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Comparative Gas Chromatography-Mass Spectrometry Analysis of Nigella sativa Varieties Romanian, Sudani, Bangladeshi, Hama(Syrian)

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Abstract: ***This study investigates the phytochemical differences between four varieties of Nigella sativa (black seed) which from family Ranunculaceae cultivated in Romania which is native, Sudan, Bangladesh, and Hama (Syria) using gas chromatography-mass spectrometry (GC-MS). The focus is on quantifying key bioactive compounds-thymoquinone, p-cymene, α-phellandrene, and longifolene-recognized for their therapeutic properties. The analysis reveals significant variation in the concentration of these compounds across the different geographical origins, with the Romanian variety exhibiting the highest thymoquinone content. These findings highlight the impact of geographical origin on the chemical composition and potential therapeutic value of Nigella sativa seeds, offering valuable insights for industrial and pharmaceutical applications.***

Method and Material: Seeds from each region were collected with unified harvest timing (April, 8 am morning), cleaned, shade-dried, and ground. For extraction, 60 g of seeds per sample were soaked in 400 ml hexane at 23–26°C for three days. The extracts were filtered using nylon filter paper (0.45 µm) and treated with activated charcoal (0.25%) for purity. GC-MS analysis was performed using a Shimadzu GCMS-QP 2010 Plus system, with calibration and validation via standard compounds and the NIST11s.lib spectral library. Linearity and recovery tests confirmed high analytical accuracy ($R^2 > 0.999$, recovery 98.3–101.6%, RSD < 1.2%).

Results: GC-MS analysis showed clear phytochemical differences between the varieties. The Romanian sample had the highest thymoquinone concentration (27.9%), followed by Hama (Syrian) (16.2%), Sudanese (4.3%), and Bangladeshi (2.2%). Statistical analysis (ANOVA and Kruskal-Wallis) confirmed significant differences between origins ($p < 0.001$). Toxicity predictions indicated that p-cymene (LD50 = 3 mg/kg) is the most toxic among the analysed compounds, while thymoquinone and others are within safe limits for food and therapeutic use.

Conclusion: The study demonstrates that the phytochemical profile of Nigella sativa varies significantly with geographical origin, particularly regarding thymoquinone content. The Romanian variety is richest in thymoquinone, suggesting superior therapeutic potential, while other varieties differ in their dominant compounds. These results underscore the importance of origin in selecting Nigella sativa varieties for medicinal and industrial use and call for further research using advanced analytical methods to optimize economic and health benefits1.

Keywords: GC-MS, nigella sativa, Geographical variation

Abbreviations: NSNigella sativa, MW molecular weight, GC-MSGas Chromatography-Mass Spectrometry

I. INTRODUCTION

Black seed (Nigella sativa) has a long history in traditional and prophetic medicine(Ibn-Qaiyim al-Ğauzīya 2003),valued for its antioxidant, anti-inflammatory, and anticancer and for asthma treatment and antidiabetic properties(Ahmad et al. 2013), primarily due to active compounds like thymoquinone and p-cymene (Ahmad et al. 2013)In This research I aim to compare between phytochemical differences of nigella sativa from four different Geographical origins of Nigella sativa cultivated in Bangladesh, Hama(Syria), and Romania(native) and Sudan, using GC-MS analysis to determine variations in active compounds ,The most important active compounds with therapeutic activity include thymoquinone, p-cymene, α-phellandrene, longifolene, among others found in volatile oils with therapeutic properties. Although valuable articles compare different geographical origins of Nigella sativa such as Saudi Arabia(Farhan, Salih, and Salimon 2021), India(Ahmad et al. 2013), and Turkey(Erdoğan, Yilmazer, and Erbaş 2020), and by using prediction system and database like Superperd ,Protox III we can add significantinformation about important therapeutic indications and Toxicity profile ,

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this subject represents a research gap that researchers have called for further exploration through broader analytical studies and specially use different analytical methods and different methodology procedure to know the real phytochemical content of different geographical type of nigella sativa ,objectives related to economic and therapeutic uses of Nigella sativa as well as its agriculture and choice which origin is more nutritionally and therapeutically, The geographical differences between Bangladesh, Hama(Syria,)and Romanian and Sudan are significant, and many industries and exports depend on them. It is necessary to know the phytochemical content of each geographical variety to invest this information inenhancing exports while aiding industrialized countries in selecting varieties rich in phytochemical compounds

A. Background

Nigella sativa (black seed) has a long history in traditional and prophetic Medicine(Ibn-Qaiyim al-Ğauzīya 2003), valued for its antioxidant, anti-inflammatory, anticancer, antiasthmatic, and antidiabetic properties(Ahmad et al. 2013),primarily due to active compounds like thymoquinone and p-cymene and other important compounds .

