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Comparative Screening of Phytochemical Compounds from Selected Clones of *Eucalyptus tereticornis*

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ABSTRACT
Eucalyptus tereticornis, commonly called as Eucalyptus, is a relatively fast growing tree upto 45 m tall or taller, bark is smooth, whitish, peeling in irregular thin sheets or large flakes. It belongs to the family myrtaceae. The wood contains 6-12% tannin and the bark 3-15%.It is one of the most important tree species in plantation forestry. The whole plant is also medicinally important as it contains enormous number of phyto-constituents which helps in curing ailments. The GC - MS analysis results of selected clones ofEucalyptus tereticornis (F-7, FC&RI-106 and ITC-1) bark showed that the compounds like hexadecanoic acid, methyl ester, quercetin and rhamnazin were present in all the three clones. Among the phytochemicals, the compound quercetin is found to be a natural colouring pigment which can be used in textile dyeing. The screened phytochemicals are known to possess anti-inflammatory, anti-microbial and anticancerous properties.
Keywords: *Eucalyptus tereticornis, bark, hexane extraction, GC-MS, phytochemicals, quercetin, rhamnazin, anti-microbial and anti-inflammatory*

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INTRODUCTION

Phytochemicals are naturally present in the plants and shows biological significance by playing an essential role in the plants to defend themselves against various pathogenic microbes by showing antimicrobial activity (inhibition or killing mechanisms). The secretion of these compounds varies from plant to plant; some produce more and some produce in minimal quantity. Sometimes they can be harmful and sometimes they can be very helpful [1]. India has a rich biodiversity and it is not only one of the world's twelve megadiversity countries, but also one of the eight major centres of origin. It has approximately 4,90,000 plant species of which about 17,500 are angiosperms; more than 400 are domesticated crop species and almost equal number in their wild relatives [2]. India is rich in natural wealth and there is ample scope to explore and revive the application of phytochemicals from plant kingdom. With regard to this, *Eucalyptustereticornis*, commonly called as Eucalyptus, is a relatively fast growing tree upto 45 m tall or taller, bark is smooth, whitish, peeling in irregular thin sheets or large flakes. The wood contains 6-12% tannin and the bark 3-15%. *E. tereticornis* occurs over a wide range of climatic conditions and principally in open-forest formation with a number of other eucalyptus and on river flats or hill slopes with alluvial or sandy to gravelly soils. The great adaptation capability, luring economics and ecological requirements of the area convinced the farmers to take up eucalyptus plantation in eucalyptus as well as non eucalyptus zone. It is considerably drought resistant but is susceptible to frost [3].Eucalyptus bark is one of the most important sources of yellowish-brown colourant. In addition, eucalyptus is a source for a multitude of known and unknown commercial properties, such as medicinal drugs, natural insecticides and non-timber products. The important compounds found in the *Eucalyptus* bark are Eriodictyol, Naringenin, Quercetin, Rhamnazin, Rhamnetin and Toxifolin, apart from tannins of which some are colourants [4]. The main objective of the present study is to identify the chemical constituents present in the clones of *Eucalyptustereticornis.*

MATERIAL AND METHODS

BARK COLLECTION

The bark was collected from the three selected clones of *Eucalyptus tereticornis* maintained at Forest College and Research Institute, Mettupalayam without harming the trees.

1. Clone F-7
2. Clone FC&RI-106
3. Clone ITC-1

WASHING AND DRYING

The collected bark samples were washed thoroughly with water and shade dried up to moisture content 9 – 11.5 % under room temperature with constant turning to inhibit fungal growth. The dried bark was later crushed to obtain coarse powder for easy extraction using soxhlet apparatus.

PREPARATION OF HEXANE EXTRACT

Exactly 5.0 grams of the crushed bark samples of eucalyptuswere extracted with 25 mL of hexane in an automated soxhlet apparatus (SOXTEC 2043 FOSS). The extraction was performed at 60^0 C for 2 hours and 30 minutes completing three cycles. All the phytoconstituents were extracted from the bark at the end of the third cycle. The extract was then dried at room temperature and stored at 4^0C in air tight sterile vials in the refrigerator.

