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Extraction and Chemical Modification of Important Terpenoids from Eucalyptus globulus Leaves

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ABSTRACT

Eucalyptus globulus leaves have been known to contain important terpenoids with potential pharmaceutical applications. In this study, we aimed to extract and chemically modify important terpenoids. The extraction of terpenoids was carried out using hydro distillation methods, and the chemical modification was performed by using acetylation reactions. The extracted and modified terpenoids were then characterized using various analytical techniques, including Gas Chromatography - Mass Spectrometry (GC - MS). The major constituents of the essential oil of *Eucalyptus globulus* are Eucalyptol having Rt = 9.415 min and was found to be 28.09 %; (-) - Globulol and α - terpineol having Rt = 13.966 min and 10.990 min and was found to be 10.66% and 6.81%. The modified terpenoids confirms the presence of monoterpene and sesquiterpene hydrocarbons such as 3-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl); Trans-5-Isopropyl-6,7-epoxy-8-hydroxy-8-met; Gama-eudesmol; 9H-Cycloisolongifolene,8-oxo- having Rt = 10.451, 13.492, 14.211, 15.142 and was found to be 2.18 %, 1.56 %, 2.88 %, 9.98 % (**SAMPLE 1);** and Isobornyl acetate; Ylangene having Rt = 14.632, 15.706 and was found to be 2.35 %, 1.69 % (**SAMPLE 2).** Overall, this study highlights the partially transformation of 1, 8 cineol into new products as shown by GC - MS.

Keywords: *Eucalyptus globulus*, Terpenoids, Hydro distillation, Chemical Modification, GC - MS

INTRODUCTION

Eucalyptus globulus is a fast-growing tree species that is native to Australia but is now widely cultivated throughout the world for its commercial and medicinal value. The leaves of *Eucalyptus globulus* are a rich source of terpenoids, which are a diverse class of natural compounds with a wide range of biological activities[1]. Terpenoids from *Eucalyptus globulus* have been shown to have antimicrobial[2], anti-inflammatory[3], and antioxidant[4] properties, among other activities, making them promising candidates for the development of natural products for various applications.

Extraction and chemical modification of important terpenoids from *Eucalyptus globulus* leaves is a topic of great interest in the field of natural products research. Several extraction methods have been used to isolate terpenoids from *Eucalyptus globulus* leaves, including hydro distillation[5], solvent extraction[6], and supercritical fluid extraction[7]. Chemical modifications of terpenoids, such as esterification[8] and oxidation[9], have also been explored to enhance their biological activity or to develop novel derivatives.

In this study, we aimed to extract and chemically modify important terpenoids from *Eucalyptus globulus* leaves. Hydro distillation was used for the extraction of terpenoids, and acetylation reactions were performed for their chemical modification. The extracted and modified terpenoids were characterized using analytical technique, such as Gas Chromatography - Mass Spectrometry (GC - MS).

The essential oil of *Eucalyptus globulus* leaves is known to contain various terpenoids, including Eucalyptol, (-) - Globulol, and α – terpineol[10] etc. Previous studies have reported the potential pharmaceutical applications of these terpenoids due to their bioactive properties. However, the chemical modification of these terpenoids to develop novel derivatives has not been extensively explored.

This study aimed to fill this gap in knowledge by partially transforming Eucalyptol into new products, as shown by GC - MS analysis. The modified terpenoids identified in this study included monoterpene and sesquiterpene hydrocarbons such as 3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl); Trans-5-Isopropyl-6, 7-epoxy-8-hydroxy-8-met; Gama-eudesmol; and 9H-Cycloisolongifolene, 8-oxo-. The results of this study have the potential to contribute to the development of new natural products that could have applications in various industries, including pharmaceuticals, cosmetics, and food.

Overall, this study contributes to the growing body of knowledge on the potential pharmaceutical applications of terpenoids from *Eucalyptus globulus* leaves. The chemical modification of terpenoids could lead to the development of novel derivatives, offering new opportunities for natural product drug discovery.

METHODOLOGY

Collection of plant material

The plant material viz, *Eucalyptus globulus* was procured from the Saharanpur region (Uttar Pradesh). Plant was got authenticated from Indian Institute of Integrative Medicine (CSIR) Jammu 180002.

Extraction of volatile oil

The fresh mature leaves (250 g) were completely immersed in water overnight, then hydro distilled for three hours for complete extraction of essential oil, using a commercial Clevenger apparatus. The light yellowish green coloured oil was obtained. The oil samples obtained from hydro distillation were freed from moisture by adding anhydrous sodium sulphate and absolute oil samples were obtained. The resulting oils was collected, preserved in a sealed amber glass sample tube and stored at 4°C under refrigeration until analysis.

Chemical Characterization of *Eucalyptus globulus* oil by GC - MS

The extracted oil from leaves (accurately weighed) by hydro distillation using Clevenger apparatus was analyzed by GC - MS. GC - MS revealed the presence some major compounds like Eucalyptol (28.09 %), Gamma - Terpinene (2.42 %), L – alpha - Terpineol (6.81 %), Alloaromadendrene (1.83 %), Epiglobulol (1.87 %), (-) - Globulol (10.66 %), Isolongifolene (2.09 %).