B. Problem Statement

Despite previous studies comparing *Nigella sativa* from different geographical origins such as Saudi Arabia, India, and Turkey, there remains a research gap regarding the phytochemical differences among these origins using advanced analytical methods like GC-MS. This study aims to fill this gap by examining the variation in active compounds between *Nigella sativa* from four geographical locations: Bangladesh, Hama (Syria), Romania (native), and Sudan, more research in nigella sativa origins and determine percentages of important active compounds and their therapeutic activity and toxicity is a valued information for nutritional and pharmaceutical applications.

C. Objectives

This study aims to:

- Determine Phytochemical Differences: Use GC-MS to analyze variations in active compounds such as thymoquinone, p-cymene, α-phellandrene, longifolene, among others, between different *Nigella sativa* origins.
- Evaluate Economic and Therapeutic Uses: Utilize information on phytochemical content to enhance exports and guide industrialized countries in selecting varieties rich in active compounds.
- Identify the Most Nutritious and Therapeutic Origin: Compare different origins to determine which is superior in nutritional and therapeutic value.

D. Significance

This research is crucial for enhancing the economic and therapeutic pharmaceutical uses of *Nigella sativa*. It can contribute to improving exports and guiding industries in selecting the most beneficial varieties for more income. Additionally, it aids in understanding how geographical differances affect phytochemical composition, like Romanian (native)compare to Hama Syria and Sudan and Bangladesh, opening new avenues in herbal medicine.

E. Computational Analysis

To further assess the therapeutic potential and toxicity of the eight compounds, including thymoquinone and p-cymene, we will utilize computational tools such as SuperPred and Protex III. These tools will help predict the most likely toxic compound among the eight and evaluate the potential health impacts of these compounds, providing a comprehensive understanding of their therapeutic and toxicological profiles.

II. EXPERIMENTAL

A. Materials and Method

- Specifications requested when purchasing samples:Unification of harvest time ,it should at Aprile in the morning before 8 am . Four types of Nigella sativa were purchased from local stores— Hama city in Syria, in Turkey from a Konya city,and Dhaka city in Bangladesh and Sudani fromKhartoum city,After purchasing and preparing the seeds (cleaning, drying, grinding), For sample preparationthey were washed and dried for three days in the shade and purified from all impurities.

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- Standard Materials : Thymoquinone 98% and α-Pinene and β-Pinene 95% Sigma Aldrich,KremsChem for p-Cymene 99%,Merck Millipore for α-Phellandrene 60% and the rest compounds taken from the system library

B. Characterization Methods

Sample preparation

The four samples were in good condition. Grinding was done first by carefully weighing them on a sensitive balance in the laboratory, where we weighed 60 grams for each type separately, then the special seed grinder was used Secura Electric grind , After grinding, we use them directly . Hexane (Rahim et al. 2022)is used as a solvent, and the amount of NS is 60 grams and the solvent is 400 ml. The experiment takes three overnight while maintaining a temperature of 23-26 C. then the extract is placed in a dark bottle in the refrigerator, waiting for the rest of the steps(Jha and Sit 2022).

Filtration Process: The filtration step involves using nylon filter paper (pore size: 0.45 µm) multiple times to remove colour that could interfere with GC-MS analysis. All extraction, filtration, and storage processes must be conducted at low temperatures to prevent chemical decomposition due to heat

C. Activated Charcoal discolorations:

Use of activated charcoal(Chetima et al. 2024) is used with extreme caution and in small concentrations (0.25% of the total volume of the extract) so that the active substances in the extract do not stick to it, but we took this step to obtain a very pure sample for use in GC-MS

Data Collected: The GC-MS analysis provided detailed information on the concentration of active compounds like thymoquinone, p-cymene, and other phytochemicals in each variety of NS. This helped correlate their chemical composition.

