RESEARCH ARTICLE

Exploring Antimicrobial Phytocompounds in *Tanacetum dolicophyllum*: A GC-MS Profiling Approach

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ABSTRACT

Tanacetum dolicophyllum, known locally as tansies, exhibitng a plethora of challenging and noteworthy pharmacological properties. Aerial parts of *T. dolicophyllum* were utilized for extract preparation, and then their *in-vitro* antimicrobial activity was assessed against various organisms employing both the well diffusion method and MIC assay. The ethyl acetate extract of *T. dolicophyllum* was analyzed using gas chromatography-mass spectrometry (GC-MS) investigation to determine phytocomponents and investigate their antimicrobial activity. The result demonstrated that ethyl acetate extract exhibited good antimicrobial activity. The extract showed the highest efficacy against *Klebsiella pneumoniae*, *Streptococcus agalactiae*, *S. pyogenes*, *Escherichia coli*, and *Candida albicans*. Analysis revealed the presence of 37 components in the *T. dolicophyllum* extract, as determined by GC-MS investigation. The most abundant compounds include pentacosane, constituting 17.31 of the peak area% with a retention time (RT) of 24.603, and molecular formula $C_{25}H_{52}$; 2(3H)-Furanone, dihydro-3-[(7-methoxy-1,3-benzodioxol-5-yl)methyl]-4-[(3,4,5-trimethoxyphenyl)methyl]- accounting for 6.43 peak area% with a RT of 31.829 and, molecular formula $C_{23}H_{26}O_8$; 4-(1,3-benzodioxol-5-ylmethyl)dihydro-3-[(3,4,5-trimethoxyphenyl)methyl]- representing 5.76 peak area%, RT 28.409 and, molecular formula $C_{22}H_{24}O_7$; Yangambin present at 5.44% of peak area, with an RT of 29.513 and, molecular formula $C_{24}H_{30}O_8$; Chrysantenyl 2-methuylbutanoate constituting 4.60 of peak area%, RT 18.825, and molecular formula $C_{15}H_{24}O_2$; Phytyl tetradecanoate with 3.22 area%, RT 31.284, and formula $C_{34}H_{66}O_2$; and Tricosane accounting for 1.44 peak area%, with a RT of 18.228, and molecular formula $C_{23}H_{48}$. These compounds exhibit various medical potentials, suggesting the ethyl acetate extract of *T. dolicophyllum* may have diverse pharmacological applications.

Keywords: Tansies, Pentacosane, Ethyl acetate, Antimicrobial, GC-MS analysis.

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INTRODUCTION

The genus *Tanacetum* comprises around 150 species known for their medicinal properties. Various species within this genus have been recognized for their potential in treating conditions such as menstrual cramps, headaches, ulcers, and fever.[1,2] A broad range of pharmaceutical activities considering antioxidant, antimicrobial activity, anti-cancer, anti-insecticidal, and antitumor was shown by the extracts of the genus *Tanacetum*.[3,4]

One notable species *Tanacetum dolicophyllum* (Asteraceae), presents numerous intriguing pharmacological properties. Conventionally, *T. dolichophyllum* has been used in the Uttarakhand and Ladakh regions for its fragrant and incense material.[5]

The local people of Ladakh have long utilized this species in ancient medicines. However, there is a notable lack of information regarding the *in-vivo* and *in-vitro* effectiveness of *T. dolichophyllum*. This learning aims to inspect *in-vitro* antifungal and antibacterial activities of phyto-extracts isolated from *T. dolichophyllum* highlighting the significant potential of *T. dolichophyllum* as sources of antimicrobial drugs.

The current research focuses on identifying the phytoconstituents in *T. dolicophyllum* using GC-MS investigation.[6] There has remained a rising attention on characterizing organic compounds in plants. This research aims to isolate bioactive compounds from *T. dolicophyllum* and evaluate their pharmacological potential. It also involves characterizing these compounds through GC-MS analysis.[7] Specifically, the study investigated the phytochemical components existing in the ethyl acetate extract of *T. dolicophyllum* via the GC-MS analysis. This approach

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is utilized to better understand and validate the medicinal characteristics associated with the plant.

MATERIALS AND METHODS

Plant Gathering and Authentication

T. dolicophyllum plant specimens were gathered from the Choskore area of Panikhar in the Kargil region of Ladakh. Furthermore, the botanical authenticity of the flora was identified by taxonomists at the Botanical Survey of India, based in Jodhpur, India.

