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EVALUATION OF ANTIMICROBIAL POTENTIAL OF WILD MARIGOLD (*TAGETES MINUTA*) ESSENTIAL OIL ON *SALMONELLA* AND *E. COLI*

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ABSTRACT

The increasing prevalence of antibiotic-resistant foodborne pathogens, such as *Salmonella* and *E. coli*, necessitates the exploration of natural alternatives. *Tagetes minuta*, a medicinal plant, has been recognized for its antimicrobial properties. This study aimed to evaluate the antimicrobial activity of *T. minuta* essential oil against *Salmonella* and *E. coli*. The essential oil was extracted from flowers and analyzed using GC-MS. The major compounds identified included Aromadendrene 2, Beta Atlantone, cis-Caryophyllene, cis-Ocimene, cis-Ocimenone, Delta.3-Carene, Dihydrotagetone, D-Limonene, and Tagetone. The essential oil was tested using the agar well diffusion method. However, no significant inhibitory activity was observed against *Salmonella* and *E. coli*. These findings suggest that the specific composition of the essential oil, particularly the lower content of cis-Ocimene, may influence its antimicrobial activity. Further research is needed to explore the factors affecting the antimicrobial properties of *T. minuta* essential oil and to identify optimal extraction methods to maximize its potential as a natural antimicrobial agent.

Keywords: Antimicrobial activity, E. coli, Marigold, Salmonella, Tagetes minuta

Introduction

Salmonella and *E. coli* are becoming a challenge in food industry during the last few years due to the wide-spread distribution. Extensive utilisation of antibiotics in food industry against foodborne pathogens or food models has resulted in additional antibiotic resistance which has grown into an imminent threat for public health. There has been a growing global interest about therapeutic values of natural products. Nature has presented to humanity the gift of vast therapeutic antimicrobial agents of plant origins.

Tagetes sp. commonly known as marigold belongs to family *Asteraceae* consists at least of 56 species (Soule, 1994). It is a plant that is native to America, but it has naturalised in other nations in Asia, Africa, and Europe. (Babu & Kaul, 2007) (Politi *et al.*, 2018). *T. minuta* is a widely spread plant in Uganda that grows primarily during the rainy season. It is utilised for a variety of purposes around the country. T. *minuta* is an aromatic essential plant with a wide range of biological functions (Batish *et al*., 2007). Marigold extracts contain a variety of chemicals with distinct characteristics, including phenylpropanoids, carotenoids, flavonoids, thiophenes, and others. (Marotte *et al*., 2004). Previous research has shown that *T. minuta* essential oil (EO) inhibits *Aspergillus niger* and *Candida albicans*, as well as Gram-positive bacteria, revealing that *T. minuta* exhibits cytotoxic (Shirazi *et al*., 2014) and anti-inflammatory properties. The main aim of this study is to assess antimicrobial potential of wild marigold (*tagetes minuta*) essential oil on *Salmonella* and *E. coli.*

Materials and Method

This study was conducted in the Department of Industrial Microbiology, Jacob Institute of Biotechnology and Bioengineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj. The oils extracted from the flower of *Tagetes minuta* were procured from the district Kishtwar, Jammu and Kashmir. Pure cultures of bacteria were procured from the Microbial Culture Collection Bank (MCCB) of the Department of Industrial Microbiology, JIBB, SHUATS, Prayagraj**.** The GC-MS analysis was out sourced from CSIR - Central Institute of Medicinal and Aromatic Plants (CIMAP) Lucknow, Uttar Pradesh.

Agar well diffusion assay of essential oil, Muller Hinton agar plates were used for testing antimicrobial potential. It was determined by the zone of inhibition formed using the well diffusion method. The inoculum was swabbed uniformly over the agar medium and wells were cut using a sterile cork borer of 6 mm diameter, well of 50 µL volume of essential oil was poured in concentration of 25%, 50% and 100% of essential oil. After these plates were allowed to remain for 1 hour at room temperature, to facilitate diffusion of the oils across the surface and then were incubated for 24 hours at 37°C. The zone of inhibition around each well was calculated in millimetres and the assay was carried out three times for oil (NCCLS, 2000). Ciprofloxacin, an antibiotic was used as positive control.