GC-MS Procedure :Brand: Shimadzu(Centre for Microscopy, Characterisation & Analysis 2018) ,Model: GCMS-QP 2010 Plus ,Brief description: The GCMS-QP2010 Plus shows the highest sensitivity ever reached in a GCMS system. Ideal for rapid analysis of trace components, the GCMS-QP2010 Plus is the ideal instrument for complex organic mixtures in environment(Stein and Scott 1994),Column Oven TemperatureColumn SH-Rxi-5Sil MS (Shimadzu), length :30 ,internal Diameter (ID) :0.25micrometer ,Film Thickness: 0.25 micrometre ,Carrier Gas: Helium at a flow rate of 1.0 mL/min , Injection Volume and Mode: Spitless injection of 1 µL sample

Oven Temperature Program: Initial temperature of 60°C held for 2 minutes

Ramp at a rate of 10°C/min to a final temperature of 280°C

Hold at final temperature for 10minutes,Pressure 600 bar

D. MS Conditions:

Ionization mode: Electron Impact (EI) ,Ion source temperature: 200°C ,Mass range scanned: m/z 50–550Solvent Delay(min) 4 mint , library NIST11s.lib contribute to the identification of compounds ,The **NIST11s.lib** contributes to compound identification in GC-MS by providinglarge database of reference mass spectra for spectral matching ,retention index data for cross-verifying compound identity,enhanced accuracy through combined evaluation of mass spectra and retention indices, NIST11s.lib it is a comprehensive collection of mass spectral data compiled by the National Institute of Standards and Technology (NIST) in collaboration with the Environmental Protection Agency (EPA) and the National Institutes of Health (NIH). It includes over 243,000 spectra for approximately 213,000 chemical compounds, as well as additional databases for replicate spectra, retention indices, and MS/MS data. This database is widely used for identifying unknown compounds by comparing their mass spectra to those in the database. The accompanying NIST Mass Spectral Search Program (Version 2.0g) provides tools for efficient searching, retrieval, and analysis of this data (Share 1976)

This dual approach ensures more reliable identification of compounds in complex mixtures, as demonstrated in your sample analysis where multiple hits were confirmed with high similarity indices and corresponding retention indices Calibration of GCMS:All GCMS analyses were performed at UM university laboratories in Kuala Lumpur.

E. Calibration of GCMS analysis:

Example for calculations We calculated the concentrations of standard Thymoquinone 10mg/ml of hexane and diluted to prepare 1,2.5,5,10,20,50 mg/ml samples to prepare linearity test and recovery test, I should add 0.5 mg/ml of diethyl phthalate as internal standard, R^2 was equal 0.9993 results demonstrated a strong linear relationship FIG 3 ,confirming the methods validity for quantitative applications with the studied range(Y et al. 2024)

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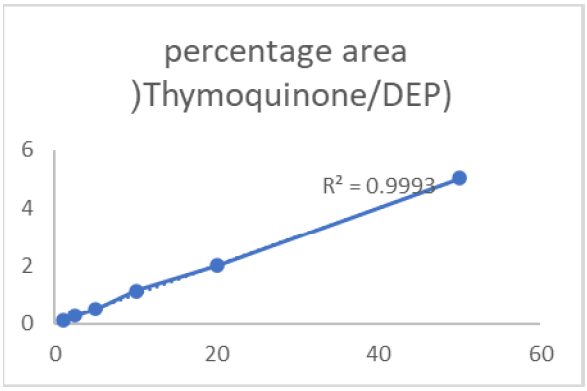