Preparation of Plant Extract

Following collection, the plant materials underwent thorough washing and sterilization before being shade-dried and then powdered finely. About 90 g of the pulverized sample was placed into soxhlet extractor for the extraction process. Various solvents, counting benzene, water, petroleum ether, ethanol and ethyl acetate, were used for extraction. Dimethyl sulphoxide (DMSO) served as the liquified solvent for these extracts.[8] The extraction process was carried out using a Soxhlet apparatus with a temperature gradient maintained through a time period of 48 hours. The extracts were collected by means of an oven set at room temperature to acquire dried extract. The dried extract was then used for evaluating antimicrobial activity and GC-MS investigation to determine and characterize the components present in the extract. This method ensured efficient extraction of phytochemicals from the plant material, allowing for further analysis of its chemical composition and potential bioactivities.[9]

Microbial Culture

In this research, a total of five test organisms were selected, including *Klebsiella pneumoniae* (MTCC432), *Streptococcus agalactiae* (ATCC13813), *S. pyogenes* (MTCC1924), *Escherichia coli* (MTCC730), and *Candida albicans* (MTCC7315). The antimicrobial activity was conducted for each extract using a disc diffusion method.[10,11]

Antimicrobial Activity of Extracts

The antimicrobial action of various extracts of *T. dolicophyllum* was evaluated using the well diffusion method. The microorganisms were injected into nutrient stock and overnight incubated for 24 hours, adjusting the turbidity to match 0.5 McFarland standards, and then cultured with standard microbial culture broth. About 50 mg/mL of plant extracts were equipped in DMSO. Using a sterile 6 mm cork-borer, 6 wells were created in the inoculated media. Each well was filled with 50 µL of plant extracts. For bacterial strains and fungal isolates, amikacin (30 µg) and nitrofurantoin (300 µg) were used as a positive control, 1-mg/mL of cyclohexylamine was used. DMSO served as the solvent/negative control.[11,12]

The plates were kept for about half an hour on room temperature to enable diffusion, followed by incubation for 24 hours. For the establishment of a pure zone indicating antimicrobial activity, the plates were examined. The ZOI was measured in millimeters.[13]

Evaluation of MIC of extracts of *T. dolicophyllum*

The minimum inhibitory concentration (MIC) was observed by the broth dilution method. Amikacin served as a positive control.[12-15]

GC-MS investigation of *T. dolicophyllum*

The GC-MS investigation was conducted by means of Shimadzu GCMS equipped with a Turbo Mass quadrupole mass spectrometer.[16,17] Plant extract's components identification was dependent on a comparison of mass spectra from the Mass Spectral Database; NIST14 library.[18]

Statistical Analysis

Both the antimicrobial assays were performed in triplicates. Results are stated as mean ± SD.

RESULTS

Using the soxhlet extraction method, five different extracts were obtained. These extracts were subsequently tested for their antimicrobial activity against various organisms commonly associated with infectious organisms. The study demonstrated that each plant extract exhibited different levels of antimicrobial effectiveness against all the test organisms.

Antimicrobial Activity

Among the five plant extracts tested, the ethyl acetate extract was analyzed as the furthermost effective. It demonstrates a zone of inhibition (ZOI) towards fungal strains for both gram-ve and +ve bacteria. The ethanol extract was operative against both gram-ve and +ve bacterial strains but did not reveal a little antifungal action. The extracts of petroleum ether exhibited the smallest ZOI against *K. pneumoniae*, and showed no antifungal action against *C. albicans*. The benzene and water extracts showed no antibacterial action against either gram-ve and +ve bacterial strains only, although the benzene and water extract displayed minimal antifungal activity (Table 1).

MIC testing was conducted for those extracts that demonstrated a ZOI and sensitivity to the tested organism in the preceding antimicrobial assessment *via* well diffusion method. Ethyl acetate extract demonstrated the highest antimicrobial activity. The MIC of ethyl acetate extract was 5 mg/mL against the gram +ve bacterium *S. agalactiae* and *S. pyogenes*. For gram -ve bacteria, the MIC values meant for *E. coli* and *K. pneumoniae* were 7 and 10 mg/mL. MIC values of ethyl acetate extract against *C. albicans* was 9 mg/mL. The ethanolic extract was active against *S. pyogenes* and *K. pneumoniae* through MIC rates of 14 and 12 mg/mL, respectively. The petroleum ether extract was effective against *K. pneumoniae* with MIC values 42 mg/mL; it did not exhibit antifungal activity. The benzene and water extracts did not exhibit antibacterial activity but show least antifungal activity (Table 2).

