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ESTIMATION OF PHYTOCHEMICAL CONSTITUENTS IN LEAF EXTRACT OF *EUCALYPTUS TERETICORNIS* CLONES

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ABSTRACT

A field trail was conducted to study the chemoprofiling of various phytochemical constituents present in different clones (No. 10, 413, 2040 and 2151) of *Eucalyptus tereticornis* leaves extract. The study was performed at village Majitha near Bhedaghat district Jabalpur (M.P.) India. Amongst the different clones, clone No. 413 had significantly higher leaf extract of 12.07 and 14.40 per cent with methanol and ethanol, respectively, as compared to other clones. Phytochemical analysis exhibited the presence of tannins, alkaloids and phenols in leaves extracted with methanol and ethanol extractant. Solvent ethanol was more suitable for extraction of various phytochemical constituents present in the leaf extract of *Eucalyptus tereticornis.* Clone No. 2151 recorded appreciably higher yield of 3.88 and 3.52 per cent of flavonoids in leaves extracted with methanol and ethanol, respectively, whereas Phenol was found to be 2.55 per cent in clone No. 413 followed by 2.41 per cent in clone No. 10 with ethanol.

KEYWORDS : ***Eucalyptus tereticornis*** **clones, medicinal plant, phytochemicals, leaves extracts**

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INTRODUCTION

Medicinal and aromatic plants are gifts of nature which contain hundreds or thousands of metabolites.[1] World Health Organization (WHO)[2] has defined medicinal plants as plants that contain properties or constituents that can be used for therapeutic purposes or those that synthesise metabolites to produce useful drugs.[3] Herbal medicines have become more popular in the treatment of many diseases having belief that green medicine is safe, easily available and with less side effects.[4] Eucalyptus is one of these plants and 300 species of this genus contain volatile oil in their leaves containing several metabolites which, may be used for various purposes. Secondary metabolites of plants serve as defence mechanisms against predation by many microorganisms, insects and herbivorous.[5] Plant based natural constituents can be derived from any part of the plant which may contain active components. The most important of these substances include flavonoids, saponins, tannis, steroids, terpenoids, alkaloids, cardiac glycosides, phenols and reducing sugars, etc.[6] The present investigation was aimed to study the phytochemical constituents in the leaves extract of *Eucalyptus tereticornis* clones.

MATERIALS AND METHODS

Collection and sampling of plant material

The field experiment was taken up at village Majitha where various clones of *Eucalyptus* were grown. Four clones of *Eucalyptus tereticornis* were selected to study the phytochemical constituents in the leaf extracts. Fresh leaves of the clones were collected, washed thoroughly under running tap water, dried in shade and then in hot air oven at 40-50^{0}C for 24-48 hours and homogenized for various analysis.[7] The crude extract was extracted with Soxhlet apparatus after making thimble for extraction with ethanol and methanol solvent.[8] The phytochemical screening of leaves extracts obtained from different clones of *Eucalyptus tereticornis* were screened using the method of Horborne (1973),[9] Trease and Evan (1989).[10] The ethanol and methanol extracts of leaves were subjected to qualitative and quantitative test using standard procedures to identify various plant constituents as follows:

Primary phytochemical screening

Flavonoids

Flavonoids were tested by heating 1 g powdered leaf sample with 10 ml ethyl acetate over a steam bath (40-50^{o}C) for 5 minutes; filtrate was treated with 1 ml dilute ammonia. A yellow colouration demonstrated presence of flavonoids.

Saponins

In 1 ml of leaf extract, 3ml of distilled water was added and shaken vigorously for 2 minutes. Frothing started which persisted on warming was an evidence for the presence of saponins.

Tannins

The presence of tannin was confirmed by boiling 0.5 g leaf powder sample in 20 ml distilled water, followed by the addition of 3 drops of 5% $FeCl_3$ to the filtrate, brownish- green or blue-black colour indicated the presence of tannins.

Steroids

1ml of the leaf extract was mixed with 2-3 ml of chloroform and equal concentrated Sulphuric acid was carefully poured along the sides of the test tube, two different layers were formed, the upper layer turns red and sulphuric acid layer showed yellow with green fluorescence indicated the presence of steroids.

Terpenoides

The leaf extract (1 ml) is mixed with 2-3 ml of chloroform and 2-3 ml of concentrated sulphuric acid carefully poured along the sides of the test tube to form two different layers. Formation of reddish-violet colour indicates the presence of terpenoids.