FIG 3

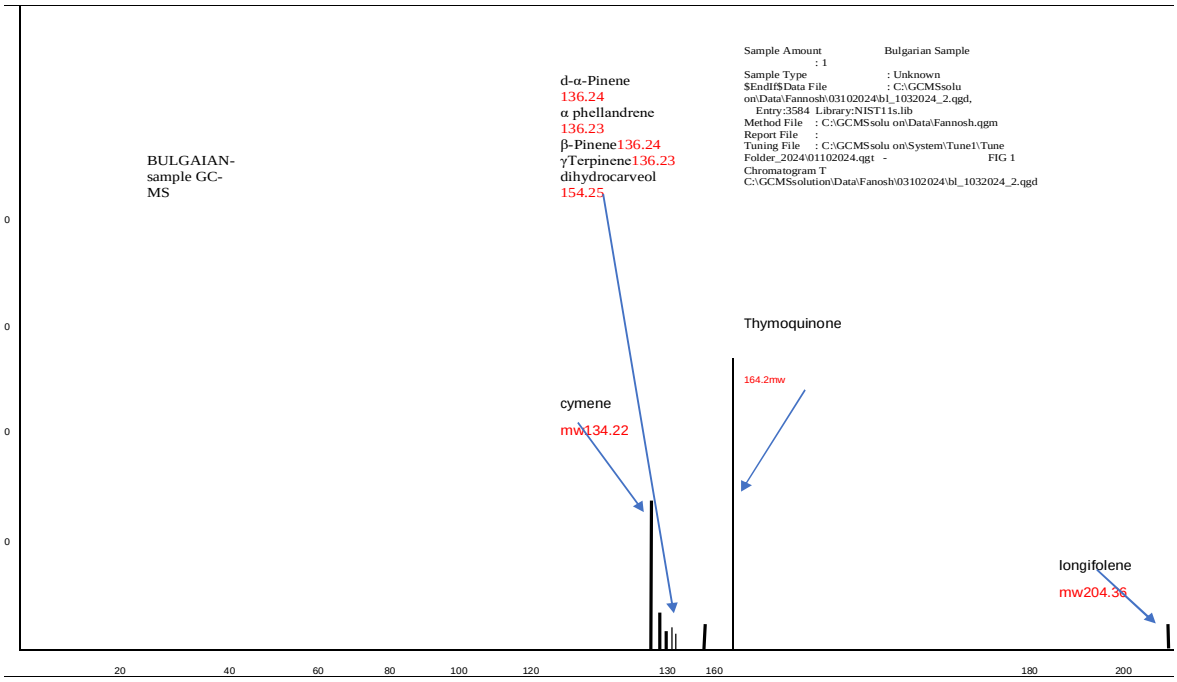
Linearity test done for all compounds P cymene , α-Phellandrene , d-α-Pinene , β-Pinene , γ-Terpinene, Dihydrocarveol, Longifolene ,

The linearity and recovery of the eight aromatic compounds were evaluated using GC-MS over a concentration range of (0.01–0.5 mg/ml) it was repeated three times. The results showed excellent linear response for all compounds with correlation coefficient (R^2) values higher than 0.999, confirming the accuracy and reliability of the analytical method in detecting the target compounds.

The recovery test was conducted to validate the accuracy and reliability of the analytical method using GC-MS. Recovery percentages ranged between 98.3% and 101.6%, which are within the acceptable range according to AOAC standards (85–115%)(n.d.). The relative standard deviation (RSD) values were below 1.2%, indicating high precision and reproducibility of the method

III. RESULTS AND DISCUSSION

GC-MS Phytochemical Analysis*Table(1.4)*: Romanian Nigella sativa showed the highest thymoquinone concentration at 27.9% (41,850 ppm), followed by Hama (Syrian) at 16.2% (28,650 ppm), Sudan at 4.3% (6,450 ppm), and Bangladeshi at 2.2% (3,300 ppm). The total sample concentration was 150,000 ppm, derived from 60 mg of Nigella sativa seed powder dissolved in 400 ml hexane solvent



(Romanian *Sample*)FIG4

A. Calculation of compounds concentrations:

Concentration by mg /ml= $\frac{\text{Mass of powder (mg)}}{\text{Volum of solvent (ml)}}$

in our study we use just 60 g of nigella sativa seed in 400ml in hexan as solvent

First conversion of 60g to mg =60000mg , in the experiment I just use 1 ml for GC-MS analysis , that gives$\frac{60}{400}$ = 150 mg/ml to convert to

Ppm , 1mg/ml = 1000 ppm , 150000 ppm final sample concentration

Concentration compound= = $\frac{\text{peak area}}{100}$ $total\ concentration$, in table 1.3 the concentration by ppm for each compound.

