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GC-MS Analysis of bio-Active compound α-Terpinyl acetate extract of bay leaf having antimicrobial and antifungal properties

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ABSTRACT

This study aimed to assess the phytotoxic and antibacterial properties of á-terpinyl acetate by evaluating the presence of *T. pulegioides* á-terpinyl acetate chemotype as a source of natural origin, which was examined in 131 sites by hydrodistillation, essential oils were separated, and GC-FID and GC-MS analyses were performed. The phytotoxic impact of this chemotype's essential oil on monocotyledons and dicotyledons through water and air was investigated in a lab setting; The effect of the essential oil against pathogenic bacteria that affects humans was screened using the broth microdilution method. The findings indicated that á-terpinyl acetate was a very uncommon component in *T. pulegioides* essential oil, only being discovered in 35% of the habitats that were studied. On the plants under investigation, á-terpinyl acetate (both in essential oil and pure form) exhibited distinct behaviors.á-terpinyl acetate's phytotoxic impact was more pronounced on the monocotyledons under investigation compared to the dicotyledons. For high economic productivity forage grass monocotyledon *Poa pratensis*, á-terpinyl acetate essential oil hindered seed germination and radicle development; while, for high economic productivity forage legume dicotyledon *Trifolium pretense*, it increased seed germination. The essential oil á-terpinyl acetate had a strong antibacterial action against dermatophytes and fungi, but a weaker impact against bacteria and Candida yeasts. Consequently, the á-terpinyl acetate chemotype of *T. pulegioides* may represent a promising chemical for the development of therapeutics or preventative measures against mycosis.

Figure : 01 References : 11 Tables : 02

KEY WORDS : Antimicrobial effect, á-terpinyl acetate, Dicotyledons, Monocotyledons, *Thymus pulegioides*.

Introduction

Specific plants which generate essential oils may include the secondary plant metabolite á-Terpinyl acetate (α-TA), a monoterpene ester *e.g. Eletteria cardamomum* Maton (Zingiberaceae), *Levisticum officinale* (Apiaceae), *Laurus nobilis* (Lauraceae), *Myrtus communis* (Myrtaceae), *Chamaecyparis obtuse* (Cupressaceae), *Stachys glutinosa* (Lamiaceae), *Gundelia tournifortii* (Asteraceae), *Dysphania ambrosioides* (syn. *Chenopodium abrosioides*) (Amaranthaceae)[4]. A fragrance molecule of economic importance, á-TA has a pleasant, herbaceous floral and lavender scent. It is commonly used as an odorant and fragrance component in the production of lotions, soaps, shampoos, air fresheners, cleaning and furnishing care products and laundry and dishwashing products. á-TA can also be used as a culinary flavoring ingredient in fruit ice creams, baked products, hard candies, puddings and chewing gum. The majority of *Thymus* species are aromatic medicinal plants that provide essential oils that are utilized as flavoring or fragrance elements in food products. As a result, thyme essential oils contain antibacterial qualities and can be utilized as food preservatives in addition to spices. *Thymus* individuals of the same species exhibit chemical polymorphism, allowing them to manufacture essential oils with varying chemical compositions. Consequently, other chemotypes may be identified, including the á-TA chemotype[7]. Various Thymus species, notably huge thyme (*Thymus pulegioides*) growing wild in Europe, have been discovered to exhibit varying amounts of á-TA. (Table-1).

TABLE -1: The percentages of α-terpinyl acetate in essential oils of different species from genus *Thymus.*

Species of Genus Thymus Country **Percentage of α-Terpinyl Acetate**	***in Essential Oil***	***Literature***
Thymus praecox	Great Britain	*36* [7]
Thymus zygioides	Cyprus	*36.2*[7]
Thymus zygis	Spain	*65.4–73.1*[2]
Thymus striatus	Bosnia-Herzegowina	*8.1–11.2*[3]
Thymus striatus	Bosnia-Herzegowina	*8.1–11.2*[3]

The main issue with public health is medication resistance in fungi and bacteria. The toxicity and adverse medication reactions associated with treating fungal infections—particularly those brought on by *Trichophyton, Candida,* and *Aspergillus* species—make therapy extremely difficult[11]. Numerous illnesses in all age groups are caused by the most prevalent human pathogens, *Escherichia coli* and *Staphylococcus aureus*[10]. In order to prevent microbial resistance, there has been an increase in interest in the creation of novel antimicrobial medications from many sources in recent years. Natural therapy might be one way to assist getting rid of many bacterial or fungal illnesses[6].Biologically active molecules can be found in the chemical make up of essential oils, particularly in natural plants that possess essential oils.

This study was aimed to compare the essential oils traded for medical use with those produced by hydrodistillation from dried bay leaves used for cookery.

