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Comparative Extraction Using Steam and Simultaneous Extraction Techniques, Chemical Profiling, and Antibacterial Activity of Essential Oil from *Tagetes erecta* and *Tagetes patula*

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Abstract – The extraction of essential oil can be done in several ways, with some emphasizing quality and others emphasizing quantity. Essential oil components of *Tagetes erecta* and *Tagetes patula* were analyzed and compared using two common extraction techniques, i.e., steam distillation (SD) and simultaneous distillation extraction (SDE), coupled with GC-MS for their chemical composition and antimicrobial activity. In both species, the highest % yield was obtained by the SD with yields of 0.05% and 0.12%, respectively. Thus, the comparative study of extraction methods unveiled SD as the best method for the extraction of essential oil, providing a high yield with a greater percentage of major compounds. The GC-MS analysis unveiled 24 different compounds in *T. erecta* and 27 compounds in *T. patula*. SD of *T. erecta* and *T. patula* revealed a higher concentration of β-Caryophyllene with compositions of 37.25% and 65.47%. A comparative analysis of the antibacterial activity of essential oil extracted using SD revealed that *T. erecta* is effective against all four tested bacterial strains, i.e., *Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae and Salmonella Typhi*. Notably, the largest zone of inhibition (ZOI) was observed against *K. pneumoniae* (12 mm) for *T. erecta*. In contrast, *T. patula* demonstrated activity against two bacterial strains, i.e., *K. pneumoniae* and *S. typhi,* with a maximum ZOI of 11 mm, observed against *K. pneumoniae*. The stronger antibacterial activity of *T. erecta* oil might be due to the synergistic interaction of the most abundant bioactive compounds obtained through SD.
Keywords – *Tagetes*, Marigold, Steam distillation, Simultaneous distillation extraction, GC-MS, Antimicrobial activity

Introduction

Tagetes is an important genus of the Asteraceae family, also referred to as Marigold and is originally found in Mexico and Guatemala, although it is currently grown all over the globe due to its decorative use.[1] Out of 55 species of *Tagetes*, *T. patula* L. (French marigold) and *T. erecta* L. (African marigold) are commonly available species in Nepal.[2] In Nepal, *Tagetes* flowers are mostly cultivated for decorative and religious duties. However, the use of *Tagetes* extends beyond ornamentation; it holds significant importance in folk medicine for curing various conditions, including wounds, fever, scabies, digestive and nervous disorders, dental problems, skin infections, hepatic abnormalities, and ulcers.[3,4] *Tagetes* is an important source of lutein ester, a nutrient vital for eye health as well as guarding against age related muscular degeneration.[5,6] The essential oil of *T. erecta* contains different bioactive chemicals, like β-caryophyllene, piperitone, and piperitenone.[7] At the same time, the major compounds found in *T. patula* are piperitone, terpinolene, and β-caryophyllene, which exhibit anti-inflammatory, anticancer, insecticidal, antioxidant, and antibacterial properties.[4,8,9] Essential oil of *T. erecta* and *T. patula* benefit is not only limited to the therapeutic agent but also have great significance in the cosmetic and perfume industry as a flavoring agent.[10] Major chemical constituents in *Tagetes* include tagetin, polyphenols, quercetin, quercetagetin, tannins, steroids, flavonoids, and saponins.[10,11] β-caryophyllene is widely known to reduce acute and chronic pain through endo-

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cannabinoid and opioid systems and shows neuro-protective, cardioprotective, cytotoxic, and hypolipidemic activities as well.[12]

Despite their wide application, scope, and use, the quality and quantity of essential oils extracted from these species are low enough to compete in a global commercialized market. To make its essential oil competitive in the global market, it is crucial to increase the chemical content by choosing the right extraction process. Hence elaborate study is necessary as extraction techniques, geographic area, cultivation process, storage, plant species, and other factors can have an impact on the yield and composition of the essential oil. The choice of an effective extraction technique can significantly improve the quality and composition of the essential oil.[13] Various extraction techniques are utilized to extract essential oil. However, the most widely used techniques are steam distillation (SD) and simultaneous distillation extraction (SDE). Both SD and SDE are the common and popular methods for isolating volatile compounds from plant materials.[14,15]