Results and Discussion

Nine chemicals that had various retention time frames and area percentages were detected in the *Tagetes minuta* oil's GC-MS chromatogram, which showed nine peaks in Table 2. The major compounds present in *Tagetes minuta* oil were Aromadendrene 2 (1,1,7-trimethyl-4-methylidene-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulene) (2.07%), Beta Atlantone (6-methyl-2-[(1R)-4-methylcyclohex-3-en-1-yl] hepta-1,5-dien-4-one) (4.79%), cis-Caryophyllene ((1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene) (4.92%), cis-Ocimene (cis-β: (3Z)-3,7-Dimethylocta-1,3,6-triene) (5.28%), cis-Ocimenone ((Z)-2,6-Dimethylocta-2,5,7-trien-4-one) (7.75%), Delta.3-Carene (3,7,7-Trimethylbicyclo [4.1.0] hept-3-ene) (9.89%), Dihydrotagetone (2,6-dimethyloct-7-en-4-one) (15.06%), D-Limonene (1-Methyl-4-(prop-1-en-2-yl)cyclohex-1-ene) (17.13%), Tagetone (5,7-Octadien-4-one, 2,6-dimethyl-, (Z)-) (33.12%) respectively with different properties as mentioned in Table 2.

According to Sharifi-Rad *et al.* (2017), essential oils may have antibacterial properties. A 50% antimicrobial content (v/v) was found in the oil from flower of *Tagetes minuta.* However, during a 24-hour incubation period, the strains' antimicrobial activity against *Salmonella* and *E. coli* showed no inhibitory zone which clearly shows that oil extracted only from the flower of *Tagetes minuta* may not work as antimicrobial agent against *Salmonella* and *E. coli* Table 1. According to Previous studies done by Shirazi *et al.* (2014) aerial part of tagetes minuta is showing antimicrobial activity against *salmonella* and *E. coli* due the less amount of cis-Ocimene (cis-β: (3Z)-3,7-Dimethylocta-1,3,6-triene) (5.28%) found in oil in contrast to previous research by (Héthélyi *et al.,* 1986) factors which are responsible for variation chemical composition are growing method, environmental factor, development stage of plant and pollination method. which can change the quality of the oil (Kumar *et al.,* 2020) therefore more research is needed in future to identify the responsible factor which inhibits *Salmonella* and *E. coli* whereas, positive control Ciprofloxacin antibiotic show 23±0.8 mm clear zone. Ciprofloxacin has been the treatment of choice for enteric fever for over a decade (Capoor *et al.,* 2007).

Conclusion

The experiment conducted to assess the antimicrobial activity of *Tagetes minuta* against *Salmonella* and *E. coli* showed no significant result may be due to the lack of chemical compound which is responsible for the inhibition of the growth of *Salmonella and E. coli.* Further research is needed to identify the specific compounds and mechanisms involved in the antimicrobial activity of *Tagetes minuta* essential oil.

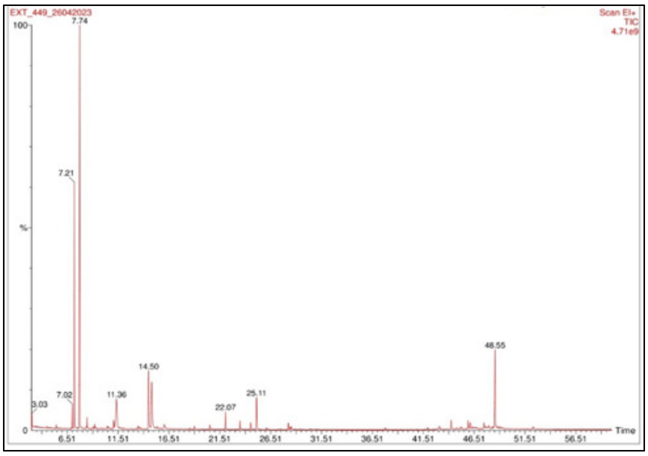

Fig. 1 : GC-MS graph of *Tagetes minuta*

Fig. 2 : Screening of *Tagetes minuta* against A (*E. coli*) and B (*Salmonella*)

Table 1 : Screening of essential oil against bacteria

Cultures	TM	Ciprofloxacin
E. coli	2.3 ± 0.3	23 ± 0.8
Salmonella	1 ± 0.5	22 ± 0.5

Values are represented as mean ± standard error of three determination (n=3). Values followed by different superscripts are significantly different ($p \leq 0.05$) within rows and within columns.