GC - MS ANALYSIS

The chemical composition of the hexane extract of eucalyptus bark samples were analysed using Thermo GC - Trace Ultra Ver: 5.0 and Thermo MS DSQ II fitted with a DB 35 - MS capillary standard non - polar column (30 m, ID: 0.25 mm and film thickness of 0.25 µm). 0.5 µl of hexane extract was injected for analysis and Helium was used as a carrier gas at 1 mL/ min. The instrument was set as follows, Injector port temperature set to 250^0 C, source kept at 220^0 C. The oven temperature was programmed from 70^0 C to 260^0 C at the 6^0 C/ min rate. The MS was set to scan from 50 - 650 Da. The MS also had inbuilt pre - filter which reduced the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum, NIST4 and WILEY9 containing more than five million references.

IDENTIFICATION OF COMPOUNDS

Interpretation of mass spectrum of GC - MS was done using the database of National Institute Standard and Technology (NIST4) and WILEY9 [5]. The spectrum of the unknown component was compared with the spectrum of the known components stored in the inbuilt library.

RESULTS

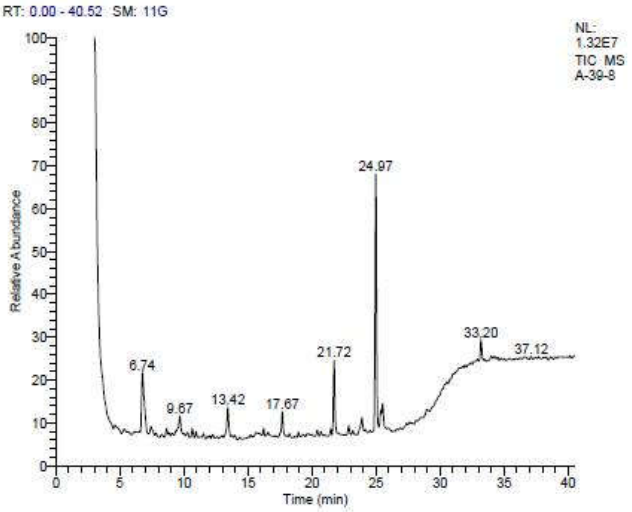
CHEMICAL COMPOSITION OF CLONE F-7

The results pertaining to GC-MS analysis led to the identification of number of compounds from the hexane extract of *Eucalyptus tereticornis* bark. The phytochemical compounds found in clone F-7 is presented in Table 1. The prevailing major components of clone F-7 were Cetene(4.55 %), Hexadecanoicacid(9.3 %), Octadecanoic acid (5.83 %), Quercetin (20.34 %) and Rhamnazin (17.71 %).The minor compounds were found to be Digitoxin,1-Butoxy-2-ethoxyethane, Z-7-Pentadecanol, Lucenin 2and Pyranthrene.The chromatograph of hexane extract from eucalyptus clone F-7by GC - MS is given in Figure 1.

Table 1. Phytochemicals identified in Eucalyptus clone F-7 bark extract

S.No.	RT (min)	Compound name	Molecular formula	Molecular weight	Area (%)
1.	5.33	1-Butoxy-2-ethoxyethane	$C_8H_{18}O_2$	146	0.87
2.	11.52	Digitoxin	$C_{41}H_{64}O_{13}$	764	0.54
3.	13.42	Cetene	$C_{16}H_{32}$	224	4.55
4.	21.72	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	9.3
5.	24.97	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	31.60
6.	25.51	Octadecanoic Acid	$C_{19}H_{38}O_2$	298	5.83
7.	27.39	Z-7-Pentadecenol	$C_{15}H_{30}O$	226	0.56
8.	27.60	Quercetin	$C_{18}H_{16}O_7$	344	20.34
9.	28.96	Rhamnazin	$C_{17}H_{14}O_7$	330	17.71
10.	32.91	Lucenin 2	$C_{27}H_{30}O_{16}$	610	0.55
11.	34.23	Pyranthrene	$C_{30}H_{16}$	376	0.57