In the past, SD was widely used for extraction due to its environmentally friendly and reduced operational cost, as it used water as a solvent. However, water substantially diluted the volatile component, which was conquered by the SDE, where an organic solvent is used.[13,16–21] Each method has its advantages as well as disadvantages; therefore, the obtained oil was analyzed using the GC-MS technique to enhance the quality and quantity of essential oil. A systematic comparison of steam distillation (SD) and simultaneous distillation extraction (SDE) for *Tagetes* species remains unexplored, particularly regarding the resulting chemical profiles and their corresponding biological activities. Such an investigation is critical for establishing an optimized, standardized extraction protocol that maximizes both yield and the concentration of key bioactive constituents, thereby enhancing the commercial viability and therapeutic relevance of *Tagetes* essential oils.

This study provides a systematic evaluation of steam distillation and simultaneous distillation extraction to determine their efficiency in optimizing both the yield and the bioactive quality of *Tagetes* essential oils. While SD proved more effective for overall yield, SDE with specific solvents enabled targeted recovery of individual compounds. The novelty of this work lies in its integrated approach directly linking extraction outcomes to antibacterial efficacy, thereby establishing an evidence-based framework for selecting methods based on application-specific goals, such as maximizing yield, enriching particular constituents, or enhancing bioactivity.

Experimental

Chemical – Hexane, dichloromethane (DCM), diethyl ether, ultra-pure water from the Milli-Q water purification system, and acetone. The solvents were selected based on three criteria: common application in volatile compound isolation, a range of polarities to compare extraction efficiency, and immiscibility with water. All the chemicals used were of analytical grade.

Plant collection and sample preparation – The plant specimens (Flowers) of *T. erecta* L. and *T. patula* L. were collected from Tansen, Palpa, located at an altitude of 1350 m above sea level (msl) (27° 52’ 2.32” N latitude and 83° 32’ 48.12” E longitude). The collected samples were identified by the botanist at the Central Department of Chemistry, Kritipur, Nepal. The accession codes for these specimens are Palpa 101 and Palpa 201, respectively. The samples were shade-dried for two weeks to achieve a moisture content of less than 10%, ground into a powder, and kept in an airtight container for further use.

Extraction of essential oil – The essential oil from two species of *Tagetes* was extracted using two distinct techniques, SD and SDE, following the reported protocol.[19,22] In the process of extracting hexane, diethyl ether, and DCM were used as solvents.

Steam distillation – 200 g of *Tagetes* flower (*T. erecta* and *T. patula*) were kept in a perforated flask positioned above a round-bottom flask containing 2.5 litres of milli-Q water, and the condenser was connected to the other open end. The apparatus was heated to boil the water, initially reaching the boiling temperature at 100°C, and maintained for 4 hours. The steam formed travels through the sample into the condenser, where it condenses along with the essential oil. The resulting oil and water mixture was collected in a specially designed separating vessel. The two layers were then separated based on their density. Thus, collected oil was transferred into vials containing sodium sulfate until further use for GC-MS analysis.

