and retain significant water content. Parameters such as viscosity, polymer concentration, and essential oil concentration were optimized to ensure the stability and effectiveness of the nano-hydrogel. The nanoscale dimensions (1-100 nm) of the hydrogel particles increased the surface area-to-volume ratio, improving interaction with target sites and enhancing drug loading capacity. Essential oil encapsulation, through physical entrapment and chemical conjugation, provided controlled release and protection from environmental factors. The antifungal efficacy of the nano-hydrogel was assessed against pathogens like Candida albicans via in vitro tests, measuring parameters such as minimum inhibitory concentration (MIC) and zone of inhibition. Results indicated significant antifungal activity, with variations in biomass and essential oil yields observed across different months and locations. Gas Chromatography-Mass Spectroscopy (GC-MS) analysis revealed seasonal fluctuations in the chemical composition of essential oils. Both antibacterial and antifungal assays demonstrated the enhanced efficacy of the nano-hydrogel formulation, showcasing its potential as a viable antifungal treatment.

Keywords: Mentha piperita, nano-hydrogel, essential oil, antifungal activity, biocompatible polymers, encapsulation, controlled release, minimum inhibitory concentration (MIC), Gas Chromatography-Mass Spectroscopy (GC-MS), Candida albicans.

INTRODUCTION

Mentha piperita essential oil is used in the careful formulation of a nano-hydrogel for antifungal activity. The method starts with the selection of suitable polymers to produce a durable gel-like material. The three-dimensional networks of polymer chains that make up hydrogels are capable of absorbing and holding onto a lot of water. The selected polymers for this formulation need to have certain qualities, like biocompatibility, which guarantees the hydrogel is safe to use in biological systems without producing negative reactions. Furthermore, after the hydrogel is applied, variables like viscosity are critical in defining its consistency and spreadability [1]. Furthermore, to acquire the ideal properties of the nano-hydrogel, boundaries including the concentration of polymers, natural ointment, and some other augmentations are improved all through the detailing system. This involves figuring out the best proportion of polymer to essential oil to provide maximal effectiveness while preserving the hydrogel's stability and integrity [2]. Furthermore, meticulous control is

exercised over variables such as pH, temperature, and mixing conditions to guarantee uniformity and repeatability in the formulation process. Because the particles involved have nanoscale dimensions, integrating nanotechnology into the composition of the hydrogel containing Mentha piperita essential oil brings about a number of benefits. The designation "nano" denotes the hydrogel particles' nanometer size, which normally ranges from 1 to 100 nanometers. Among the many benefits of nano-hydrogels is their higher surface region to-volume proportion, which advances better cooperation with target areas and raises the viability of the typified medicinal balm. Besides, in view of their high porosity and potential to hold more dynamic restorative parts in similar volume than customary hydrogels, nano-hydrogels give further developed drug stacking limit [3].

In order to manufacture the nano-hydrogel containing Mentha piperita essential oil for antifungal activity, essential oil encapsulation is a necessary step. The procedure entails encasing the essential oil inside the hydrogel matrix in order to attain regulated release and extended efficacy against fungi [4]. Encapsulation maximises the medicinal efficacy of the essential oil by preventing degradation, improving stability, and regulating the kinetics of release. There are various ways to encapsulate, such as chemical conjugation or physical entrapment inside the polymer matrix. During the formulation process, the essential oil is dispersed throughout the hydrogel matrix to achieve physical entrapment. The molecules of the essential oil are encapsulated by the hydrogel's polymer chains, which effectively traps them inside the gel network [5]. By using this technique, the essential oil is distributed uniformly throughout the hydrogel and the release of the active components is regulated over time. Physical entrapment also serves to protect the essential oil from light, heat, and oxygen, three environmental conditions that might weaken its efficacy [6]. As an alternative, chemical conjugation entails covalently attaching the molecules of the essential oil to the hydrogel's polymer chains. The encapsulated essential oil is more stable and bioavailable thanks to this process, which forms strong chemical connections between the oil molecules and the polymer matrix. Chemical conjugation can also make it easier for the essential oil to be delivered specifically to the right places in the body, which will increase its healing benefits [7].

An important stage in creating the nano-hydrogel formulation with Mentha piperita essential oil is assessing its antifungal efficacy. In order to evaluate the manufactured hydrogel's effectiveness against fungal pathogens—like Candida albicans, which are frequently linked to fungal infections—in vitro tests must be conducted [8]. In order to assess the hydrogel's antifungal activity, many parameters are examined when it is exposed to the fungal strains under carefully controlled circumstances. The minimum inhibitory concentration (MIC), or the least concentration of the hydrogel expected to stop the development of the contagious microorganisms, is one of the fundamental rules evaluated [9]. This aids in assessing how well the nano-hydrogel formulation inhibits the growth of fungi. To determine how much the hydrogel inhibits the formation of fungal colonies surrounding the application site, the zone of inhibition is also evaluated. A bigger zone of inhibition suggests that the hydrogel has more antifungal efficacy [10].

MATERIAL AND METHODS

1. Site Selection and Plant Material Collection

Two plant species from the Lamiaceae family — Mentha longifolia and *M. piperita* — were picked for additional review. The review was done in the exploration labs of Shoolini College's School of Organic and Natural Sciences in Solan [11].

2. Data Collection

Three sites, namely Giripul (Distt. Sirmour), Khairi (Distt. Sirmour), and Bajhol (Distt. Solan) along the range of mid hills of North West Himalaya were chosen for the collecting of M. longifolia samples. Each sampling location had three 1 m × 1 m quadrats set up, and 45 plants/quadrat (135 plants/3 quadrats) were gathered for each of the four months of one growth season (April, May, June, July). Using a stratified random sample technique, the plants were taken from both the quadrat's centre and its edges. Cultivated *M. piperita* plants were gathered from the Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni nursery. Since *M. piperita* was only gathered from one location, it served as a comparative parameter for M. logifolia [12].

Between April and July, the middle of each month was used to gather plants of both Mentha spp. (M. longifolia and *M. piperita*) in the early morning before 12:30 pm. For additional research, the uprooted plants were gathered in polyethylene bags and stored in an ice box. 45 of the 135 plants at each location were utilised to produce biomass, and the remaining 90 plants were used to extract oil [13].

3. Sample Collection

On the basis of plant morphology and information from books and research publications, M. longifolia plants were identified in the field. The M. longifoila species that were assembled were consequently affirmed or distinguished at the Y.S. Parmar College of Agriculture and Ranger service in Nauni, Solan [14]. Under UHF-Herbarium No. 13571, distinguished M. longifolia plants were submitted to the herbarium of Y.S. Parmar College of Agriculture and Ranger service, Nauni. Extra acquired plant examples of M. longifolia and *M. piperita* were sent in to the Shoolini College Herbarium at the School of Organic and Natural Sciences in Solan [15].