Chemical Modification of *Eucalyptus globulus* oil

For the chemical modification of *Eucalyptus globulus* oil, reactions were carried out using organic solvents like acetic anhydride and glacial acetic acid and a drop of mineral acids such as HCl and H_2SO_4. Reactions were carried out at ambient temperature for 4 hours. That reactant was further washed with alkali and water so that it could be free from acid. Furthermore the work deals with the synthetic modification of the active constituents of the oil into some analogues like Eucalyptol (1.58 %), Isobornyl acetate (2.35 %), Ylangene (1.69 %), Caryophyllenyl alcohol (5.16 %), (-) – Isolongifolol acetate (11.82 %); Eucalyptol (0.95 %), 3-

Cyclohexen-1-ol,1-methyl-4-(1-methylethyl) (2.18 %), trans-5-Isopropyl-6,7-epoxy-8-hydroxy-8-met (1.56 %), Caryophyllenyl alcohol (3.30 %), Gama-eudesmol (2.88 %), 9H-Cycloisolongifolene,8-oxo- (9.98 %).

RESULT and DISCUSSION

The oil was extracted from the Eucalyptus leaves by hydro distillation method and the result was shown in Table 1. From the Table 1, it was observed that Eucalyptol is the major constituent having Rt = 9.415 min and was found to be 28.09 %. Another major constituents are (-) - Globulol and α - terpineol having Rt = 13.966 min and 10.990 min and was found to be 10.66 % and 6.81 %. The result of modified oil indicate the partially transformation of 1, 8 cineol into new products as shown by GC - MS. From Table 2 **(For sample 1)**, it was clear that 1,8-cineol changed into terpene hydrocarbon and further rearranges to form 3-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl); Trans-5-Isopropyl-6,7-epoxy-8-hydroxy-8-met; Gama-eudesmol; 9H-Cycloisolongifolene,8-oxo- having Rt = 10.451, 13.492, 14.211, 15.142 and was found to be 2.18 %, 1.56 %, 2.88 %, 9.98 %. It is difficult to predict the formation of other products [Caryophyllenyl alcohol] mechanism. From Table 3 **(For sample 2)**, it was clear that 1, 8-cineol changed into terpene hydrocarbon and further rearranges to form Isobornyl acetate; Ylangene having R t= 14.632, 15.706 and was found to be 2.35 %, 1.69 %. It is difficult to predict the formation of other products [Caryophyllenyl alcohol; (-) - Isolongifolol acetate] mechanism.

Table 1: Peak Report TIC of *Eucalyptus globulus* oil (Parent Oil)

Peak#	R. Time	Area	Area%	Name	Base m/z
5	9.415	12290623	28.09	Eucalyptol	43.05
6	9.651	1058818	2.42	Gamma - Terpinene	93.10
10	10.990	2979449	6.81	L – alpha - Terpineol	59.10
12	12.966	799153	1.83	Alloaromadendrene	161.15

14	13.802	820154	1.87	Epiglobulol	82.10
16	13.966	4663868	10.66	(-) - Globulol	43.05
17	14.032	912144	2.09	(-) - Isolongifolene	109.15

Table 2: Peak Report TIC of Chemically Modified Oil (Sample 1)

Peak#	**R.Time**	**Area**	**Area%**	**Name**	**Base m/z**
1	9.423	123603	0.95	Eucalyptol	43.05
2	10.451	283227	2.18	3-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl)	81.10
10	13.492	203142	1.56	Trans-5-Isopropyl-6,7-epoxy-8-hydroxy-8-methyl	43.05
12	13.926	428997	3.30	Caryophyllenyl alcohol	111.10
14	14.211	374485	2.88	Gama-eudesmol	189.10
18	15.142	1298891	9.98	9H-Cycloisolongifolene,8-oxo-	157.10

Table 3: Peak Report TIC of Chemically Modified Oil (Sample 2)

Peak#	R.Time	Area	Area %	Name	Base m/z
1	11.758	175953	1.58	Eucalyptol	43.00
3	14.632	261516	2.35	Isobornyl acetate	95.10
6	15.706	187831	1.69	Ylangene	120.10
9	17.439	573508	5.16	Caryophyllenyl alcohol	111.05
12	18.326	1315187	11.82	(-) Isolongifolol acetate	161.10

CONCLUSION

Our results showed that the major constituents of the essential oil of *Eucalyptus globulus* are Eucalyptol having Rt = 9.415 min and was found to be 28.09 %; (-) - Globulol and α - terpineol having Rt = 13.966 min and 10.990 min and was found to be 10.66 % and 6.81 %.

Chemical modification of parent oil was carried out using organic solvents such as acetic anhydride, glacial acetic acid, and mineral acids such as HCl and H_2SO_4 separately at an ambient temperature and the chemical product were analyzed by GC-MS which confirms the presence of monoterpene and sesquiterpene hydrocarbons such as 3-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl); trans-5-Isopropyl-6,7-epoxy-8-hydroxy-8-met; Gama-eudesmol; 9H-Cycloisolongifolene,8-oxo- having Rt = 10.451, 13.492,

14.211, 15.142 and was found to be 2.18 %, 1.56 %, 2.88 %, 9.98 % (**SAMPLE 1);** and Isobornyl acetate; Ylangene having Rt = 14.632, 15.706 and was found to be 2.35 %, 1.69 % (**SAMPLE 2).**

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