Simultaneous distillation extraction – For SDE, a modified Likens-Nickerson apparatus was employed. A 5 L round-bottom flask containing 200 g of plant material and 2.5 L of Milli-Q water was connected to the left arm. The right arm was connected to a 500 mL round-bottom flask containing 200 mL of organic solvent (solvent-to-sample ratio of 1:1 v/w). The chilled circulating coolant (isopropanol) was maintained at −5°C. Both flasks were heated independently to their boiling points using heating mantles. The vapours from both flasks met in the central condensation chamber, where they condensed and formed two immiscible layers. The design of the apparatus

allowed continuous recirculation of both phases; the aqueous phase returned to the plant flask, and the organic phase returned to the solvent flask, ensuring continuous extraction over the 4 hours. To minimize solvent loss, all joints were securely sealed with PTFE sleeves, the condenser efficiency was verified, and the system was maintained under slight positive pressure. After extraction, the solvent phase was collected, dried over anhydrous sodium sulfate, and concentrated under reduced pressure (40°C) using a rotary evaporator.

$$\% \text{ Yield} = \frac{\text{Dry weight of essential oil}}{\text{Dry weight of the sample}} \times 100\%$$

GC-MS analysis – The composition of the concentrated essential oils of *Tagetes* species was analyzed using gas chromatography-mass spectroscopy. An Agilent 7890A GC system (Agilent, USA) equipped with an Agilent HP-5 (5% phenylmethylsiloxane) MS fused silica capillary column (30 m × 250 μm × 0.25 μm) was used to chromatograph the concentrated oil, and an Agilent MS 5975C was used to continue the qualitative examination of the essential oil. The spectrometer was operated in electron impact mode at 70 eV utilizing helium as a carrier gas with a flow rate made constant at 1.0 mL/min, split ratio of 75:1, and head pressure of 6.777 psi.

1 μL of the extracted essential oil was diluted in hexane at a ratio of 1:10 v/v for GC analysis. The column temperature was initially set at 35°C for 5 minutes and then ramped at 5°C/min to 100°C, 7°C/min to 150°C, and 10°C/min to 205°C for a total time of 33.64 min. The sample was fed into a GC inlet, maintaining a column flow rate of 1 mL/min and purge flow of 3 mL/min. The ion source temperature was maintained at 220°C and 235°C as the contact temperature. Mass spectra were scanned at a speed of 666, from *m/z* 25–500. The retention indices were calculated by using a standard hydrocarbons mixture (C5-C32) from LGC group Dr Ehrenstorfer, United States. Lastly, by comparing retention indices and mass spectral fragmentation patterns (with over 90% similarity index). The constituent present in the essential oil was identified by cross-referencing MS with the NIST library database.

Preparation of microbial culture media – 13 g of LB powder (Sisco Research Laboratories Pvt. Ltd., India) were dissolved in one liter of distilled water to create the liquid broth (LB) medium. The mixture was autoclaved for 25 minutes at 121°C and 15 psi pressure. After cooling to 40–50°C, the sterilized medium was transferred into 15 mL falcon tubes that had been previously sterilized (5 mL each). Bacterial seed was co-cultured in each tube independently using the prepared medium, and the tubes were incubated for a full day.

Preparation of MH media plates – One liter of distilled water was used to dissolve 39 g of Mueller-Hinton (MH) agar powder (Sisco Research Laboratories Pvt. Ltd., India) to create the MH agar plates. The mixture was autoclaved for 25 minutes at 121°C and 15 psi pressure. After cooling to 40–50°C, the sterilized medium was transferred onto 25 mL sterile petri dishes.

Antibacterial assay – The antibacterial activity of the essential oils was evaluated using the agar well diffusion method. Briefly, 100 μL of a freshly prepared bacterial suspension was spread evenly onto the surface of Mueller-Hinton agar plates. Wells (6 mm in diameter) were punched aseptically into the agar. Subsequently, 100 μL of each essential oil sample, dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL, was introduced into the designated wells. Appropriate controls were included: DMSO and deionized water (DW) served as the negative control, and kanamycin (5 μg/well) served as the positive antibiotic control. All assays were performed in triplicate (n = 3). The plates were incubated at 37°C for 24 h, after which the zones of inhibition (ZOI) were measured in millimetres.