4. Biomass Yield Assessment

To calculate the biomass yield, 15 plants or quadrats were collected from each sampling location. After several tap washes, blotting paper was used to dry these and remove any remaining soil. These were divided into pieces so that a weighing machine could readily weigh them and determine their fresh weight. For an additional ten days, these were let to air dry at room temperature. After that, dried plants were weighed in the same manner as before. The total biomass was computed subsequent to the fresh and dry weight measurements. Every month was handled in the same way. To estimate biomass, the following formula was used g/m^2 [16]:

$$\text{Biomass for 1m} \times \text{1m} = \frac{Dry\ Weight \times 100 \times 100}{Size\ of\ the\ quadrate\ taken}$$

5. Isolation Of the Essential Oil

Utilizing a clevenger gear and Clevenger's strategy (1928), the medicinal ointments of both Mentha spp. (M. longifolia and *M. piperita*) were separated from each site in picked months. A sum of 90±5 plants were separated for oil from one area. After a few tap washes, smearing paper was utilized to dry these and eliminate any excess soil [17]. To remove the oil, 100 g of new leaves were isolated, gauged, and utilized. These were placed in a round-base flagon with 1000 cc of refined water and warmed to 100°C utilizing the clevenger hardware. Following three hours of extraction, the natural balm was gathered and the hydrosol was isolated. Oil creation was determined consistently. The got oil tests were then used for organic action characterisation and GC-MS investigation [18].

Figure 1: Extraction of oil through clevenger apparatus

6. Oil Yield Assessment

For the evaluation of essential oil yield, the Maridass et al. (2008) and Hagos et al. (2017) methods were applied. The following formula was used to determine each sample's oil yield in percentage terms [19]:

$$Oil\ yield = \frac{W1}{W2} \times 100$$

Where, W1 = net weight of oil (g)
W2 = total weight of fresh leaves (g)

7. GC-MS Analysis

Following the procedure of Okut et al. (2017), the natural oils of M. longifolia and *M. piperita* were exposed to Gas Chromatography-Mass Spectroscopy (GC-MS) at Punjab College's Focal Instrumentation Lab in Chandigarh. The TG 5MS hairlike section (30 m x 0.25 mm i.d., 0.25 µm film thickness) was utilized in the GC/MS device (Thermo Follow 1300GC matched with Thermo TSQ 8000 Triple Quadrupole MS) for the subjective examination of the oil tests. Before examination, the medicinal balm was weakened 1/10 in n-hexane (v/v). The motivation behind the auto sampler was to infuse oil. At 250˚C was the injector temperature [20]. The section temperature was intended to go from 60˚ to 250˚C. Subsequent to holding the temperature at 60˚C for two minutes, it developed steadily to 200˚C at a pace of 15˚C each moment for five minutes, and at long last to 220˚C at a pace of 15˚C each moment for five minutes. A 1.5 ml/minute stream pace of helium was utilized as the transporter gas, and mass spectra were gathered in check mode. Seventy eV was the ionization voltage [21]. The split proportion was 20 to 1. The temperature of the particle source was 230˚C, while the connection point was 250˚C. Three minutes was the solvent cut time. An example of 0.1 µl was utilized for the examination. In light of their retention time (Rt) estimated with reference, the parts of oil were distinguished. Coordinating the parts with the MS-information bank (NIST 2.0 electronic library) filled in as the reason for distinguishing proof) [22].

8. Screening Of Essential Oil for Biological Activities

Antimicrobial assay

Four bacterial and three parasitic strains — two gram-positive kinds of Bacillus subtilis and Staphylococcus aureus, two gram-negative types of Escherichia coli and Klebsiella pneumoniae, one contagious kind of Fusarium oxysporum, and two yeast kinds of Candida albicans and Saccharomyces cerevisiae — were picked to assess the antimicrobial exercises.

The Shoolini College in Solan, California's Staff of Biotechnology and Applied Sciences gave the microbial strains as a whole [23].

Agar disc diffusion method

The antibacterial action of Mentha spp. (M. longifolia and *M. piperita*) medicinal ointment against microorganisms (both gram positive and negative strains) and yeast was resolved utilizing the Kizil et al. (2010) strategy. Bacterial strains and yeast (0.5 McFarland standard) were added to NA and YPD agar, individually. Six-millimeter sterile channel paper circles were impregnated with various concentrations (1:10 in DMSO) at the accompanying proportions: 1.5 mg/ml, 2.0 mg/ml, 2.5 mg/ml, and 3.0 mg/ml. The circles were then put on the immunization plates. Positive controls included Fucanozole for yeast and Ampicillin for microscopic organisms [24]. Following two hours of standing at 4°C, the tainted plates were brooded for 24 hours at 37°C for microorganisms and 48 hours at 30°C for yeast. Utilizing the HiMedia anti-microbial scale, the restraint zone breadths were estimated in millimeters. Triple testing was utilized for each test [25].

Poison food technique

The process for creating poison food was carried out by slightly altering Ramaiah and Garampalli's (2015) methodology. Conical flasks holding 25 ml of sterilised potato dextrose agar were filled with the various oil concentrations (1.5 mg/ml, 2.0 mg/ml, 2.5 mg/ml, and 3.0 mg/ml) from the stock solution (1:10 oil dilution in DMSO) and then placed into petri plates [26]. Each concentration is applied to a different sterilised medium. The centre of the petri dish held an actively growing 6 mm diameter F. oxysporum mycelium disc from 6–7-day old culture. Negative control plates were those devoid of essential oil. At 27°C, the infected plates were incubated. Three sets of the antifungal test were conducted. After the mycelium was incubated for seven days, its radial growth was assessed. A negative control group was used to compare the outcomes [27]. The following calculation was used to determine the percentage of the fungus that was inhibited in the treatments;

$$Percent\ inhibition = \frac{C - T}{C} \times 100$$

Where, T is the pathogen's radial expansion when essential oil is present, and C is the colony radius in the control plate.

Dry weight method

With a couple of minor changes, the antifungal movement was resolved utilizing Premanath and Devi's (2011) strategy. Six mm in measurement effectively creating F. oxysporum mycelium plates from societies that were six to seven days old were added to individual test tubes containing sterile potato dextrose stock [28]. Following that, different oil concentrations (1.5 mg/ml, 2.0 mg/ml, 2.5 mg/ml, and 3.0 mg/ml) were added to the test tubes from the stock arrangement (1:10 oil weakening with 100 percent DMSO) and brooded for seven days at 30ºC. As a negative control, test tubes without any trace of medicinal ointment were utilized. How much noticeable mycelia development in the cylinders showed how dynamic the oil was. Utilizing Whatmann No. 1 channel paper, the growth mycelia from the cylinders above were isolated. To get a reliable weight, the channel paper was permitted to dry at 60ºC. By considering the control and test mycelial dry loads, the development hindrance of the growth was assessed [29]. The accompanying recipe was utilized to decide the rate development hindrance:

$$\%growth\ inhibition = \frac{Control - test}{Control} \times 100$$

Determination of the minimum inhibitory concentration

MIC was carried out using, with a small modification to the protocol, the method of Silvério et al. (2013). The CLSI-recommended broth microdilution method was employed to ascertain MIC values. testing for antimicrobial activity were conducted in Nutrient Broth, antifungal

testing in Potato Dextrose Broth, and yeast tests in YPD Broth. For both bacteria and fungi, the inoculum densities were roughly 0.5 McFarland standards. After that, 10 µl of each microbial suspension was applied to each well [30]. As a negative control, the final row with just the antimicrobial agent serial dilutions free of microorganisms was utilised. As positive controls for bacteria, fungus (F. oxysporum), and yeast, respectively, ampicillin, hygromicine B, and fluconazole were utilised (10 mg/ml). Microtiter plates were incubated for 18–24 hours at 30°C for bacteria and 48 hours at 25°C for yeast and F. oxysporym. The lowest oil concentration that prevents bacteria from growing visibly was determined to be the minimum inhibitory concentration (MIC) following the incubation time [31].