Figure 1. Chromatograph of hexane extract of Eucalyptus clone F-7 barkby GC-MS



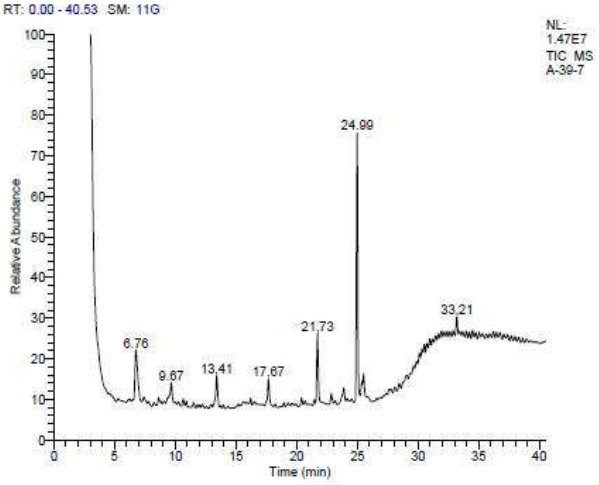
CHEMICAL COMPOSITION OF CLONE FC&RI-106

The active compounds of *Eucalyptustereticornis*clone FC&RI-106 are presented in Table 2. The results showed similarity with the results of clone F-7, containing major compounds such as Hexadecanoic acid (9.21 %), Quercetin (18.68 %) andRhamnazin (15.84 %).Apart from the above compounds, clone FC&RI-106 contained Methyl stearate and epi-PhotocitralA as additional compounds.Clone FC&RI-106 also constituted minor chemical compounds such asSynaptogenin B, Phytofluene, 3-n-Pentadecyl-2,4-dinitrophenol, Toxaphene and Flurandrenolide.The chromatograph of hexane extract from eucalyptus clone FC&RI-106by GC - MS is given in Figure 2.

Table 2. Phytochemicals identified in Eucalyptus clone FC&RI-106 bark extract

S.No.	RT (min)	Compound name	Molecular formula	Molecular weight	Area (%)
1.	21.73	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	9.21
2.	24.99	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	29.71
3.	25.51	Methyl stearate	$C_{19}H_{38}O_2$	298	6.76
4.	27.62	Synaptogenin B	$C_{30}H_{46}O_4$	470	0.95
5.	27.81	Quercetin	$C_{18}H_{16}O_7$	344	18.68
6.	28.90	Rhamnazin	$C_{17}H_{14}O_7$	330	15.84
7.	30.11	Phytofluene	$C_{40}H_{62}$	542	0.75
8.	31.93	epi-Photocitral A	$C_{10}H_{16}O$	152	6.28
9.	34.50	3-n-Pentadecyl-2,4-dinitrophenol	$C_{21}H_{34}N_2O_5$	394	0.88
10.	34.75	Toxaphene	$C_{10}H_8Cl_8$	408	0.64
11.	36.77	Flurandrenolide	$C_{24}H_{33}FO_6$	436	0.62

Figure 2. Chromatograph of hexane extract of Eucalyptus clone FC&RI-106 barkby GC-MS



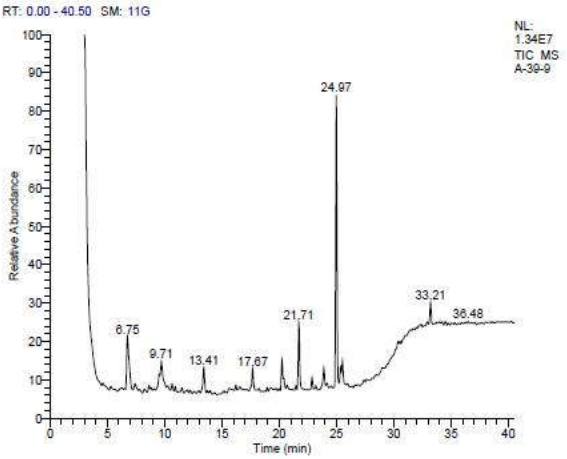
CHEMICAL COMPOSITION OF CLONE ITC-1

The chemical compounds in clone ITC-1 were found to be n-Pentadecanol (3.08 %), Hexadecanoic acid (8.17 %), Dioctyl phthalate (2.67 %), Quercetin (22.96 %) and Rhamnazin (19.58 %) (Table 3).Stephanine, Homotrypticene, N-Demethyl-buchenavianine, Methyl abietate and Thiirane, octyl constituted the minor compounds in clone ITC-1. The chromatograph of hexane extract from eucalyptus clone ITC-1by GC - MS is given in Figure 3.