ompound	Molecular Weight (g/mol)		Retention Time (min)	Initial Time (i-time)	Final Time (f-time)	Area (%)	ppm
hymoquinon	164.2	Romanian	4.8	4.8	4.9	27.9	41850
		Bangladesh	4.7	4.7	4.8	19.1	28650
		Hama(,Syrian)	5	5	5.1	2.2	3300
		Sudan	5.2	5.2	5.3	4.3	6450
-Cymene	134.22	Romanian	7.4	7.4	7.5	16.2	24300
		Bangladesh	7.5	7.5	7.7	12.8	19200
		Syrian	7.4	7.4	7.6	10.66	1599
		Sudan	7.5	7.5	7.8	45.6	68400
hellandrene	136.23	Romanian	4.9	4.9	5	2.75	4125
		Bangladesh	4.8	4.8	5	3.1	4650
		Syrian	4.8	4.8	4.9	9.59	14385
		Sudan	4.7	4.7	4.8	5.5	8250
l-α-Pinene	136.24	Romanian	7.5	7.5	7.6	1.06	1590
		Bangladesh	7.85	7.8	7.9	0.98	1470
		Syrian	7.85	7.8	7.9	2.25	3375
		Sudan	7.6	7.6	7.8	1.85	2775
-Pinene	136.24	Romanian	8.3	8.2	8.5	0.66	990
		Bangladesh	8.25	8.2	8.3	0.85	1275
		Syrian	8.25	8.2	8.3	1.9	2850
		Sudan	8.3	8.3	8.3	1.6	2400
-Terpinene	136.23	Romanian	8.7	8.7	8.8	0.55	825
		Bangladesh	8.7	8.6	8.8	0.74	1110
		Syrian	8.5	8.5	8.6	0.81	1215
		Sudan	8.6	8.6	8.7	0.89	1335
ihydrocarvol	154.25	Romanian	9.3	9.3	9.4	0.88	1320
		Bangladesh	9.2	9.2	9.3	0.23	345
		Syrian	9.2	9.2	9.3	3.88	5820
		Sudan	9.3	9.3	9.3	0.25	375
ongifolen	204.36	Romanian	24.5	24.5	24.6	0.88	1320
		Bangladesh	25.3	25.3	25.5	0.45	675
		Syrian	24.5	24.5	24.5	1.62	2430
		Sudan	25.5	25.5	25.5	2.2	3300

TABLET 1

B. Statistical test of the results:

N = 10, and in the Table 1.1 the Mean and SD is provided for all volatile compounds results.**One-way ANOVA**displayed significant differences in levels among the four geographical origins **F(3, 36) = 587.5, p < 0.001**, (p should be<0.05), and **KruskalWallis test** statistic of ≈**36.6** with **3** degrees of freedom, corresponding to a p-value **p<0.001**,This result indicates that there are statistically significant differences(Asimi et al. 2022),(De Vivo, Balivo, and Sarghini 2023).

C. Toxicity of the Compounds:

P-cymene has an LD50 of 3 mg/kg, making it the most toxic compound in this list. It is classified as Class 1, indicating the highesthazard level,α phellandrene is class 6 after that Thymoquinone andthe rest of active constituents are Class 5, that gives impression that we can use it as food or of treatment with some reassurance even if the results are from a prediction program, so that we can then focus on this aspect to use it in the pharmaceutical and nutritional aspects

The findings of this study underscore phytochemical variations in Nigella sativa related to Geographical variations .

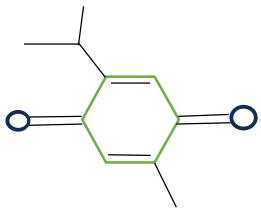
COMPOUND NAME	LD50	CLASS
Thymoquinone	2400 mg/kg	5
P cymene	3 mg/kg	1
α phellandrene	5700 mg/kg	6
d-α-Pinene	5000 mg/kg	5
β-Pinene	4700 mg/kg	5
γ-Terpinene	2500 mg /kg	5
Dihydrocarveol	5000 mg /kg	5
Longifolene	5000 mg /kg	5

TABLE 2

Geographical differences: Between Romania, Bangladesh, Hama (Syria), and Sudan, there are differences in weather, soil, irrigation methods, and temperatures. All of this was translated into the results that were collected and analyzed.

Among the Four types studied, the Romanian variety demonstrated the highest followedby the Hama Syrian andBangladeshi varieties.