9. Statistical Tools

PRISM 5.0 and MS-EXCEL software were used to calculate the data's mean and standard deviations and perform statistical analysis [32].

RESULT AND DISCUSSION

1. Biomass Assessment

Biomass assessment of M. longifolia and *M. piperita* in the months from April- July

The highest biomass of the plants that were taken from each site in July was shown by the findings of the biomass evaluation of M. longifolia and *M. piperita*. Regarding M. longifolia, the site with the highest biomass was Bajhol (4.18±0.77), while the site with the lowest biomass was Khairi (0.88±0.13). The two-way ANOVA method was used in the statistical analysis for the biomass assessment, along with the Bonferroni multiple comparison tests. Nonsignificant variation was found in the biomass gathered from the locations Giripul vs. Khairi, Giripul vs. Nauni, and Khairi vs. Nauni, according to statistical analysis of the data. In every month that was chosen, there were notable differences between the samples from Khairi and Bajhol. Giripul vs. Bajhol samples from April and May, as well as Bajhol vs. Nauni samples from May, also yielded noteworthy findings.

Table 1: Biomass (g/m^2) of M. longifolia and *M. piperita* from April - July Month

Biomass (g/m^2)				
Months	**M. longifolia**			***M. piperita***
	Giripul	**Bajhol**	**Khairi**	**Nauni**
April	1.18±0.13	1.91±0.41	0.87±0.14	0.63±0.25
May	1.60±0.42	2.42±0.54	0.95±0.23	1.48±0.26
June	2.25±0.27	3.31±0.53	1.46±0.30	1.53±0.55
July	2.33±0.56	4.17±0.78	1.91±0.16	1.85±0.73

Table 2: Bonferroni post-test showing biomass comparison

Months	**Row Factor**	**Difference**	**T**	**P-Value**	**Summary**
Giripul vs Khairi	April	-0.306	0.860	P > 0.05	ns
	May	-0.656	1.830	P > 0.05	ns
	June	-0.788	2.205	P > 0.05	ns
	July	-0.425	1.184	P > 0.05	ns
Giripul vs Bajhol	April	0.730	2.045	P > 0.05	ns
	May	0.800	2.233	P > 0.05	ns
	June	1.080	3.107	P > 0.05	*
	July	1.855	5.197	P <0.01	***
Giripul vs Nauni	April	-0.574	1.605	P > 0.05	ns
	May	-0.135	0.376	P > 0.05	ns
	June	-0.705	1.966	P > 0.05	ns

	July	-0.460	1.288	P > 0.05	ns
Khairi vs Bajhol	April	1.040	2.900	P > 0.05	*
	May	1.455	4.060	P < 0.01	**
	June	1.870	5.225	P < 0.001	***
	July	2.285	6.380	P < 0.001	***
Khairi vs Nauni	April	1.040	2.900	P > 0.05	ns
	May	1.455	4.060	P > 0.05	ns
	June	1.871	5.225	P > 0.05	ns
	July	2.285	6.380	P > 0.05	ns
Bajhol vs Nauni	April	-0.265	0.743	P < 0.01	**
	May	0.528	1.455	P > 0.05	ns
	June	0.086	0.240	P < 0.001	***
	July	-0.037	0.104	P < 0.001	***

**- More significant (P<0.05), Ns –Non Significant (P>0.05)

Percentage oil yield

The output of essential oils for M. longifolia and *M. piperita* was found to rise from April to July in every test site. The location Giripul yielded the maximum amount of M. longifolia oil (0.56%) in July, whereas the site Khairi yielded the lowest amount (0.09%) in April. Conversely, with *M. piperita*, the highest oil output of 0.63 percent was recorded in July and the lowest yield of 0.26 percent in April.

Table 3: Essential oil yield (%)

Essential oil yield (%)				
Months	**M. longifolia**			***M. piperita***
	Giripul	**Bajhol**	**Khairi**	**Nauni**
April	0.25±0.00	0.2±0.00	0.09±0.00	0.26±0.00
May	0.45±0.00	0.27±0.00	0.11±0.00	0.45±0.00
June	0.50±0.00	0.29±0.00	0.16±0.00	0.59±0.00
July	0.55±0.00	0.35±0.00	0.17±0.00	0.64±0.00

Table 4: Bonferroni post-test showing oil yield (%) comparison

Months	**Row Factor**	**Difference**	**T**	**P-Value**	**Summary**
Giripul vs Khairi	April	-0.150	31.80	P < 0.001	***
	May	-0.330	70.00	P < 0.001	***
	June	-0.330	70.00	P < 0.001	***
	July	-0.390	82.70	P < 0.001	***
Giripul vs Bajhol	April	-0.040	8.48	P < 0.001	***
	May	-0.170	36.06	P < 0.001	***
	June	-0.210	44.55	P < 0.001	***
	July	-0.220	46.66	P < 0.001	***
Giripul vs Nauni	April	0.020	4.245	P < 0.001	***
	May	-0.135	2.121	P > 0.05	ns
	June	0.010	19.10	P < 0.001	***
	July	0.090	14.85	P < 0.001	***
Khairi vs Bajhol	April	0.070	23.45	P < 0.001	***
	May	0.110	34.00	P < 0.001	***
	June	0.120	25.45	P < 0.001	***
	July	0.170	36.00	P < 0.001	***
Khairi vs	April	0.170	36.00	P < 0.001	***

Nauni	May	0.340	72.11	P < 0.001	***
	June	0.420	89.10	P < 0.001	***
	July	0.460	97.50	P < 0.001	***
Bajhol vs Nauni	April	0.060	12.74	P < 0.001	***
	May	0.180	38.20	P < 0.001	***
	June	0.300	63.65	P < 0.001	***
	July	0.290	61.55	P < 0.001	***

2. Phytochemical Study

The total chemicals found in the essential oils of M. longifolia and *M. piperita*, which were gathered in different months (from May to July) from locations Giripul, Khairi, Bajhol, and Nauni, respectively, were studied using the GC-MS technique for phytochemical analysis.