Alkaloid

Alkaloid detection in 1g powdered leaf sample with 5 ml each of methanol and 2N HCl was tested with Meyer's reagents. The samples filtrate having turbidity or precipitation indicated the presence of alkaloids.

Cardiac glycosides

The extraction was done by taking 2g of leaf sample with 10 ml methanol to identify cardiac glycosides. 5 ml of this extract was further treated with 2 ml glacial acetic acid and a drop of 5 per cent ferric chloride solution, transferring to surface of 1 ml concentrated H_2SO_4 forming a reddish brown ring at the junction of the two liquids confirming the presence of cardiac glycosides.

Phenols

To identify the Phenol, leaves extract was mixed with 2ml of 5% $FeCl_3$ solution. A violet-brown colour was developed, which indicate the presence of phenols.

Reducing sugar

Taking 0.5ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution were added and heated, the brick red precipitate formed indicated presence of reducing sugar.

Quantitative estimation of the phytochemicals

Estimation of total phenolic content

The amount of phenol in leaves extracts with methanol and ethanol was determined by Folin-Ciocalteu reagent method with some modifications. [11]2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of 2% solution of disodium carbonate (Na_2CO_3) were added to 1ml of leaf extract. The resulting mixture was allowed to stand for 15 minutes at room temperature. The absorbance of the sample was measured at 765nm. Gallic acid was used as standard (1 mg/ml). All the tests were performed in triplicates. The results were determined from the standard curve and expressed as gallic acid equivalent to (mg/g of extracted constituent).

Estimation of total flavonoid content

Aluminium chloride colorimetric method was used to determine flavonoid content. 1 ml of leaf extract sample was mixed with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water and stabilized at room temperature for 30 minutes. The absorbance was measured at 420 nm, Quercetin was used as standard (1 mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and expressed as quercetin equivalent to (mg/g of extracted constituent).[11]

RESULTS AND DISCUSSIONS

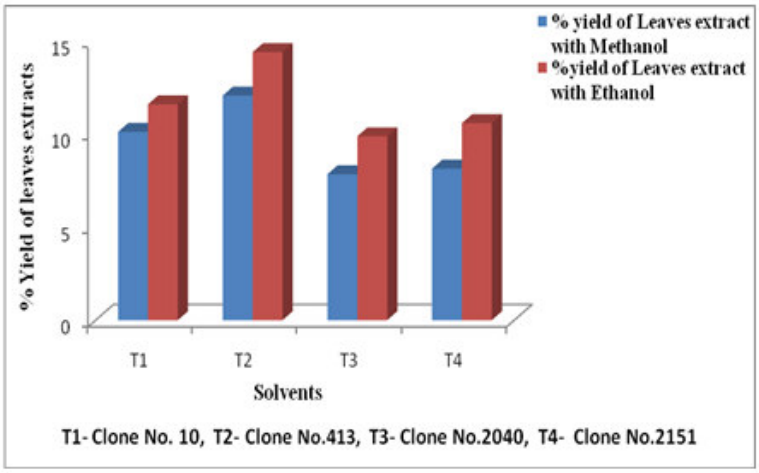
Table 1

Crude extract of different clones of Eucalyptus tereticornis obtained by using Methanol and ethanol extract

Eucalyptus Clones	% Yield of crude extract	
	Methanol	Ethanol
Clone No. 10	10.12	11.60
Clone No. 413	12.07	14.40
Clone No. 2040	7.84	9.88
Clone No. 2151	8.14	10.59
SEM ±	**0.170**	**0.177**
CD at 5%	**0.522**	**0.545**

Graph 1

Percent yield of leaves extracts of various Eucalyptus clones with Methanol and Ethanol



Clones of *Eucalyptus tereticornis*

The data presented in Table 1 indicate percent yield of crude extract with methanol and ethanol extractants in various clones of *Eucalyptus tereticornis* (Graph 1). The maximum yield of crude extract 14.40% and 12.07% with ethanol and methanol respectively, were noted in clone No. 413 which was significantly superior than the other clones. Clone No. 2040 registered the lowest crude extract yield of 7.84 and 9.88 per cent with methanol and ethanol extractants, respectively.