1.Thymoquinone : boiling point 230.00 to 232.00 °C , Smile structure : CC1=CC(=O)C(=CC1=O)C(C)C ,
IUPAC: 2-methyl-5-propan-2-ylcyclohexa-2,5-diene-1,4-dione
[19] is most important active compound ,

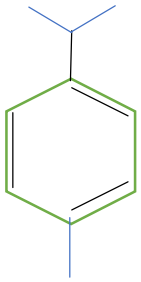


researcher can uses SUPERPERD system ,this prediction system can gives a lot of therapeutic effect of chemical compound by interacting with related Target(**Hypertension** , *probability* 93.24%), (Kabir et al. 2020),(Goyal et al. 2017),(Banerjee et al. 2024),(Erdoğan, Yilmazer, and Erbaş 2020).

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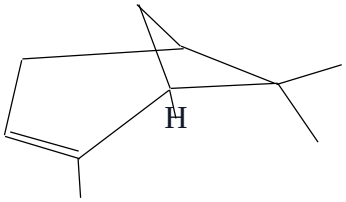
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2. P. Cymene : boiling point 176.00 to 178.00 °C smile structure : CC1=CC=C(C=C1)C(C)C
IUPAC : 1-methyl-4-propan-2-ylbenzene,



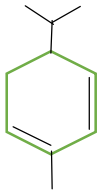
important therapeutic effect by Superperd (Multiple sclerosis 88.71%),(Farhan, Salih, and Salimon 2021) ,(Tiji et al. 2021),(Ravi et al. 2023),(Asimi et al. 2022)

3. d-α-Pinene : boiling point smile structure : CC1=CCC2CC1C2(C)C, IUPAC : 2,6,6-trimethylbicyclo[3.1.1]hept-2-ene,



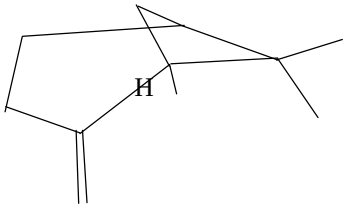
it is has interested therapeutic effect like healing of wound properties (De Vivo, Balivo, and Sarghini 2023) also including neuroprotective effects(Salehi et al. 2019)and by Superperd (Attention deficit hyperactivity disorder 94.94%)

4.α phellandrene : smile structure : boiling point CC1=CCC(C=C1)C(C)C , IUPAC 2-methyl-5-propan-2-ylcyclohexa-1,3-diene,



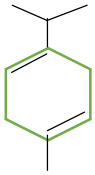
Has many effects and Superperd (Hypertension 85.78%)(De Vivo, Balivo, and Sarghini 2023),(Kim et al. 2025),(Pinheiro-Neto et al. 2021),(Seethalakshmi, Sarumathi, and Sankaranarayanan 2024),(Shaukat et al. 2023)

5.d.β-Pinene: smile structure :boiling point165–167 °CCC1(C2CCC(=C)C1C2)C ,IUPAC: 6,6-dimethyl-2-methylidenebicyclo[3.1.1]heptane ,it has Therapeutic properties , by Superperd (Salehi et al. 2019), (Glioma 84.0%) (Salehi et al. 2019)



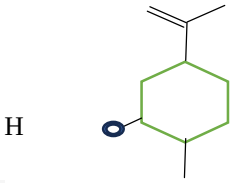
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6.γ-Terpinene:boiling point183 °CSmile structure : CC1=CCC(=CC1)C(C)C, IUPAC : 1-methyl-4-propan-2-ylcyclohexa-1,4-diene



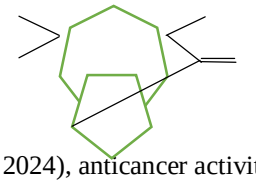
The therapeutic properties by Superperd is (Angina pectoris 85.35%) (Ramalho et al. 2015).

7.Dihydrocarveol:smile structureboiling point 224–225°CCC1CCC(CC1O)C(=C)C ,IUPAC: 2-methyl-5-prop-1-en-2-ylcyclohexan-1-ol



,Therapeutic properties by Superperd is(Acute and chronic heart failure 86.89%) (Salas-Oropeza et al. 2021),(Chetima et al. 2024)

8.Longifolene smile structure : boiling point 254 °C CC1(CCCC2(C3C1C(C2=C)CC3)C)C,IUPAC: 3,3,7-trimethyl-8-methylidenetricyclo[5.4.0.0^{2,9}]undecane



Therapeutic proparties has Antiproliferative action(Li et al. 2024), anticancer activities(Grover et al. 2022),(Radice et al. 2022) and by Superperd is (Glioma 91.11%)

This information is given, although it is limited and requires further analysis of all types of NS planted and geographically distributed, but it provides valuable information about the phytochemical content and adds depth of information to benefit from it economically and medicinally. Therefore, more research is required as future work on the geographical differences of NS, taking into consideration the most effective and accurate analytical method.