Evaluation of the phytoconstituents by GC-MS

The composites found in the ethyl acetate extract of *T. dolicophyllum*'s parts were recognized by means of GC-MS (Figure 1). GC-MS revealed various components with

Table 1: ZOI (mm) of plant extracts of *T. dolicophyllum*

Test organism	*Plant extracts*				
	Benzene	*Water*	*Petroleum ether*	*Ethyl acetate*	*Ethanol*
S. agalactiae	-	-	-	20.0 ± 1.0	-
S. pyogenes	-	-	-	20.0 ± 0.7	13.0 ± 0.5
E. coli	-	-	-	19.0 ± 0.7	-
K. pneumoniae	-	-	08.0 ± 0.6	14.0 ± 0.6	12.0 ± 0.7
C. albicans	09.0 ± 1.0	09.0 ± 0.5	-	19.0 ± 0.5	-

(-) indicates no antimicrobial activity

Table 2: MIC of various plant extracts of *T. dolicophyllum*

Test organism	*MIC values (mg/mL)*				
	Benzene	*Water*	*Petroleum ether*	*Ethyl acetate*	*Ethanol*
S. agalactiae	-	-	-	5	-
S. pyogenes	-	-	-	5	14
E. coli	-	-	-	7	-
K. pneumoniae	-	-	42	10	12
C. albicans	49	55	-	9	-

(-) indicates no antimicrobial activity

different retention times, indicating diverse structures and pharmacological properties. The MS analysis of the constituents' elution periods further confirmed these differences. As larger compounds disintegrate into smaller components, peaks appear at different m/z ratios. The identification of compounds corresponding to these peaks was achieved using a data library. Additionally, the molecular formula and weight of 37 biomolecules were noticed in the aerial parts of *T. dolicophyllum* extracts. Table 3 provides a summary and chemical structures of the phytoconstituents.

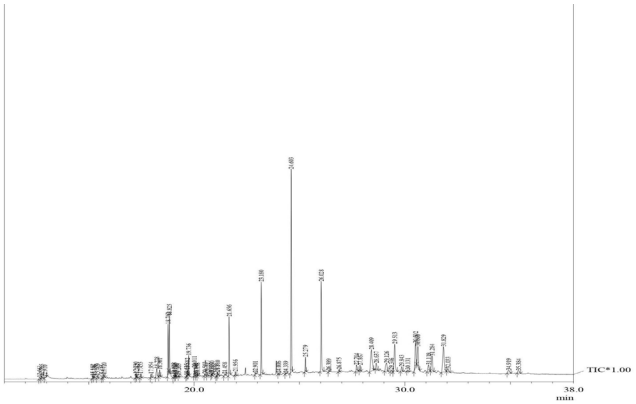


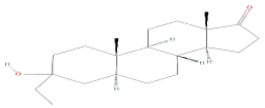
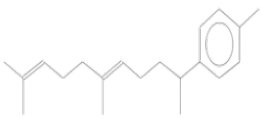
Figure 1: GC-MS of the extract of ethyl acetate isolated from *T. dolicophyllumz*

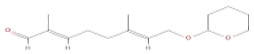
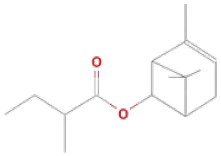
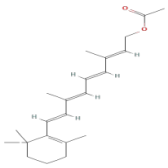
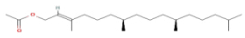
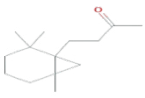
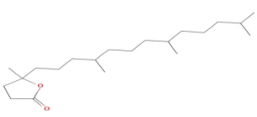
DISCUSSION

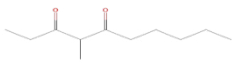
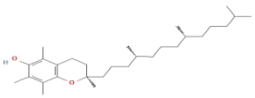
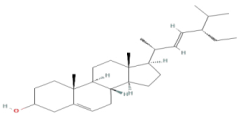
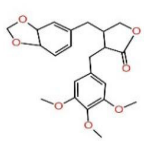
Antibiotic resistance remains a significant challenge for the healthcare sector worldwide, affecting both processing and formed countries. The rise and dissemination of MDR pathogens have considerably undermined the effectiveness of present-day antibacterial therapies.[19,20] This research was conducted to evaluate the antimicrobial action of various plant extracts isolated from *T. dolicophyllum*. The ethyl acetate extract demonstrated significant antibacterial activity against different bacterial and fungi isolates. Other extracts exhibited limited effectiveness, as determined by their respective MIC values.[21,22] A lesser MIC value shows that the least drug is requisite for inhibiting the maturation of an organism. Drugs with the lowest MIC values are potential antimicrobial agents.[23] The extract of ethyl acetate *T. dolicophyllum* demonstrated maximum action against five pathogens *K. pneumoniae* (MTCC432), *S. agalactiae* (ATCC13813), *S. pyogenes* (MTCC1924), *Escherichia coli* (MTCC730), and *Candida albicans* (MTCC7315). The significant activity demonstrated by the extract of ethyl acetate, *T. dolicophyllum* suggest that it could serve as an important alternative in combating antibiotic resistance. The extract of ethanol exhibited antimicrobial action towards *S. pyogenes* (MTCC1924) and *K. pneumoniae* (MTCC432). Although the ethyl acetate extracts exhibited strong antibacterial and antifungal potency, further evaluation through GC-MS study is warranted to determine and characterize the bioactive compound obligated for this act.

