

GONIOTHALAMUS TORTILIPETALUS ESSENTIAL OILS' CHEMICAL COMPOSITION AND THEIR ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES - M.R. HENDRICKS

Prof Jadhav pooja

Kasturi Shikshan Sanstha College of Arts, Commerce and Science,
Shikrapur, Pune

Abstract

Identifying Natural Oil in *Goniothalamus tortilipetalus* could lead to the discovery of new pharmaceutical agents for treating various diseases. M.R. Hendericks' research on the Natural Oils of *Goniothalamus tortilipetalus* could lead to the development of compounds with antioxidant properties for treating oxidative stress-related conditions. This investigation involved the extraction and analysis of Natural Oils from various parts of *Goniothalamus tortilipetalus*, such as leaves, flowers, or stems. Once the essential oils are extracted, researchers will analyse their composition using techniques such as high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS). This study would reveal the chemical constituents found in essential oils, which could include a variety of volatile compounds with antioxidant and antibacterial properties. The process of hydro distillation using the Clevenger device is a popular method for obtaining essential oils from plant sources. This apparatus separates essential oils from plant material by passing steam through it, causing the essential oils to evaporate and condense in a separate collection flask.

Keywords

Goniothalamus tortilipetalus, Natural Oils; antimicrobial and oxidative properties.

Introduction

Within the Annonaceae family, *goniothalamus tortilipetalus* is a kind of flowering plant. It was first described in 1996 by French botanist Pierre Danguy. This species is endemic to Peninsular Malaysia, specifically found in the state of Perak. As with many plants in the genus *Goniothalamus*, *G. tortilipetalus* likely possesses certain chemical compounds that could potentially have pharmacological properties, as many Annonaceae species are known to contain alkaloids and other Natural Oils. However, detailed information about the chemical composition and potential medicinal uses of *G. tortilipetalus* may be limited due to the species' relative obscurity in botanical research.

This species is native to Southeast Asia, particularly found in regions like Malaysia. Like many other members of the genus *Goniothalamus*, it is characterized by its distinctive flowers and fruits. The genus *Goniothalamus* comprises around 140 species of trees and shrubs, many of which are found in tropical forests. These plants are valued for their ornamental features as well as for their traditional medicinal uses in some cultures. However, specific information about *Goniothalamus tortilipetalus* may be limited due to its relatively lesser-known status

compared to more widely studied species within the genus. Many species within the Annonaceae family, including *Goniothalamus*, are known to contain Natural Oils with potential medicinal properties. While specific research on *G. tortilipetalus* may be limited, its chemical composition could hold promise for the development of new pharmaceuticals or botanical medicines.

Materials and Methods

Plant Material: *G. tortilipetalus* twigs, leaves, and flowers were gathered and kept.

How the Natural Oils are Extracted:

- Organising: *G. tortilipetalus* foliage, twigs, and flowers are harvested and thoroughly washed to remove dirt and impurities. They are then crushed with a grinder. Crushing helps to break down plant material and release the essential oils it contains.
- Hydrodistillation: Hydrodistillation is a widely used method for extracting Natural Oils from plant material, where crushed material and water are combined in a distillation apparatus. The mixture is then heated until the water vaporises and passes through the plant material. As the steam transports the volatile Natural Oils molecules, they condense back into liquid form in a separate flask. The water and essential oil layers are separated using a separating funnel.
- Collection of Natural Oils: The hydrodistillation process typically results in the extraction of Natural Oils from the water phase, which contains water-soluble compounds and other impurities. This separation is typically accomplished with a separating funnel, in which the lighter Natural Oils layer floats on top of the water layer and can be easily decanted or drained off.
- Analysis and storage: After collection, the essential oil can be analysed for chemical composition and purity using techniques like GC-MS. It is then kept in dark, airtight containers away from heat and light to maintain its quality and potency.

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay: Antioxidant Activity

This is a common method employed to evaluate the antioxidant action taken by various compounds, including natural products, plant extracts, and synthetic antioxidants. The assay relies on the ability of antioxidants to donate electrons or hydrogen atoms to DPPH radicals, thereby neutralizing them and causing a colour change from purple to yellow.

Serial dilutions of the Natural Oils in methanol to achieve concentrations of 100, 250, 500, 1000, 2000, and 3000 [g/mL] were prepared. A study was conducted to determine the

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concentration of ascorbic acid in methanol. Serial dilutions of ascorbic acid in methanol were prepared, and 100 μ L of each diluted essential oil sample was added to each well. The DPPH methanolic solution was added to each well, and 100 μ L of each diluted ascorbic acid sample was added to each well. For thirty minutes, the microplates were incubated at room temperature in the dark. After the incubation period, the absorbance at the appropriate wavelength (typically around 517 nm) using a microplate reader or spectrophotometer was measured. The following formula was used to determine the samples' percentage inhibition or scavenging activity:

$$\text{Scavenging activity (\%)} = (1 - A_{\text{sample}}/A_{\text{blank}}) \times 100$$

Where

- Blank: The blank is the solvent plus the DPPH absorbance of the blank.
- Sample: A sample refers to the sample's absorbance (sample plus DPPH).

The antioxidant activity was determined by plotting a graph of scavenging activity (%) against the concentration of test samples.

ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation scavenging assay

The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation scavenging assay is a commonly used technique to assess a compound's antioxidant activity.