GC-MS analysis of M. longifolia essential oil

The ongoing review's discoveries uncovered that 34 distinct parts were viewed as in M. longifolia's oil consistently. From April to July, a huge variety in the synthetic sythesis of oil was noted for every month. The substance cosmetics and relative overflow of the few parts found in the natural balm extricated from Mentha piperita are uncovered by the GC-MS examination of the oil. The rate relative wealth of every synthetic found in the rejuvenating ointment tests that were accumulated in the long periods of April, May, June, and July is shown in Table 4. variable months show variable concentrations of certain chemicals. Bicyclic organic compound borneol, which makes about 8% of the makeup of essential oils, has the highest relative abundance in April. Its abundance does, however, decline in the following months. There is a modest rise in the concentration of thymol, another aromatic molecule with antibacterial effects, in June as compared to May. The main component of the essential oil is piperitone oxide, a cyclic ketone that peaks in relative abundance in April at 44%. The relative abundances of other substances, such as carvone oxide, carvone, and caryophyllene, likewise vary throughout the course of the month. Interestingly, some molecules are only found in particular months, such as cis-p-mentha1(7),8-dien-2-ol and cyclohexanol, indicating seasonal fluctuations in the chemical makeup of the essential oil.

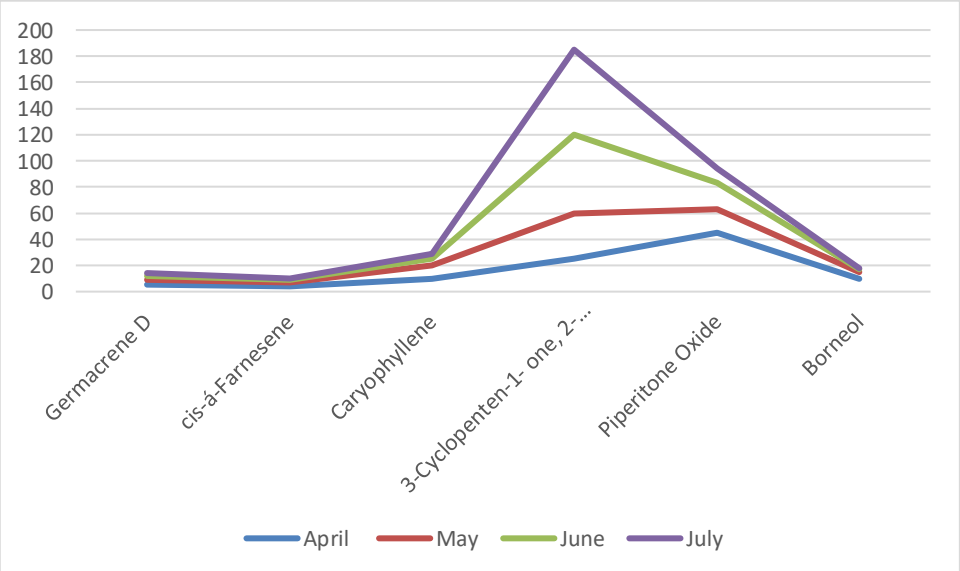
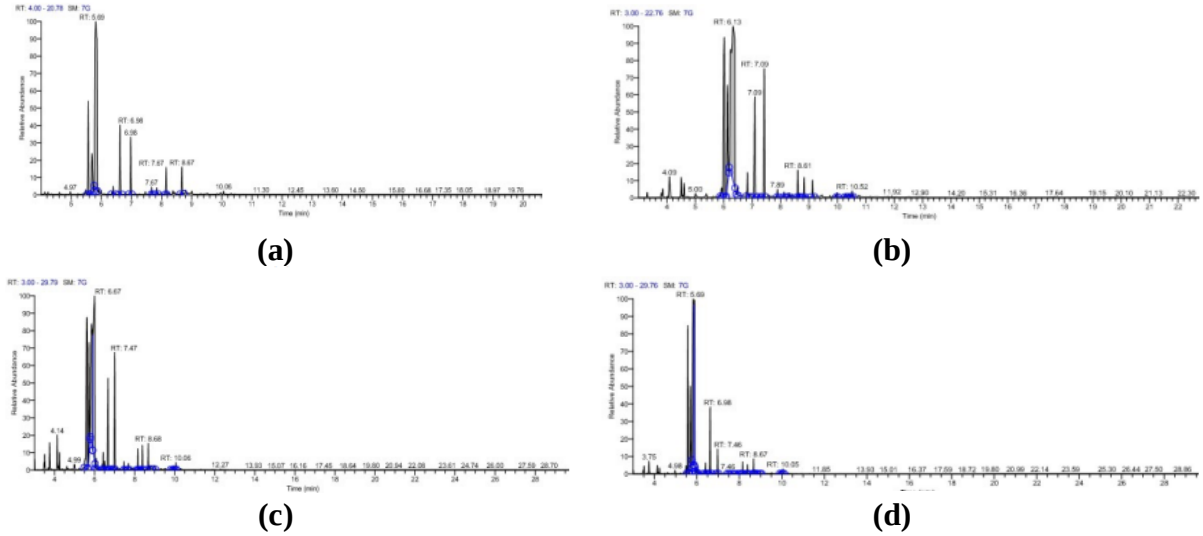


Figure 2: Increasing and decreasing trend of phytocompounds observed in essential oil

Table 5: GC-MS analysis of essential oil

Compounds	Chemical formula	Molecular weight	Months			
			April RT %	May RT %	June RT %	July RT %
Borneol	$C_{10}H_{18}O$	154	8	4.55	1.35	6.20
Cyclohexanol, 1- methyl-4-(1-methylethyl)-	$C_{10}H_{20}O$	156	-	-	0.40	-
3-Cyclohexen-1- ol, 4-methyl-1-(1-methylethyl)-, (R)-	$C_{10}H_{18}O$	154	-	-	0.30	0.60
Thymol	$C_{10}H_{14}O$	150	-	-	0.80	0.28
1HBenzocyclohepte ne,4,4a,5,6,7,8,9, 9a-octahydro-4amethyl-, trans	$C_{12}H_{20}$	164	1.35	-	-	-
à-Terpineol	$C_{10}H_{18}O$	154	-	1.75	0.78	1.15
2-Cyclohexen-1- ol, 2-methyl-5-(1- methylethenyl)-, cis	$C_{10}H_{16}O$	152	-	-	-	0.15
2-Allyl-4- methylphenol	$C_{10}H_{12}O$	148	-	-	-	0.45
Benzene, 1- methoxy-2-(1-methylethenyl)-	$C_{10}H_{12}O$	148	-	-	0.42	-
cis-p-mentha1(7),8-dien-2-ol	$C_{10}H_{16}O$	152	-	-	0.46	0.90
2-Hexenoic acid, 3,4,4-trimethyl5-oxo-, (Z)-	$C_9H_{14}O_3$	170	1.22	-	-	-
Piperitone oxide	$C_{10}H_{16}O_2$	168	44	17	19	11
Carvone oxide,	$C_{10}H_{14}O_2$	166	-	0.96	6.70	1.15
(-)-Carvone	$C_{10}H_{14}O$	150	0.95	-	-	-
Thymol	$C_{10}H_{14}O$	150	0.90	-	-	-
(+) isopiperitenone	$C_{10}H_{14}O$	150	-	1	1.30	1.68
2H-1- Benzopyran, 3,4,4a,5,6,8ahexahydro2,5,5,8atetramethyl-, (2à,4aà,8aà)-	$C_{13}H_{22}O$	194	0.66	-	7	12
Phenol, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{14}O$	150	-	1.75	0.90	0.60
6-Isopropenyl-3-methoxymethoxy -3-methyl-cyclo hexane	$C_{12}H_{20}O_2$	196	0.8	7.5	-	-
D-Verbenone	$C_{10}H_{14}O$	150	-	-	-	1.50
3-Cyclopenten-1- one, 2-hydroxy3-(3-methyl-2- butenyl)-	$C_{10}H_{14}O_2$	166	26	35	60	62
(-)-á-Bourbonene	$C_{15}H_{24}$	204	-	0.25	-	-