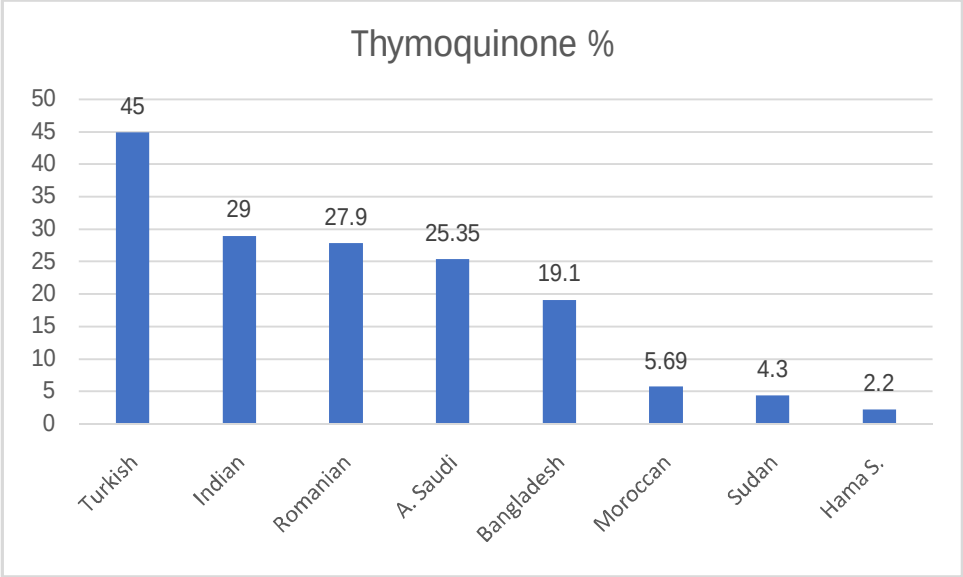
Compound	Turkey[8],[34]	India[9],[11]	Saudi Arabia(Farhan, Salih, and Salimon 2021)	Morocco[35]	Extraction Method	Analysis Method
Thymoquinone	45%	29%-	25.35%	5.69 %	Soxhelt method	HPLC
p-Cymene	29.0%	41.0%	31.50%	37.76%		GC-MS
Phellandrene	0.97%	2.30%			Grinding and overnight mix with solvent	GC-MS
Longifolene	1.54%	0.80%	3.0%	1.50%		GC-MS
αPinene	1.5%	1.20%	2.80%	2.20%		GC-MS-O
βPinene	1.9%	2.92%	1.88%	2.18%	Hydrodistillation	GC-MS-O
Terpinene	0.2%	1.80%	0.88%	0.69%	Hexane extraction,Overnight	GC-MS

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IV. CONCLUSION

This study comparesbetween Four variations of nigella sativa of different geographical origins and use GCMS analysis study to determine the phytochemical variations among *Nigella sativa* varieties from Bangladesh, Hama(Syria), and Romania and Sudan , emphasizing the impact of geographical origin on phytochemical compositions that which may effect on therapeutic effects The Romanian variety exhibited the highest thymoquinone content (27.9%), followed by Hama(Syrian) (16.2%) and Sudani was 4.3% and Bangladeshi was (2.2%) sample,When we compare the results of previous research on the Turkish, Saudi, Indian and Moroccan types, it becomes clear that ***FIG 2*** the Turkish type,(Erdoğan, Yilmazer, and Erbaş 2020),(Salehi et al. 2019) is the highest 45%, followed by the Indian 29%[9],[11], followed by the Romanian27.9%, followed by the arabia Saudi [14]25.35% , followed by the Bangladeshi 19.1% , followed by the Moroccan 5.69 %[35], followed by the Sudan4.3% , followed by the Syrian Hama 2.2% , establishing a clear link between cultivation in different placesand bioactive compound profiles.