Results and Discussion

Variations in the percentage yield of essential oil were obtained from SD and SDE for two species of *Tagetes*, as shown in Table 1. For both species, SD offers higher

Table 1. Percentage of yield obtained from two *Tagetes* species using SD and SDE

S. No	Plant materials (Scientific Name)	Dry sample weight (gram)	Percentage Yield (%)			
			Steam Distillation (SD)	Simultaneous Distillation Extraction (SDE)		
				Hexane	Diethyl ether	DCM
1	*Tagetes erecta* (Flowers)	200	0.05%	0.04%	0.03%	0.05%
2	*Tagetes patula* (Flowers)	200	0.12%	0.06%	0.11%	0.03%

yields, with 0.05% for *T. erecta* and 0.12% for *T. patula*. However, in SDE, dichloromethane offers a higher yield compared to the other two solvents. Similarly for *T. patula*. Diethyl ether offers a higher yield of essential oil.

The yellow-colored oil with different % yields was obtained from *T. erecta*. The GC-MS technique was used for the identification of chemical constituents by comparing the MS using the NIST library. A total of 24 compounds were identified in four different essential oils (Table 2). However, the percentage composition varied with the technique and solvent used. In SD, β-caryophyllene (37.25%) and germacrene D (19.17%) were identified as major compounds, whereas in SDE, in all three solvents, β-caryophyllene (20.94% average) stood out as a major compound. Out of the three solvents used in SDE, DCM and diethyl ether showed a higher yield of β-caryophyllene (21.96%). Also, the higher yield of β-linalool (23.75%) and piperitone (6.09%) was obtained using DCM as a solvent. Similarly, α-terpineol (7.46%), caryophyllene oxide (9.02%), and Z-geraniol (6.59%) were in higher amounts when diethyl ether was used as a solvent, as shown in Fig. S1.

GC-MS showed that the yellow-colored essential oil of *T. patula* obtained from two extraction techniques consists of 27 compounds (Table 3). The present study showed that more major compounds can be extracted using the SD. Compounds like β-caryophyllene (65.47%), β-cubebene (9.35%), caryophyllene oxide (6.86%), and (Z)-β-farnesene (4.09%) were obtained at higher yields when SD was used for oil extraction. The identified compounds were cross-checked with the standard retention indices of the NIST Library database Fig. S1.

Table 2. Chemical profiling of *T. erecta* flower essential oil obtained from steam distillation and simultaneous distillation extraction using GC-MS

S. No	Compound Name	Retention time (min)	Calculated retention indices	Theoretical retention indices	SD (%)	SDE		
						Hexane (%)	Diethyl ether (%)	DCM (%)
1	β-Linalool	16.541	1104	1104	7.04	18.61	16.11	23.75
2	Phenyl ethyl alcohol	16.960	1118	1118	-	-	1.04	-
3	Chrysanthenone	17.249	1127	1128	-	2.82	-	-
4	Borneol	18.542	1168	1168	-	0.80	1.18	-
6	3-Methyl undecane	18.689	1172	1171	-	2.42	-	-
7	4-Terpineol	18.940	1180	1180	-	1.00	-	0.88
8	*p*-Cymen-8-ol	19.258	1189	1189	0.55	1.06	1.35	1.18
9	α-Terpineol	19.411	1193	1192	2.42	4.43	7.46	6.57
10	Eucarvone	20.284	1224	1143	1.42	0.83	-	0.53
11	Piperitone	21.326	1261	1263	3.91	2.21	2.84	6.09
12	*Z*-Geraniol	21.380	1263	1245	2.22	2.78	6.59	4.33
13	2-Methoxy-4-vinyl phenol	22.906	1314	1315	-	0.53	0.78	-
14	Piperitenone	23.540	1348	1349	0.82	-	-	-
15	β-Caryophyllene	25.292	1427	1437	37.25	18.90	21.96	21.96
16	α-Caryophyllene	25.946	1459	1460	1.84	-	-	-
17	(*Z*)-β-Farnesene	25.984	1461	1457	1.64	0.90	1.61	0.76
18	β-Cubebene	26.512	1487		-	-	4.04	-
19	Germacrene D	26.535	1488	1485	19.17	1.69	4.04	1.69
20	α-Farnesene	27.054	1518	1511	0.71	-	-	-
21	*δ*-Cadinene	27.425	1542	1541	0.86	0.71	-	-
22	Spathulenol	28.548	1566	1566	2.65	2.02	4.77	2.70
23	Caryophyllene oxide	28.641	1620	1613	2.58	3.33	9.02	3.05
24	α-Cadinol	29.841	1666	1663	0.54	0.22	0.75	-
	Percentage (%)				85.62	65.26	84.72	73.49