Cyclohexane, 1- ethenyl-1-methyl-2,4-bis(1- methylethenyl)-, [1S-(1à,2á,4á)]-	$C_{15}H_{24}$	204	-	0.30	-	-
2-Cyclopenten-1- one, 3-methyl-2- (2-pentenyl)-, (Z)-	$C_{11}H_{16}O$	164	-	0.30	-	-
2-Cyclopenten-1- one, 2-(2-butenyl)-4- hydroxy-3- methyl-, (Z)-	$C_{10}H_{14}O_2$	166	-	0.40	-	-
Caryophyllene	$C_{15}H_{24}$	204	9	9.3	4.6	4.4
cis-á-Farnesene	$C_{15}H_{24}$	204	3.2	2.5	1.30	1.28
1,4,7,- Cycloundecatrien e, 1,5,9,9- tetramethyl-, Z,Z,Z	$C_{15}H_{24}$	204	-	0.83	-	-
Germacrene D	$C_{15}H_{24}$	204	5.4	3	2.5	2.2
Cyclohexane, 1- ethenyl-1-methyl-2-(1- methylethenyl)- 4-(1-meth ylethylidene)	$C_{15}H_{24}$	204	-	-	-	0.18
ç-Elemene	$C_{15}H_{24}$	204	-	-	0.3	-
(-)-Spathulenol	$C_{15}H_{24}O$	220	-	-	0.3	-
Caryophyllene oxide	$C_{15}H_{24}O$	220	2.7	9.5	0.6	0.76
à-Cadinol	$C_{15}H_{26}O$	222	-	-	0.40	-



(a) **(b)** **(c)** **(d)**

Figure 3: GC-MS chromatograms of *M. piperita* essential oil [April (a), May (b), June (c) and July (d)]

3. Antimicrobial Activity

Antibacterial activity of essential oil

Both gram positive (B. subtilis and S. aureus) and gram negative (K. pneumoniae and E. coli) microorganisms showed development concealment because of the antibacterial activity. The accompanying passages gave a clarification of the month-to-month correlation of natural balm action against every single tried bacterium: In contrast with May and June, the most elevated zones of hindrance of M. longifolia natural ointment against B. subtilis were tracked down in the period of July. A concentration of 3.0 mg/ml of oil created the most restraint

(17±0.6 mm for May, 15±0.6 mm for June, and 16±1.0 mm for July), while 1.5 mg/ml of oil delivered the minimum zone of hindrance. The zone of hindrance of microbes was additionally displayed to develop with every month's ascent in oil concentration from 1.5 mg/ml to 3.0 mg/ml. Consistently saw a comparable pattern. The antibacterial movement of natural balm against K. pneumoniae shifted non-fundamentally all through the three months, as indicated by factual measurements. Just the June looked at. July months with a 2.5 mg/ml volume and the May versus July and June versus July months with a 3.0 mg/ml volume showed tremendous contrast. The medicinal ointment's most noteworthy hindrance zone (19±0.6 mm) against S. aureus was distinguished in July at a portion of 3.0 mg/ml, though the minimum restraint zone (14±0.6 mm) was seen in June. The rejuvenating balm showed the most noteworthy antibacterial movement against E. coli in the long periods of May and July (16±0.6 mm and 16±1.0 mm, separately), while June showed the most minimal movement (14±0.6 mm). The antibacterial action of the oil developed every month as its sum rose, as indicated by the outcomes. Except for May versus June months with 2.0 mg/ml and 3.0 mg/ml tests, the Bonferroni numerous correlation test uncovered that all pre-owned volumes had critical differences in antibacterial movement against E. coli.

Table 6: Antibacterial activity

Bacterial spp.	Months	Inhibitory zones(in mm) measured by the Hi media scale				
		50 µg/ml	1.5 mg/ml	2.0 mg/ml	2.5 mg/ml	3.0 mg/m
B. subtilis	May	20±0.6	10±0.6	12±0.6	15±0.6	17±0.6
	June	19±0.6	10±0.0	11±0.6	14±0.6	15±0.6
	July	20±1.0	11±0.6	12±0.6	15±0.6	16±1.0
K.pneumoniae	May	16±1.0	11±0.6	12±0.6	13±0.6	15±1.0
	June	16±0.6	10±0.0	12±0.0	12±0.6	14±0.6
	July	17±0.6	11±0.6	13±0.6	14±0.6	17±1.0
S. aureus	May	18±0.6	11±0.6	12±0.6	14±0.6	16±0.6
	June	17±0.6	10±0.6	11±0.6	12±0.6	14±0.6
	July	17±0.6	13±0.6	14±0.6	17±1.0	19±0.6
E. coli	May	12±0.6	11±0.6	13±0.6	15±0.6	16±0.6
	June	13±0.6	10±0.0	11±0.6	13±0.6	14±0.6
	July	13±0.6	10±0.6	12±0.6	13±0.6	16±1.0

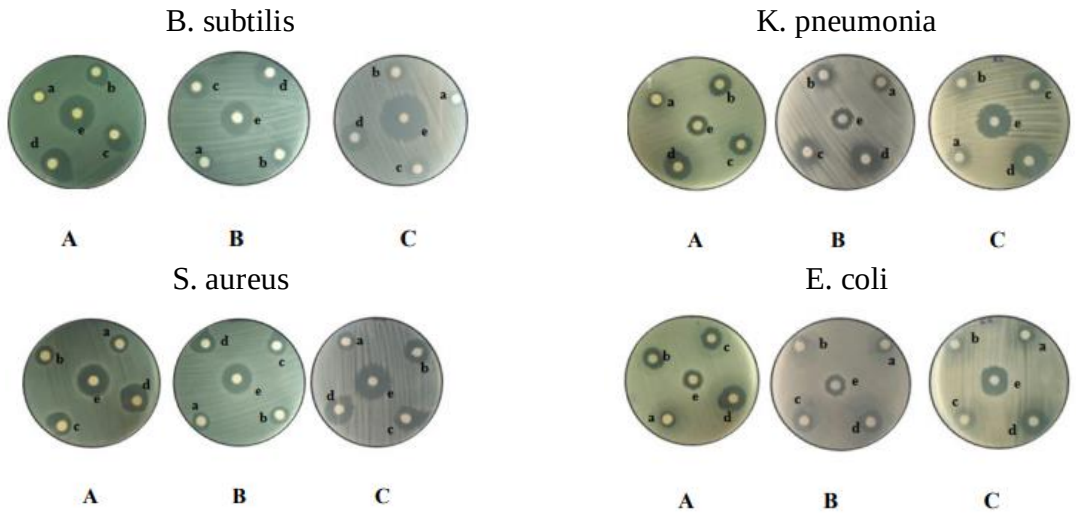


Figure 4: Antibacterial activity of essential oil in May (A), June (B) and July (C) month