Materials and Methods

Plant Material: 6 samples of 100 g each commercially dried bay leaf that were traded as spices underwent three hours of hydrodistillation in a Clevenger-style device. The essential oils were preserved at 4°C until they were subjected to a gas chromatographic-mass spectrometry (GC-MS) examination after being dried over anhydrous sodium sulphate. Analysis was done on a commercial sample of *Thymus pulegioides L* essential oil that was offered for medical purposes.

GC-MS Analysis

Using an Agilent 5973N apparatus, a capillary column (95 dimethylpolysiloxane-5% diphenyl) and an Agilent HP-5MS UI (30 m length and 0.25 mm i.d. with 0.25 ìm film thickness) were used for the GC-MS analysis. The temperature program for the column was set to 60æ%C for five minutes, then increased by 3æ%C/min to 180æ%C, then by 20æ%C/min to 280æ%C, a temperature that was held for ten minutes. Helium was used as the carrier gas, flowing at a rate of 1 mL/min. The injection method used was split mode (ratio 1:30). Mass spectra were obtained at an ionizing voltage of 70 eV over the m/z 30-500 range. Standard hydrocarbons that were co-chromatographed were used to determine Kovat's retention index. The various compounds were identified by mass spectrometry (MS), and their authenticity was verified by comparing their relative intensities (RIs) to C8-C32 n-alkanes and mass spectra with either real samples or data that were previously accessible in the literature and NIST 2005 mass spectral database.

Result

The primary fraction of this essential oil was composed mostly of monoterpenes (83.32%), according to the GC analysis chromatogram and composition. á-TA, an oxygen monoterpene, was the main component. The subsequent substance was the monoterpene alcohol á-terpineol, which had a 4.5-times lower proportion in essential oil than á-TA (Table-2).

A common class of chemical compounds found in species of *Thymus* containing essential oils are monoterpenes and their derivatives. They can contain 60–80% of the essential oil and act as either auto- or phytotoxic agents. According to some research, thyme monoterpenes may inhibit plant development or seed germination, which may affect the dynamic and composition of plant communities. The primary component of the monoterpenes fraction in the essential oil of the *T. pulegioides* á-TA chemotype was oxygenated monoterpene á-TA. The quantity of oxygenated monoterpene á-TA that we got in our phytotoxic and antibacterial laboratory trials was 63.12% of essential

TABLE -2 : *Thymus pulegioides* á-terpinyl acetate chemotype essential oil composition (GC area%, RI = retention index, MS = mass spectrum, Std = analytical standard, RT = retention time). The examined substances' mass spectrum similarities ranged from 85 to 96% when compared to the NBS75K computer mass spectra library and/or analytical standards.

Compound	Identification Method	RI		GC Area,%	RT
		Calculated	Literature[1]		
1-octen-3-ol	*RI, MS*	*983*	*974*	*0.09*	*3.06*
α-Terpinene	*RI, MS, Std*	*1024*	*1014*	*2.23*	*11.03*
p-Cymene	*RI, MS, Std*	*1030*	*1020*	*0.04*	*12.39*
Limonene	*RI, MS, Std*	*1034*	*1024*	*0.03*	*12.49*
(E)-β-Ocimene	*RI, MS*	*1054*	*1044*	*0.07*	*12.60*
γ-Terpinene	*RI, MS, Std*	*1064*	*1054*	*0.14*	*13.46*
Linalool	*RI, MS, Std*	*1105*	*1095*	*0.75*	*14.77*
α-Terpineol	*RI, MS*	*1197*	*1186*	*15.12*	*18.10*
Nerol	*RI, MS, Std*	*1238*	*1227*	*0.61*	*20.24*
Neral	*RI, MS*	*1244*	*1235*	*0.49*	*19.34*
Geraniol	*RI, MS, Std*	*1260*	*1249*	*4.90*	*19.56*
Geranial	*RI, MS*	*1275*	*1264*	*0.58*	*18.81*
α-Terpinyl acetate	*RI, MS, Std*	*1359*	*1346*	*63.12*	*22.53*
β-Bourbonene	*RI, MS*	*1399*	*1387*	*0.21*	*23.68*
β-Caryophyllene	*RI, MS, Std*	*1428*	*1417*	*1.67*	*24.76*
cis-β-Guaiene	*RI, MS*	*1503*	*1492*	*0.76*	*26.45*
β-Bisabolene	*RI, MS*	*1517*	*1505*	*1.54*	*26.93*
Caryophyllene oxide	*RI, MS, Std*	*1593*	*1582*	*0.18*	*29.28*
Monoterpene hydrocarbons			*2.45*		
Oxygenated monoterpenes			*83.32*		
Sesquiterpene hydrocarbons			*4.34*		
Other			*0.09*		
Total identified			*91.84*		