Table 3. Chemical profiling of *T. patula* flower essential oil obtained from steam distillation and simultaneous distillation extraction using GC-MS

S. No	Compound name	Retention time (min)	Calculated retention indices	Theoretical retention indices	SD (%)	SDE		
						Hexane (%)	Diethyl ether (%)	DCM (%)
1	(*E*)-β-Ocimene	14.461	1044	1045	-	0.52	-	0.79
2	β-Linalool	16.545	1104	1104	-	0.95	0.68	-
3	Chrysanthenone	17.238	1127	1125	-	2.25	1.38	0.81
4	3-Methyl undecane	18.684	1172	1171	-	2.12	-	-
5	4-Terpineol	18.929	1180	1178	-	0.55	-	-
6	Piperitone	21.329	1261	1262	-	0.55		
7	*n*-Decanoic acid	23.303	1337	1368	-	0.75	0.72	-
8	5-Isopropenyl-2-methylcyclopent-1-enecarboxaldehyde	23.550	1346	NR	-	0.82	0.56	0.83
9	Eugenol	23.926	1365	1363	-	1.20	0.56	-
10	β-Caryophyllene	25.285	1427	1423	65.47	48.89	43.70	62.13
11	(*Z*)-β-Farnesene	25.983	1461	1457	4.09	3.30	3.07	3.68
12	γ-Muurolene	26.414	1482	1481	-	1.14	-	-
13	β-Cubebene	26.512	1487	1488	9.35	2.87	5.55	9.14
14	Bicyclogermacene	26.839	1504	1505	2.10	-	1.21	2.06
15	β-Bisabolene	27.063	1514	1511	2.17	1.31	1.21	1.84
16	2,4-Bis(1,1-dimethyl ethyl)phenol	27.221	1522	1521	-	-	-	0.88
17	β-Cadinene	27.428	1533	1518	-	1.73	0.70	-
18	Spathulenol	28.552	1588	1581	2.34	4.39	4.00	2.89
19	Caryophyllene oxide	28.650	1620	1611	6.86	6.57	6.92	5.63
20	1-Hexadecene	28.711	1596	1593	-	-	-	0.89
21	4,4-Dimethyl tetracyclo[6.3.2.0(2,5).0(1,8)] tridecan-9-ol	29.567	1649	NR	0.66	1.18	0.91	0.81
22	Juniper camphor	29.862	1668	1682	0.96	-	-	-
23	Tetradecanoic acid	31.324	1771	1771	-	0.68	0.65	-
24	1-Octadecene	31.673	1797	1795	-	-	-	1.41
25	Hexahydrofarnesyl acetone	32.327	1856	1848	0.67	0.62	0.97	-
26	Linoleic acid	33.135	-	-	-	1.38	-	-
27	Thianthrene	33.277	-	-	-	2.57	0.96	0.91
	Percentage (%)				94.67	86.34	73.75	94.7

The essential oil obtained from steam distillation was evaluated for its antibacterial activity against two gram-positive bacteria, *S. aureus* and *E. faecalis* and two gram-negative bacteria, *K. pneumoniae*, and *S. typhi*, as shown in Fig. 1. PCC represents the *T. erecta*, and BC represents the *T. patula*. The antibacterial efficacy, measured by the zone of inhibition, is summarized in Table 4. *T. erecta* exhibited the 11.0 mm and 0.0 mm ZOI against *S. aureus* and *E. faecalis*, whereas *T. patula* demonstrated 10.0 mm and 0.0 mm, respectively. Against gram-negative bacteria, *T. erecta* exhibits a zone of inhibition of 12.0 mm and 11.0 mm for *K. pneumoniae* and *S. typhi*, respectively. In comparison, *T. patula* exhibits a zone of inhibition of 9.0 mm and 10.0 mm for the same bacteria.