Antibacterial activity of essential oil of *M. piperita*: Every month from May to July, the antibacterial activity of *M. piperita*'s essential oil demonstrated growth inhibition of the tested microorganisms. When the oil was utilised at a concentration of 3.0 mg/ml of volume, its highest activity was seen; at 1.5 mg/ml of volume, it was less active. In May, *M. piperita* oil showed higher zones of inhibition (19±0.6 mm) against B. subtilis than in June and July, when the zones of inhibition were nearly similar (18±1.0 mm and 18±0.6 mm, respectively). The month of May had the largest inhibition zone (17±0.6 mm) against K. pneumoniae, while the month of July had the lowest (15±0.6 mm). With the exception of 3.0 mg/ml of concentration in June vs. July, statistical analysis revealed non-significant antibacterial differences at every concentration of oil utilised between each month (Table 7). The results indicated that the month of July (17±0.6 mm) had the highest antibacterial activity against S. aureus, while the month of May (15±0.6 mm) exhibited the lowest. All of the oil quantities that were obtained exhibited non-significant fluctuations, according to the statistical analysis, with the exception of the concentrations of 1.5 mg/ml (May vs. June), 2.0 mg/ml (May vs. June), and 2.5 mg/ml (May vs. June and June vs. July), which showed significant variations.

Table 7: Antibacterial activity

Bacterial spp.	**Months**	**Inhibitory zones(in mm) measured by the Hi media scale**				
		50 µg/ml	**1.5 mg/ml**	**2.0 mg/ml**	**2.5 mg/ml**	**3.0 mg/m**
B. subtilis	May	20±0.6	14±0.6	16±0.6	17±0.6	19±0.6
	June	19±0.6	11±0.6	12±1.0	16±1.0	18±1.0
	July	19±0.6	13±1.0	14±0.6	17±0.6	18±0.6
K. pneumoniae	May	18±1.5	11±0.6	13±1.0	14±1.2	16±1.5
	June	19±0.6	12±0.6	14±0.6	15±0.6	17±0.6
	July	18±0.6	11±0.6	13±0.6	14±0.0	15±0.6
S. aureus	May	19±0.6	11±0.6	12±0.6	14±0.6	15±0.6
	June	18±0.6	11±0.6	12±0.6	14±1.2	16±0.6
	July	18±1.0	13±0.6	15±0.6	16±0.6	17±0.6
E. coli	May	15±0.6	12±0.6	13±1.0	14±0.6	15±0.6
	June	15±0.6	10±0.6	11±0.6	12±0.6	14±0.6
	July	15±0.6	11±0.6	12±0.6	14±0.6	15±0.6

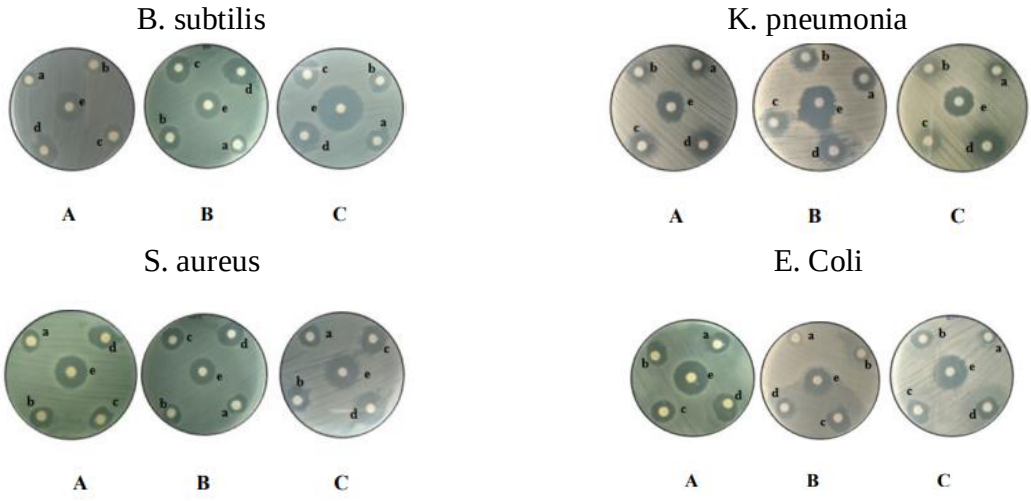


Figure 5: Antibacterial activity of essential oil of *M. piperita*

4. Antifungal Activity

Antifungal activity of essential oil *M. piperita*

The greatest antifungal action (16±0.6%) of the *M. piperita* medicinal balm against C. albicans was seen in July, though the least antifungal action (13±0.6%) was seen in May. Then again, the extricated oil from May had the most elevated antifungal action against S. cerevisiae, and the oils from June and July showed equivalent action. The consequences of the natural ointment action against Candida albicans were measurably broke down, uncovering non-tremendous contrasts between the excess months and significant contrasts between the long stretches of May and July. In like manner, factual assessment uncovered extremely irrelevant vacillations in the month to month adequacy of *M. piperita* natural oil against S. cerevisiae. Just the movement levels in May and July showed a massive contrast, at 2.5 mg/ml and 1.5 mg/ml, separately.

Table 8: Antifungal activity of essential oil of *M. piperita*

Fungal strains	Months	Inhibitory zones(in mm) measured by the Hi media scale				
		50 µg/ml	1.5 mg/ml	2.0 mg/ml	2.5 mg/ml	3.0 mg/m
C. albicans	May	12±0.6	10±0.6	10±0.6	12±0.6	13±0.6
	June	12±0.6	11±0.6	12±0.0	13±1.0	15±0.6
	July	13±0.6	12±0.6	14±1.0	16±0.6	16±0.6
S. cerevisiae	May	13±1.2	12±0.6	13±1.2	15±0.6	15±0.6
	June	14±0.6	11±0.6	12±0.6	13±1.0	14±0.6
	July	14±0.6	10±0.6	11±1.0	12±0.6	14±0.6