Table 2 : GC-MS analysis of volatile compounds in *Tagetes minuta*

Peaks	Retention time	Peak area	% Compound	Compound name
3.03	967	146.59	2.07	DELTA.3-Carene (3,7,7-Trimethylbicyclo [4.1.0] hept-3-ene)
7.02	986	146.59	4.79	D-Limonene (1-Methyl-4-(prop-1-en-2-yl) cyclohex-1-ene)
7.21	981	146.59	4.92	cis-Ocimene (cis-β: (3Z)-3,7-Dimethylocta-1,3,6-triene)
7.74	982	146.59	5.28	dihydrotagetone (2,6-dimethyloct-7-en-4-one)
11.36	998	146.59	7.75	Tagetone (5,7-Octadien-4-one, 2,6-dimethyl-, (Z)-)
14.5	941	146.59	9.89	cis-Ocimenone ((Z)-2,6-Dimethylocta-2,5,7-trien-4-one)
22.07	987	146.59	15.06	CIS-CARYOPHYLLENE ((1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo [7.2. 0]undec-4-ene)
25.11	982	146.59	17.13	aromadendrene 2 (1,1,7-trimethyl-4-methylidene-2,3,4a,5,6,7,7a,7b-octahydro -1aH-cyclopropa[e]azulene)
48.55	900	146.59	33.12	beta Atlantone (6-methyl-2-[(1R)-4-methylcyclohex-3-en-1-yl] hepta-1,5-dien-4-one)

Table 3 : Proprieties of volatile compounds found in *Tagetes minuta*

Compound name	Properties	Ref.
DELTA.3-Carene (3,7,7-Trimethylbicyclo [4.1.0] hept-3-ene)	anti-inflammatory properties	(Russo, 2011)
D-Limonene (1-Methyl-4-(prop-1-en-2-yl) cyclohex-1-ene)	Anti-inflammatory, Antioxidant, Anti-cancerous, Anti-nociceptive, Gastroprotective, Immunomodulatory, Anti-fibrotic, Anti-genotoxic	(Anandakumar *et al.,* 2020)
cis-Ocimene (cis-β: (3Z)-3,7-Dimethylocta-1,3,6-triene)	Fragrance, Decongestant, Antibacterial, Antiseptic, Interlayer dielectric, Encapsulating layer, Food preservative.	(Bazaka *et al.,* 2016)
dihydrotagetone (2,6-dimethyloct-7-en-4-one)	Anti–oxidative and anti–inflammatory	(Karimian *et al.,* 2014)
Tagetone (5,7-Octadien-4-one, 2,6-dimethyl-, (Z)-)	antifungal and antimicrobial	(Romagnoli *et al.,* 2005)
cis-Ocimenone ((Z)-2,6-Dimethylocta-2,5,7-trien-4-one)	antiseptic and antifungal	(Joshi and Sharma, 2014)
CIS-CARYOPHYLLENE ((1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2. 0]undec-4-ene)	Pain relief, Anti-anxiety, Mood enhancement, Immune system support, Inflammation reduction, Relaxation, Anti-inflammatory, Antimicrobial, Antibacterial, Antioxidant.	(Foreverest Resources Ltd, 2023)
aromadendrene 2 (1,1,7-trimethyl-4-methylidene-2,3,4a,5,6,7,7a,7b-octahydro-1aH-cyclopropa[e]azulene)	antibacterial	(Salgueiro *et al.,* 1997)
beta Atlantone (6-methyl-2-[(1R)-4-methylcyclohex-3-en-1-yl]hepta-1,5-dien-4-one)	Surfactant, Emulsifier, Membrane stabilizer (HMDB: HMDB0038187)	(Lingwood and Simons, 2010)

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