The present study demonstrates that the chemical composition and yield of essential oils from *T. erecta* and

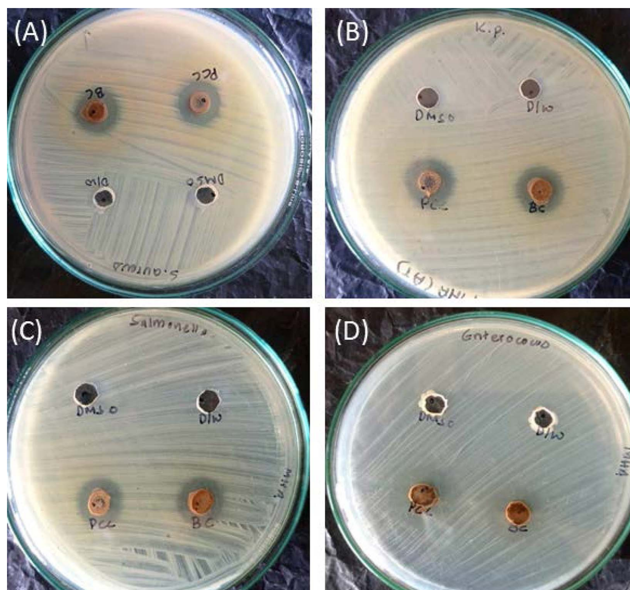

Fig. 1. Antimicrobial activity of the *T. erecta* and *T. patula* against (A) *S. aureus*, (B) *K. pneumoniae*, (C) *S. typhi*, and (D) *E. faecalis*.

T. patula are significantly influenced by the extraction technique, with SD proving superior for overall yield and recovery of key terpenoids such as β-caryophyllene. This aligns with earlier findings that SD better preserves thermally stable, non-polar volatiles compared to solvent-based methods, which may selectively extract or alter certain compounds due to solvent polarity and boiling point effects.[13,14] In our study, SD of *T. erecta* and *T. patula* yielded 0.05% and 0.12% oil, respectively, which is consistent with yields reported for other *Tagetes* species using hydro distillation.[8,23] However, the notably high β-caryophyllene content in SD extracts (37.25% in *T. erecta*, 65.47% in *T. patula*) exceeds levels commonly reported in literature for these species,[7,24] possibly due to genotypic variation, environmental factors, or the avoidance of solvent-induced compound loss.

From *T. erecta* and *T. patula*, a total of 24 and 27 chemical constituents were discovered. *T. erecta* contained significant amounts of β-caryophyllene, β-linalool, and germacrene D, while *T. patula* included significant amounts of β-caryophyllene, β-cubebene, and caryophyllene oxide. Diethyl ether can be used as a solvent in SDE to extract large amounts of caryophyllene oxide for both species. DCM solvent was thought to be the most effective for extracting piperitone from *T. erecta*. However, it is less prevalent in *T. patula*, and hexane was considered the most effective solvent for its extraction. It is interesting to note that, although using solvent-based extraction might be preferential to extracting some oxygenated monoterpenes, it has been indicated in a study[25] that using hydrodistillation, high yields of piperitone and piperitenone were obtained using *T. erecta*. This ostensible inconsistency can be explained by the fact that plant chemotype, growing environment, or minor differences in distillation parameters may also cause it, and it shows how the results of extractions can be affected by both methodological and biological factors.