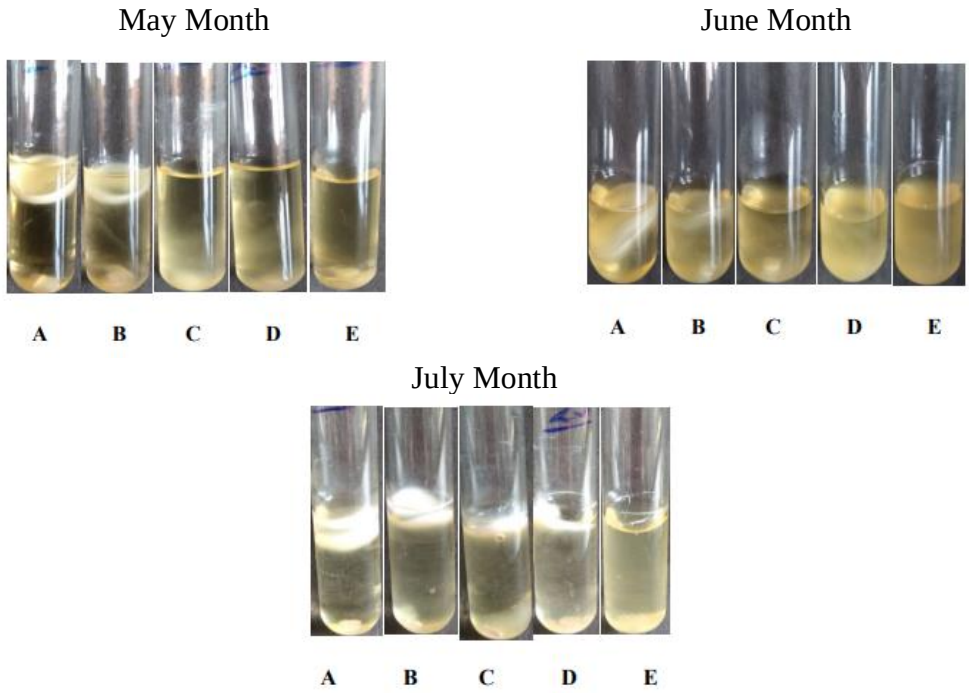


Figure 6: Antifungal activity of essential oil of *M. piperita*

CONCLUSION

The incorporation of Mentha piperita essential oil into a nano-hydrogel formulation offers a promising approach to enhancing its antifungal activity. The careful selection of biocompatible polymers enabled the creation of a hydrogel matrix capable of absorbing and retaining significant amounts of water, essential for maintaining the gel's integrity and functionality. This study underscores the importance of optimizing various parameters,

including polymer concentration, essential oil concentration, viscosity, pH, temperature, and mixing conditions, to achieve a stable and effective nano-hydrogel. The nanoscale dimensions of the hydrogel particles, ranging from 1 to 100 nanometers, significantly increased the surface area-to-volume ratio, which in turn improved the interaction of the nano-hydrogel with target sites. This characteristic is crucial in enhancing the bioavailability and efficacy of the encapsulated essential oil. Moreover, the high porosity of nano-hydrogels allows for greater drug loading capacity, making them superior to conventional hydrogels in delivering therapeutic agents. Encapsulation methods, both physical entrapment and chemical conjugation, were employed to ensure the controlled release and stability of the essential oil within the hydrogel matrix. Physical entrapment involved dispersing the essential oil throughout the hydrogel, where the polymer chains effectively trapped the oil molecules. This method provided uniform distribution and protection from environmental factors such as light, heat, and oxygen, which could degrade the oil. Chemical conjugation, on the other hand, formed covalent bonds between the essential oil molecules and the polymer chains, enhancing stability and bioavailability and potentially allowing for targeted delivery. The antifungal efficacy of the nano-hydrogel was evaluated through a series of in vitro tests against fungal pathogens like Candida albicans. Key parameters such as the minimum inhibitory concentration (MIC) and the zone of inhibition were measured to assess the hydrogel's effectiveness. The results demonstrated significant antifungal activity, with the nano-hydrogel formulation showing enhanced inhibition of fungal growth compared to non-nano formulations. In addition to antifungal activity, the study also examined the antibacterial properties of the essential oil against both gram-positive and gram-negative bacteria. The findings indicated that the essential oil, when incorporated into the nano-hydrogel, exhibited potent antibacterial activity, further broadening its potential therapeutic applications. Seasonal and locational variations in biomass yield and essential oil content were observed, highlighting the influence of environmental factors on the production and quality of Mentha piperita. Gas Chromatography-Mass Spectroscopy (GC-MS) analysis revealed significant seasonal fluctuations in the chemical composition of the essential oils, which could impact their therapeutic efficacy. This variation underscores the need for standardized harvesting and processing methods to ensure consistent quality and efficacy in the final product. The study's findings suggest that the nano-hydrogel formulation of Mentha piperita essential oil is a viable and effective approach for antifungal treatment. The enhanced stability, controlled release, and improved bioavailability of the essential oil within the nano-hydrogel matrix make it a promising candidate for further development and clinical application. Future research should focus on in vivo studies to validate these findings and explore the therapeutic potential of this formulation in real-world settings. The integration of nanotechnology into the formulation of Mentha piperita essential oil hydrogels represents a significant advancement in the field of antifungal therapies. The nano-hydrogel's superior properties, including high surface area, enhanced drug loading capacity, and controlled release mechanisms, contribute to its effectiveness. This innovative approach not only maximizes the therapeutic benefits of Mentha piperita essential oil but also opens new avenues for the development of advanced drug delivery systems for various medical applications.