Table 3. Phytochemicals identified in Eucalyptus clone ITC-1 bark extract

S.No.	RT (min)	Compound name	Molecular formula	Molecular weight	Area (%)
1.	4.61	Stephanine	$C_{18}H_{19}NO_3$	297	0.60
2.	5.30	Homotrypticene	$C_{22}H_{18}$	282	0.86
3.	8.23	N-Demethyl-buchenavianine	$C_{21}H_{21}NO_4$	351	0.47
4.	11.51	Methyl abietate	$C_{21}H_{32}O_2$	316	0.48
5.	17.67	n-Pentadecanol	$C_{15}H_{32}O$	228	3.08
6.	21.71	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	8.17
7.	24.97	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	33.50
8.	27.85	Quercetin	$C_{18}H_{16}O_7$	344	22.96
9.	28.90	Rhamnazin	$C_{17}H_{14}O_7$	330	19.58
10.	28.94	Thiirane, octyl	$C_{10}H_{20}S$	172	0.47
11.	33.21	Dioctyl phthalate	$C_{24}H_{38}O_4$	390	2.67

Figure 3. Chromatograph of hexane extract of Eucalyptus clone ITC-1 barkby GC-MS



DISCUSSION

The rise in demandto study the plant kingdom, which is one of the richest sources of promising versatile chemical compounds, is growing persistently throughout the world during the last few decades. Plants could play a great role in exploring new resources against the threat of new and recent diseases.

In the current investigation on chemical composition from the barks of Eucalyptus clones, the compound dibutyl phthalate was considered to be a contaminant in the hexane extract as the solvent leaches out the plastic material from the vials in which the extract was stored [6]. Hence the compound should be neglected from the chemical components screened.

The compoundsquercetin and rhamnazin was found to be present in all the three clones analysed, i.e. F-7, FC&RI-106 and ITC-1. The compound quercetin,a plant-derived flavonoid glycosides, has been used as a nutritional supplement and may be beneficial against a variety of diseases. Some of the beneficial effects include cardiovascular protection, anticancer, antitumor, anti-ulcer, anti-allergy, anti-viral, anti-inflammatory activity, anti-diabetic, gastroprotective effects, antihypertensive, immunomodulatory and anti-infective [7].Quercetin was also found to be a compound responsible for imparting colour to the fabrics when used as a natural dye [8]. The compound rhamnazin is commonly found in Artemisia, Alnus, Betula, Aesculus and Rhamnus spp. Rhamnazin gained wider acceptance among bio-actives for health care benefits due to its anti-oxident, anti-bacterial, anti-angiogenic and anti-tumor properties [9].All the three clones analysed were found to contain methyl ester of hexadecanoic acid which can be an effective antibacterial and antifungal agent [10]. This compound along with octadecanoic acid was also reported in the bark of many *Eucalyptus* species [11 and 12], calyx of the green *Hibiscus sabdariffa*[13], tuber parts of *Solenaamplexicaulis*[14], leaves of *Macrotylomauniflorum*[15], leaves of *Acacia nilotica*[16] and

Sinapisalba[17].Octadecanoic acid was reported to havepesticidal, antimicrobial and anti-inflammatory properties [18] and antimicrobial, antioxidant, anti-inflammatory activities[17].The phytochemical epi-photocitralA was noticed as a major compound in Clone FC&RI-106 and was also noticed in theessential oilof *Lippiacitriodora*.Epi-photocitralA is a desired natural flavour, used in beverages, confectionary, pharmaceuticals, cosmetics and perfumery industries [19].The n-pentadecanolconstituted as a major compound with an area percent of 3.08 per cent in the Clone ITC-1.Similar to the present studyn-Pentadecanol was also been reported in leaves of *Cyperusrotundus*[15] and fruit of *Elaeocar pusserratus*[20].n-Pentadecanol is used to control viscosity and dispersion characteristics in cosmetics, personal care products and pharmaceutical ingredients such as medications for the treatment of eczema.Dioctyl phthalate was found with an area percent of 2.67 per cent in Clone ITC-1 which was also observed in *Andrographis paniculata*[21] and of *Cleistanthuscollinus*[22].Dioctyl phthalate may act as a highly potential therapeutic agent for the people who are suffering from cancer [22].
Hence, Eucalyptus bark which is a rich source of secondary metabolites should be used effectively rather than just burning them up in boilers.

CONCLUSION

The eucalyptus bark which goes as waste in sectors like paper and pulp industry and composite wood industry can be effectively utilized for the extraction of useful secondary metabolites. The compound quercetin extracted from the bark of eucalyptus can also be used as a natural dye for textiles. It could be concluded from the present study that the quercetin extracted from eucalyptus bark can be used in medical textiles as it has anti-microbial property. Also, the bark could be exploited for promising versatile chemical compounds which play a great role against the threat of new and recent diseases.

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