While the essential oils of *T. erecta* and *T. patula* share key compounds such as β-caryophyllene, their profiles exhibit significant interspecific variation. *T. erecta* oil was distinguished by the presence of germacrene D and higher yields of β-linalool, whereas *T. patula* oil contained greater proportions of caryophyllene oxide and (*Z*)-β-farnesene. Notably, piperitenone, a compound reported in prior studies of *T. patula*, was absent in our extracts, underscoring the impact of chemotypic variation. While the major compounds identified here align with previous literature, their relative abundances differ considerably, a divergence likely attributable to a combination of genetic factors, environmental conditions, and extraction methodology. The prevalence of β-caryophyllene in both oils is of particular relevance, given its well-documented bioactivity, including antimicrobial, anti-inflammatory, and neuroprotective properties mediated through pathways such as CB2 receptor interaction.[26,27]

The higher antibacterial activity of *T. erecta* oil, especially against *K. pneumoniae*, can be correlated with its unique chemical profile. The SD oils of both species were rich in β-caryophyllene, while *T. erecta* oil included a wider range of significant components such as piperitone and germacrene D, which were absent or

Table 4. Antibacterial activity of *T. erecta* and *T. patula*

Strain	Reference culture	Type	Positive control (c+) (cm)	*T. erecta* (PCC) (mm)	*T. patula* (BC) (mm)
Enterococcus faecalis	ATCC 29212	Gram +ve	2.5	11.0	0.0
Staphylococcus aureus	ATCC 6538P	Gram +ve	2.5	10.0	0.0
Klebsiella pneumoniae	ATCC 700603	Gram -ve	2.4	12.0	11.0
Salmonella typhi	ATCC 14028	Gram -ve	2.6	10.0	9.0

minimal in *T. patula*. β-Caryophyllene is a well-known antimicrobial sesquiterpene capable of disrupting bacterial membranes and potentiating the activity of other agents. Its high concentration provides a baseline of activity in both oils. However, the broader-spectrum efficacy of *T. erecta* oil suggests a possible synergistic interaction.[28–29] For example, the combination of β-caryophyllene with germacrene D and oxygenated monoterpenes like piperitone may create a multi-target effect, compromising bacterial cell integrity, efflux pumps, and enzymatic functions more effectively than β-caryophyllene alone.[30] This aligns with previous studies on *Tagetes* oils, where antimicrobial potency was attributed to the combined activity of the terpenoid ensemble rather than a single compound.[29,31] The absence of piperitenone in our samples further emphasizes how chemotypic diversity and extraction technique influence not only composition but also biological activity, which contrasts with several reports highlighting piperitenone as a key antibacterial agent in *T. patula*.[12] Consequently, the enhanced activity of *T. erecta* oil is likely the result of a specific, synergistic compositional matrix optimally produced by steam distillation, rather than the concentration of a single component.

It is important to note that this study employed an agar well diffusion assay, which provides an initial indication of antibacterial potential through zone of inhibition (ZOI) measurements. While the ZOI data clearly differentiate the activity profiles of the two oils, the quantitative biological relevance cannot be fully ascertained without determining Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values. Future work should include broth dilution assays to establish MIC/MBC and further explore the mechanisms of synergy among the bioactive constituents.

In conclusion, this comparative study demonstrates that while steam distillation (SD) is optimal for maximizing overall yield and producing essential oils with broad-spectrum antibacterial activity, simultaneous distillation extraction (SDE) provides superior selectivity for enriching specific target compounds. Therefore, the choice of extraction method depends on the intended application; SD is recommended for maximizing total oil recovery and bioactivity, whereas SDE is better suited for targeted compound isolation. These findings offer practical guidance for selecting extraction protocols based on desired outcomes. Future studies incorporating statistical validation, MIC/MBC determinations, and expanded bioactivity assessments will further clarify the therapeutic potential of *Tagetes* essential oils.

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Conflicts of Interest

The authors declare no conflict of interest.

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