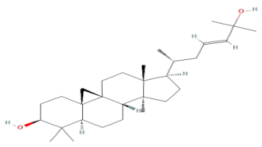
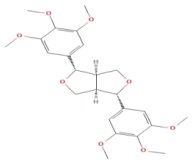
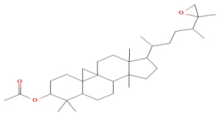
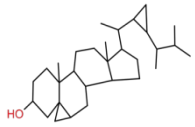
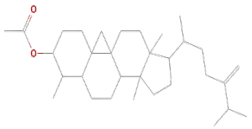
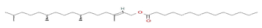
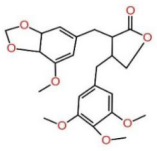
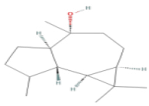
In addition to their therapeutic properties, medicinal plants offer valuable insights into a broad range of chemical components that could be developed into highly selective drugs. These plants are the potential sources of chemical compounds, providing new lead and insights for modern drug design. Ethyl acetate extract of *T. dolicophyllum* is reported to contain carbohydrates, proteins, phenols, polyphenols, tannins, coumarins, flavonoids, alkaloids, glycosides and phlobatannins. The discovery of these bioactive components highlights *T. dolicophyllum*'s potential as a promising source for developing novel healing agents. This contribution holds

Table 3: GC-MS evaluation of the leaves and stem of *T. dolicophyllum*

S.No	*Compound name*	*Retention time (minutes)*	*Peak area %*	*Molecular formula*	*Molecular weight*	*Compound nature*	*Compound structure*
1.	(2e)-3,7-Dimethyl-2,6-octadienyl 3-methylbutanoate	12.765	0.38	$C_{15}H_{26}O_2$	238	Ester	
2.	E-11-Tetradecen-1-ol trifluoroacetate	12.910	0.11	$C_{16}H_{27}F_3O_2$	308	Alcohol	
3.	1-Heptadecene	15.182	0.23	$C_{17}H_{34}$	238	Alkene	
4.	Tetracosane	15.246	0.16	$C_{24}H_{50}$	338	Alkane	
5.	Ethyl-3-hydroxyandrostan-17-one	15.409	0.32	$C_{21}H_{34}O_2$	318	Ketone	
6.	Neophytadiene	15.635	0.16	$C_{20}H_{38}$	278	Alkene	
7.	2-Pentadecanone, 6,10,14-trimethyl-	15.720	0.38	$C_{18}H_{36}O$	268	Ketone	
8.	1-Hexadecanol	17.230	0.33	$C_{16}H_{34}O$	242	Cetyl alcohol	
9.	Gerany-p-cymene	17.455	0.42	$C_{20}H_{30}$	270	Heterocyclic	

10.	(2E,6E)-2,6-Dimethyl-8-(tetrahydro-2H-pyran-2-yloxy)-2,6-octadien-1-ol	17.954	0.36	$C_{15}H_{24}O_3$	254	Heterocyclic	
11.	Tricosane	18.228	1.44	$C_{23}H_{48}$	324	Alkane	
12.	Phytol	18.361	0.82	$C_{20}H_{40}O$	296	Diterpenoid	
13.	Chrysantenyl 2-methuylbutanoate	18.825	4.60	$C_{15}H_{20}O_2$	236	Heterocyclic	
14.	Retinol, acetate	19.028	0.23	$C_{22}H_{32}O_2$	328	Vitamin A	
15.	N-Nonadecanol-1	19.095	0.20	$C_{19}H_{40}O$	284	Alcohol	
16.	Phytol, acetate	19.265	0.13	$C_{22}H_{24}O_2$	338	Phytyl acetate	
17.	4-(2,2,6-Trimethylbicyclo [4.1.0]hept-1-yl)-2-butanone	20.158	0.13	$C_{14}H_{24}O$	208	Ketone	
18.	4,8,12,16- Tetramethyl-heptadecan- 4-olide	20.503	0.23	$C_{21}H_{40}O_2$	324	Heterocyclic	