The Turkish Nigella sativa variant shows superior thymoquinone content (45%), highlighting its value for pharmaceuticals and food. Caution is advised for Sudanese (45.6% p-cymene) and Indian (41.0% p-cymene) varieties due to toxicity (LD50: 3 mg/kg). Thymoquinone's safety supports therapeutic applications. Future research should expand to other regions and standardize cultivation/extraction protocols to enhance economic and medicinal potential



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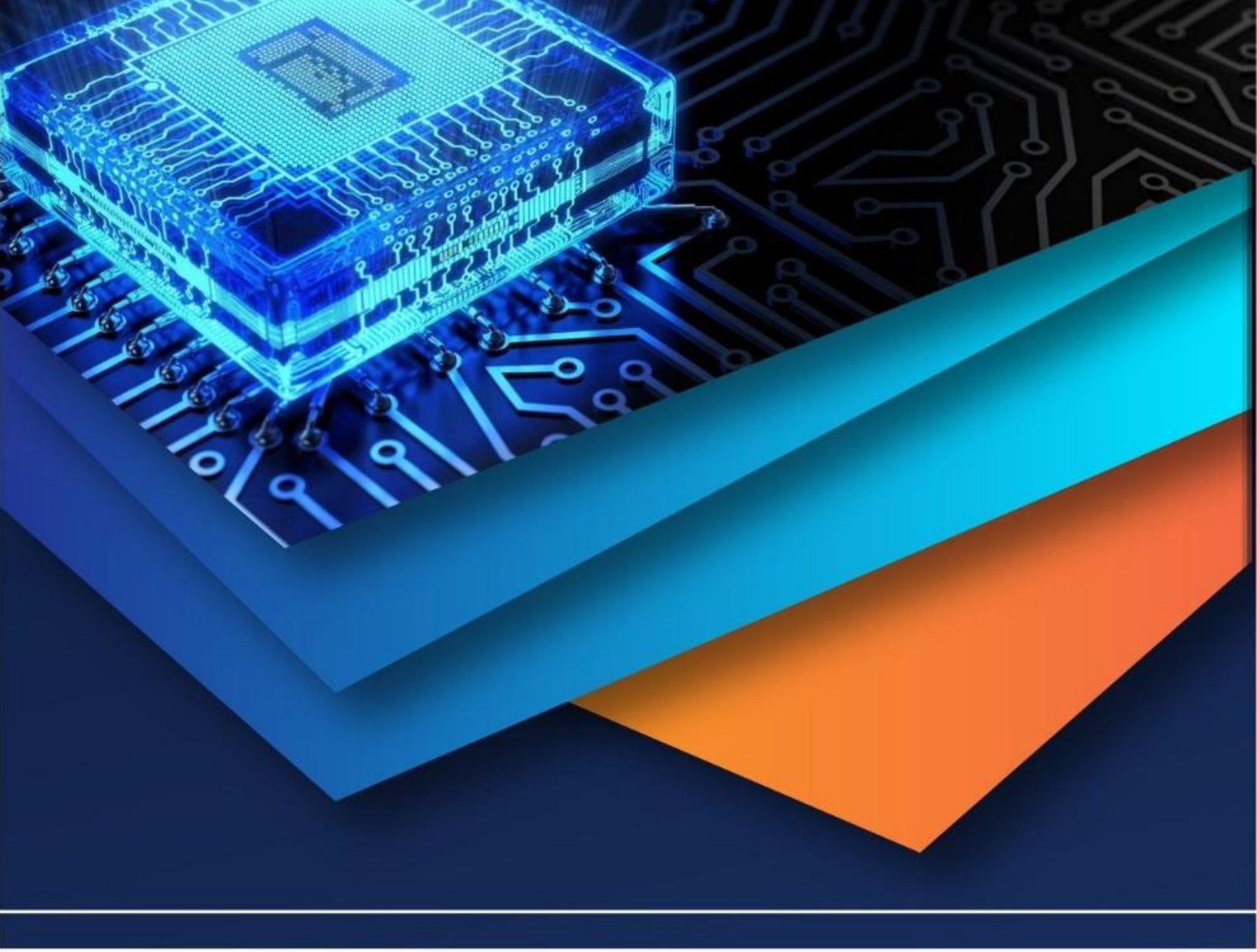
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