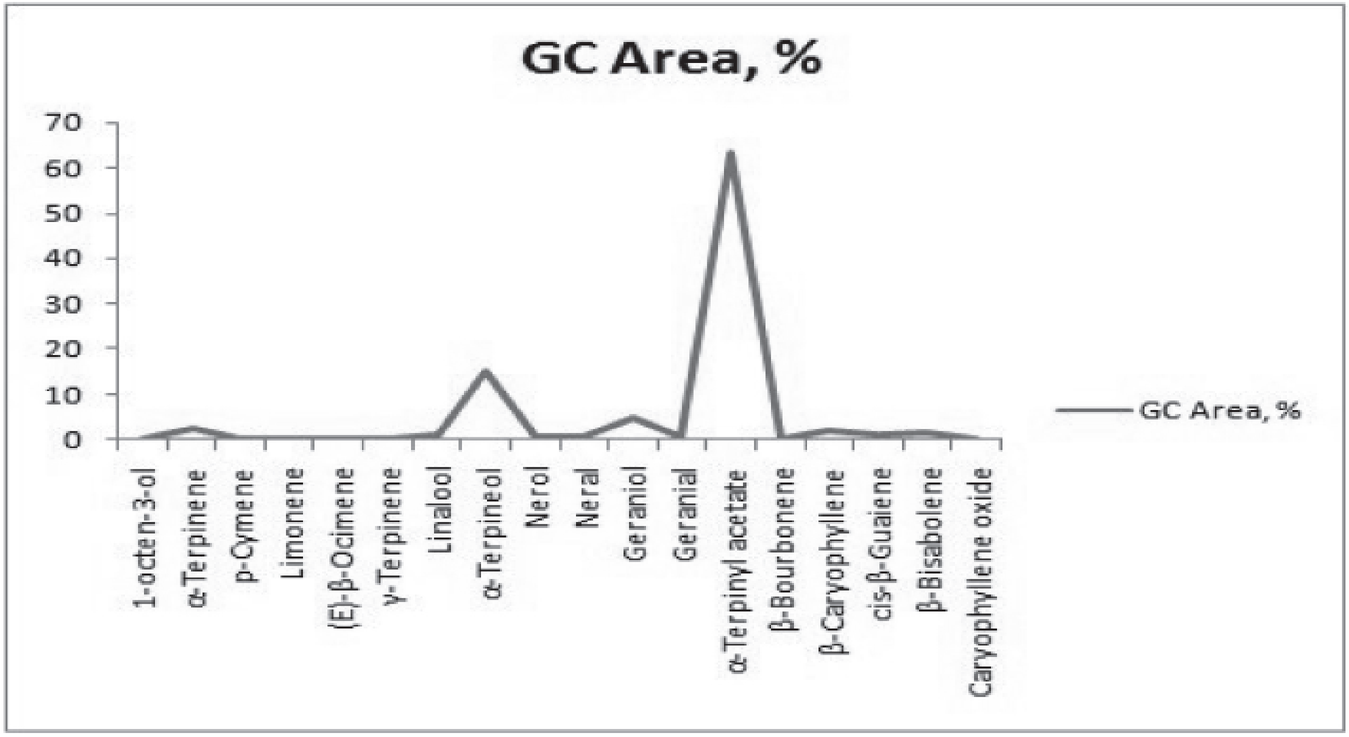

Fig. 1: α-terpinyl acetate chemotype essential oil composition GC area %

oil (Fig.1).

Conclusion

The effects of α-TA essential oil on the fungus *Aspergillus* and *Trichophyton* under investigation were quite significant, outperforming the effects of the control drug itraconazole on these microbes. Previous investigations on *Thymus tosevi*i essential oil showed a very significant antifungal action even at low doses of 0.25–1.0 µL/mL; the percentage of α-TA in these experiments was only 13.5%[9]. The substantial antifungal action of α-TA essential oil was validated by our investigation using the α-TA analytical standard. This might be related to the large amount of α-TA and perhaps the presence of additional bioactive chemical components such α-terpineol, geraniol, and α-terpinene (Table 2). Additional investigations[5,8] have also reported on the antimicrobial properties of geraniol and á-terpineol on various bacteria. It's interesting to note that, compared to fungi and dermatophytes, *Candida* yeasts exhibited a higher degree of resistance to α-TA essential oil in our research. However, they were marginally more sensitive to *C. parapsilosis* than to itraconazole. The study's overall findings demonstrated the strong antibacterial properties of both pure and *T. pulegioides* α-TA chemotype essential oil against human pathogenic pathogens.

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