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REFERENCE

1. C. Guemidi *et al.*, “Enhancement of yogurt functionality by adding Mentha piprita

phenolic extract and evaluation of its quality during cold storage," *Food Sci. Nutr.*, 2024, doi: 10.1002/fsn3.3981.
2. H. S. Nacef, A. Bouguerra, and R. Belhattab, "Antifungal activity of the endophytic Aspergillus against Candida albicans," *Biosyst. Divers.*, 2023, doi: 10.15421/012346.
3. C. Author and T. A. Abuhlega, "ORIGINAL ARTICLES Utilization of Some Essential Oils to Extend The Shelf-Life of Coated Semi Fried Nile Perch Fish Fillets during Cold Storage," 2013.
4. R. Singh, M. A. M. Shushni, and A. Belkheir, "Antibacterial and antioxidant activities of Mentha piperita L.," *Arab. J. Chem.*, 2015, doi: 10.1016/j.arabjc.2011.01.019.
5. O. Taylan, N. Cebi, and O. Sagdic, "Rapid screening of mentha spicata essential oil and l-menthol in mentha piperita essential oil by atr-ftir spectroscopy coupled with multivariate analyses," *Foods*, 2021, doi: 10.3390/foods10020202.
6. Y. X. Li, Y. B. Liu, A. Q. Ma, Y. Bao, M. Wang, and Z. L. Sun, "In vitro antiviral, anti-inflammatory, and antioxidant activities of the ethanol extract of Mentha piperita L.," *Food Sci. Biotechnol.*, 2017, doi: 10.1007/s10068-017-0217-9.
7. W. Yi, R. Chen, F. Xie, C. Xu, and W. Tian, "Anti-inflammatory and immunomodulatory properties of Mentha piperita green-formulated gold nanoparticles and its effect on ovalbumin-induced asthma and lung pathological changes in rats," *J. Exp. Nanosci.*, 2022, doi: 10.1080/17458080.2022.2033730.
8. V. K. Panthi, S. K. Jha, R. Pangeni, and K. R. Paudel, "Formulation and Development of Adapalene Topical Nanohydrogel Using Different Surfactants and Cosurfactants for Antiacne Activity: In Vitro and Ex Vivo Evaluation," *J. Nanomater.*, 2022, doi: 10.1155/2022/6889293.
9. U. Lestari, M. Muhaimin, A. Y. Chaerunisaa, and W. Sujarwo, "Improved Solubility and Activity of Natural Product in Nanohydrogel," *Pharmaceuticals*. 2023. doi: 10.3390/ph16121701.
10. Y. Dange, V. Salunkhe, S. Honmane, and K. Thorawade, "Design, Development and Optimization of 5-Fluorouracil Nanohydrogel for Improved Anticancer Therapy," *Bionanoscience*, 2024, doi: 10.1007/s12668-024-01315-1.
11. R. A. Durham, D. L. Mummey, L. Shreading, and P. W. Ramsey, "Phenological Patterns Differ between Exotic and Native Plants: Field Observations from the Sapphire Mountains, Montana," *Nat. Areas J.*, 2017, doi: 10.3375/043.037.0310.
12. D. Barrett and A. Twycross, "Data collection in qualitative research," *Evidence-Based Nursing*. 2018. doi: 10.1136/eb-2018-102939.
13. H. R. Ganesha and P. S. Aithal, "How to Choose an Appropriate Research Data Collection Method and Method Choice Among Various Research Data Collection Methods and Method Choices During Ph.D. Program in India?," *Int. J. Manag. Technol. Soc. Sci.*, 2022, doi: 10.47992/ijmts.2581.6012.0233.
14. Y. L. Lyu, S. J. Rong, F. Sun, C. Xiang, and J. Y. Li, "Management of an emergency sample collection team under the setting of whole-community severe acute respiratory syndrome coronavirus 2 nucleic acid testing," *Int. Health*, 2023, doi: 10.1093/inthealth/ihac057.
15. D. Lu *et al.*, "Advances in the noninvasive and minimally invasive sample collection for wearable electrochemical sensors," *Electroanalysis*. 2024. doi: 10.1002/elan.202300385.
16. C. M. Sastre *et al.*, "Centralised electricity production from winter cereals biomass grown under central-northern Spain conditions: Global warming and energy yield assessments," *Appl. Energy*, 2014, doi: 10.1016/j.apenergy.2013.08.035.
17. K. Ainiyah, F. Andriyani, S. Soemargono, and N. K. Erliyanti, "ISOLATION OF CLOVE ESSENTIAL OIL BY FERMENTATION PROCESS," *Konversi*, 2021, doi:

10.20527/k.v10i1.10415.
18. K. Bica, P. Gaertner, and R. D. Rogers, "Ionic liquids and fragrances – direct isolation of orange essential oil," *Green Chem.*, 2011, doi: 10.1039/c1gc15237h.
19. S. M. Adekenov, G. M. Baysarov, A. N. Zhabaeva, A. Sabitova, A. S. Adekenova, and V. V. Polyakov, "Technology for Isolation Essential Oil from the Buds of Populus balsamifera L," *Eurasian Chem. J.*, 2024, doi: 10.18321/ectj1545.
20. U. Narayanamurthy, D. Barathane, S. Karthik, and S. J. V. Anandh, "Preliminary Phytochemical and GC-MS Analysis of Marine Seaweed-Acoathophora deilei (Red alga)," *Biomed. Pharmacol. J.*, 2022, doi: 10.13005/bpj/2508.
21. S. H. Mlozi, J. A. Mmongoyo, and M. Chacha, "GC-MS analysis of bioactive phytochemicals from methanolic leaf and root extracts of Tephrosia vogelii," *Sci. African*, 2022, doi: 10.1016/j.sciaf.2022.e01255.
22. B. P. Ezhilan and R. Neelamegam, "GC-MS analysis of phytocomponents in the ethanol extract of Polygonum chinense L.," *Pharmacognosy Res.*, 2012, doi: 10.4103/0974-8490.91028.
23. E. Jaisankar, M. E. Pavithra, S. Krishna, M. Thirumarimurugan, and R. S. Azarudeen, "Dual property of chitosan blended copolymer membranes: Antidiabetic drug release profile and antimicrobial assay," *Int. J. Biol. Macromol.*, 2020, doi: 10.1016/j.ijbiomac.2019.12.038.
24. J. M. Martins *et al.*, "Spectinomycin, gentamicin, and routine disc diffusion testing: An alternative for the treatment and monitoring of multidrug-resistant Neisseria gonorrhoeae?," *J. Microbiol. Methods*, 2022, doi: 10.1016/j.mimet.2022.106480.
25. F. Mansour-Ghanaei, G. Poostizadeh, F. Joukar, and F. Siavoshi, "Efficacy of Disc Diffusion and Agar Dilution Methods in Evaluating Helicobacter pylori Susceptibility to Antibiotics," *Middle East J. Dig. Dis.*, 2022, doi: 10.34172/mejdd.2022.274.
26. C. Erhonyota, G. I. Edo, and F. O. Onoharigho, "Comparison of poison plate and agar well diffusion method determining the antifungal activity of protein fractions," *Acta Ecol. Sin.*, 2023, doi: 10.1016/j.chnaes.2022.08.006.
27. M. Jannat, S. Kiran, S. Yousaf, T. Gulzar, and S. Iqbal, "Potential Antifungal Effects of D. malabarica Assisted Zinc Oxide and Silver Nanoparticles against Sheath Blight Disease of Rice Caused by Rhizoctonia solani," *Polish J. Environ. Stud.*, 2022, doi: 10.15244/pjoes/150049.
28. L. Mannetje and K. P. Haydock, "THE DRY-WEIGHT-RANK METHOD FOR THE BOTANICAL ANALYSIS OF PASTURE," *Grass Forage Sci.*, 1963, doi: 10.1111/j.1365-2494.1963.tb00362.x.
29. S. Prakash and A. K. Jain, "Antifungal activity and preliminary phytochemical studies of leaf extract of solanum Nigrum Linn," *Int. J. Pharm. Pharm. Sci.*, 2011.
30. I. O. Chikezie, "Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method," *African J. Microbiol. Res.*, 2017, doi: 10.5897/ajmr2017.8545.
31. E. Yusuf, M. Van Westreenen, W. Goessens, and P. Croughs, "The accuracy of four commercial broth microdilution tests in the determination of the minimum inhibitory concentration of colistin," *Ann. Clin. Microbiol. Antimicrob.*, 2020, doi: 10.1186/s12941-020-00383-x.
32. I. C. Rahmansyah and L. N. Rani, "ANALISIS PENGARUH HARGA EMAS, MARGIN, INFLASI DAN KURS DOLLAR TERHADAP PEMBIAYAAN MURABAHAH EMAS DI PERBANKAN SYARIAH INDONESIA PERIODE APRIL 2015-AGUSTUS 2019," *J. Ekon. Syariah Teor. dan Terap.*, 2020, doi: 10.20473/vol7iss20203pp610-622.