19.	4-Methyl-3,5-decandione	20.617	0.33	$C_{11}H_{20}O_2$	184	Ketone	
20.	1-Eicosanol	21.458	0.26	$C_{20}H_{42}O$	298	Alcohol	
21.	Docosane, 11-decyl-	22.901	0.17	$C_{32}H_{66}$	450	Alkane	
22.	Squalene	24.006	0.13	$C_{30}H_{50}$	410	Alkene	
23.	Nonadecane	24.339	0.11	$C_{19}H_{40}$	268	Alkane	
24.	Pentacosane	24.603	17.31	$C_{25}H_{52}$	352	Alkane	
25.	Alpha-tocopherol	26.389	0.15	$C_{29}H_{50}O_2$	430	Vitamin E	
26.	Stigmasta-5,22-dien-3-ol	27.704	0.97	$C_{29}H_{48}O$	412	Alcohols	
27.	Tetracontane	27.867	0.74	$C_{40}H_{82}$	562	Alkane	
28.	4-(1,3-benzodioxo l-5-ylmethyl)dihydro-3-[(3,4,5-trimethoxyphenyl) methyl]-	28.409	5.76	$C_{22}H_{24}O_7$	400	Heterocyclic	

29.	1-[(3E)-5-Hydroxy-1,5-dimethyl-3-hexenyl]-3a,6,6,12a-tetramethyltetradecahydro-1H-cyclopenta [a] cyclopropa [e] phenanthren-7-ol	29.126	2.18	$C_{30}H_{50}O_2$	442	Heterocyclic	
30.	Yangambin	29.513	5.44	$C_{24}H_{30}O_8$	446	Heterocyclic	
31.	3a,6,6,12a-Tetramethyl-1-[1-methyl-4-(2-methyl-2-oxiranyl)pentyl]tetradecahydro-1H-cyclopenta[a] cyclopropa[e] phenanthren-7-yl acetate	29.843	0.85	$C_{33}H_{54}O_3$	498	Heterocyclic	
32.	Cyclopropa[5,6] -33-norgorgostan- 3-ol, 3',6- dihydro-	30.131	0.29	$C_{30}H_{50}O$	426	Heterocyclic	
33.	1-(4-Isopropyl-1-methyl-4-pentenyl)-3a,6,12a-trimethyltetradecahydro-1H-cyclopenta[a] cyclopropa[e] phenanthren-7-yl acetate	31.119	1.35	$C_{32}H_{52}O_2$	468	Heterocyclic	
34.	Phytyl tetradecanoate	31.284	3.22	$C_{34}H_{66}O_2$	506	Ester	
35.	2(3H)-Furanone, dihydro-3-[(7-methoxy-1,3-benzodioxol-5-yl) methyl]-4-[(3,4,5-trimethoxyphenyl) methyl]-	31.829	6.43	$C_{23}H_{26}O_8$	430	Heterocyclic	
36.	1H-cycloprop[e]azulen-4-ol, decahydro-1,1,4,7	32.033	0.75	$C_{15}H_{26}O$	222	Heterocyclic	
37.	Tridecanedial	34.919	0.81	$C_{13}H_{24}O_2$	212	Aldehyde	

substantial promise for advancing pharmaceutical research and drug development. A whole of 37 compounds were analyzed in the GC-MS study within the extracts of ethyl acetate derived from *T. dolicophyllum.* The chemical structures of the compounds in the extracts of ethyl acetate are presented in Table 3. Further investigations may lead to the abstraction of additional plant components, and understanding their structures could prove beneficial for future drug development endeavors.

CONCLUSION

In this work, the antimicrobial activities of five plant extracts isolated from *T. dolicophyllum* from Ladakh, India, were assessed using the soxhlet extraction method. The consequence demonstrated the potential antimicrobial effects of ethyl acetate extracts against gram +ve and -ve bacteria alongside fungal strains. In contrast to this, ethanolic extract was effective only against *S. pyogenes* and *K. pneumoniae.* Given the unsuitable effects of analytic medications, the plant components extracted from therapeutic plants could be valuable for drug discovery and development against several diseases. GC-MS analysis confirmed the occurrence of phytoconstituents in the ethyl acetate extract of *T. dolicophyllum*, indicating a rich composition of bioactive compounds.

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