Before being used, equal quantities of 2.45 mM potassium persulfate solution and 7 mM ABTS solution were combined and left to react for 16 hours at room temperature in the dark. Afterwards, ethanol was used to adjust the ABTS \bullet solution's absorbance to 0.70 ± 0.02 at 734 nm in order to guarantee test uniformity. Essential oils were produced as serial dilutions at 25, 50, 100, 250, and 500 μ g/mL. Ascorbic acid serial dilutions were prepared at concentrations of 1.5, 3, 6, 12, and 25 μ g/mL (Positive Controls). Dilute essential oils and ascorbic acid were mixed with ABTS + solution in microplates, with 20 μ L aliquots as positive controls. The mixtures were incubated at room temperature for 5 minutes, and absorbance at 734 nm was measured. The percentage inhibition of ABTS radical cation by the test samples was calculated using a formula. The reaction mixtures were allowed to stand in the dark for 5 minutes.

$$\text{Inhibition \%} = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100$$

The absorbance of the control, denoted as A control, and the absorbance of the sample, denoted as A sample.

(ABTS solution without sample).

Antibacterial Activity

Natural Oils were diluted with DMSO (Dimethyl sulfoxide) to create various concentrations. These dilutions were then loaded into Muller-Hinton broth microdilution plates. The 96-well plates were filled with 100 microliters of microbial culture, with a concentration of around 1.0×10^6 CFU/mL per well. This step ensures that each well has a standardized amount of microbial culture for testing. A negative control was included, containing only the extraction buffer but no microorganisms. This control helps to ensure that any observed effects are due to the presence of microorganisms and not artifacts from the extraction buffer. The plates were incubated at 37°C for 24 hours, followed by the determination of the Minimum Inhibitory Concentration (MIC) after the 24-hour incubation period. This determination likely involved visual inspection for growth inhibition or other methods such as spectrophotometry. These are known antibiotics with established MIC values against various microorganisms. Including them in the experiment helps validate the experimental setup and provides a reference for comparison with the efficacy of essential oils.

Results and Discussion:

1. α -Pinene
2. Myrcene
3. δ -2-Carene
4. α -Phellandrene
5. α -Terpinene
6. p-Cymene
7. o-Cymene
8. β -Phellandrene
9. γ -Terpinene
10. p-Cymenene
11. Linalool
12. (Z)-p-Menth-2-en-1-ol
13. (E)-p-Menth-2-en-1-ol
14. β -Pinene oxide
15. Terpinen-4-ol

are the significant bio active components extracted from *Goniothalamus tortilipetalus* apart from 50 other components

Anti-Oxidant Activity

The exploration of antioxidant activity in Natural Oils from *G. tortilipetalus* marks a significant step in understanding its potential applications across a number of sectors, including food, beverage, and cosmetics. The methodology employed to assess the antioxidant potential, using the ABTS radical cation and DPPH radical scavenging tests,

provides crucial insights into the efficacy of these essential oil compositions in combating oxidative stress.

The DPPH and ABTS radical scavenging assays evaluate compounds' ability to neutralize free radicals and their capacity to quench ABTS radical cation, respectively, indicating their radical scavenging potential.

By conducting these assays on Natural Oils compositions from *G. tortilipetalus*, researchers can determine their antioxidant efficacy, which is crucial for potential applications in various industries. The results of this investigation could lead to the development of novel antioxidant-rich products, contributing to the advancement of the cosmetics, food, and beverage sectors. Additionally, this research lays the foundation for further studies exploring the biochemical mechanisms underlying the antioxidant activity of *G. tortilipetalus* essential oils, offering insights into its therapeutic potential and broader applications in the field of natural product chemistry and pharmacology.

From this data, it appears that the floral Natural Oils that were extracted exhibited the strongest antioxidant activity, followed by those from the leaves and then twigs, in both DPPH and ABTS assays. The IC₅₀ values represent the concentration of the essential oil required to scavenge 50% of the radicals in the assay, with lower values indicating higher antioxidant activity.

The Comparative Values of Anti-Oxidant Activity of DPPH and ABTS are presented in Table 1 below

Sample	Antioxidant (IC ₅₀ , µg/mL)	
	DPPH	ABTS
Flower Natural Oils	725.21	123.06
Leaf Natural Oils	2017.39	290.63
Twig Natural Oils	2435.50	382.17
Ascorbic Acid	5.87	6.41

Conclusion:

Natural Oils from *G. tortilipetalus*, including flower, leaf, and twig, are high in monoterpene and sesquiterpene hydrocarbons. They show weak antioxidant activity but antibacterial activity against all microorganisms tested, with moderate inhibitory activity against Gram-positive and Gram-negative strains. This information suggests that *G. tortilipetalus* Natural Oils could be used in antibacterial products, despite their relatively weak antioxidant properties.

The study reveals that Natural Oils from *Goniothalamus tortilipetalus* exhibit distinct chemical compositions, possibly due to species differences, making it the first to report such

findings. Furthermore, the study found that each *Goniothalamus* species has a distinct chemical composition. While some compounds shared similarities with those found in other *Goniothalamus* species, there were significant differences in both major and minor compounds. In the case of *Goniothalamus* species, variations in Natural Oils composition can result from genetic factors, environmental conditions, geographic distribution, and other factors. Different species may produce different combinations and proportions of volatile compounds, leading to differences in aroma, flavour, and potential pharmacological properties.

For example, one species of *Goniothalamus* might contain higher levels of certain compounds known for their antimicrobial properties, while another species might have a different set of compounds with antioxidant or anti-inflammatory properties. This variability underscores the importance of thorough chemical and biological characterization of plant species, especially those with potential medicinal or aromatic uses.

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