Table 2

Chemoprofiling of leaves extracts of different clones of Eucalyptus tereticornis extracted with methanol and ethanol solvents

S. No.	Phytochemicals	Clone Numbers							
		10		413		2040		2151	
		Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
1.	Flavonoids	+	+	-	+	+	+	+	-
2.	Saponins	+	-	+	+	+	+	+	+
3.	Tannins	+	+	+	+	+	+	+	+
4.	Steroids	-	+	+	-	+	+	+	+
5.	Terpanoides	+	+	+	+	+	+	-	+
6.	Alkaloids	+	+	+	+	+	+	+	+
7.	Cardiac Glycosides	+	+	-	-	-	-	+	+
8.	Phenols	+	+	+	+	+	+	+	+
9.	Reducing Sugar	+	+	-	+	-	+	+	+

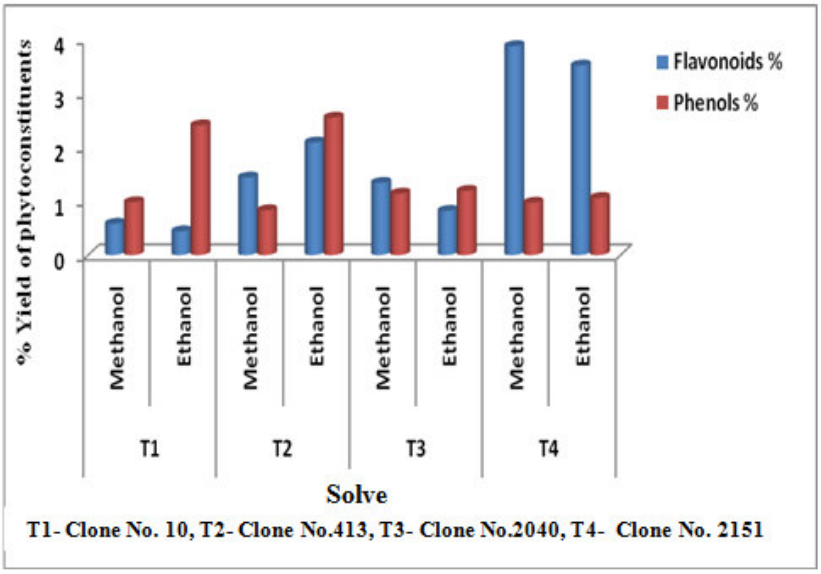
(+)Present, (-) absent

The primary screening of phytochemical constituents present in crude extract of all the clones extracted with methanol and ethanol extractants exhibited the presence of tannins, alkaloids and phenols in all the four clones. Whereas positive presence of flavonoids was recorded in clone No. 10 and 2040, steroids in No. 2040 and 2151, cardiac glycosides and reducing sugar in No. 10 and 2151. The chemical constituents saponin in the ethanolic extract of clone No. 10 and terpanoides in methanolic extract of clone No. 2151 were found to be totally absent (Table 2).

Table 3
Estimation of phytochemical constituents in leaves extract of Eucalyptus tereticornis clones with methanol and ethanol solvents

Clone No.	Solvents	Flavonoids %	Phenols %
10	Methanol	0.58	0.98
	Ethanol	0.44	2.41
413	Methanol	1.44	0.83
	Ethanol	2.09	2.55
2040	Methanol	1.34	1.14
	Ethanol	0.82	1.19
2151	Methanol	3.88	0.97
	Ethanol	3.52	1.06

Graph 2
Phytochemical constituents in leaves extracts of various clone with methanol and ethanol solvents



The data presented in Table 3 and depicted through Graph 2 revealed that the leaves extract with methanol and ethanol of various clones (No. 10, 413, 2040 and 2151) indicated the presence of flavonoids and phenols. Clone No. 2151 recorded 3.88 and 3.52 percent of flavonoids in leaves extract with methanol and ethanol, respectively, which was appreciably higher than the other clones. Whereas phenol was found to be maximum (2.55 %) in clone No. 413 followed by 2.41 percent in clone No. 10 with ethanol leaves extract. The secondary metabolites produced by medicinal plants are referred to as phytochemicals [12-14] which are divided into several categories that includes alkaloids, flavones (flavanoids, flavonols, quinones), essential oils, lectins, polypeptides, phenolic compounds, polyphenols, tannins, terpenoids, saponins, coumarins, carbohydrates, lipid proteins and nucleic acids etc.[15] have also reported similar result. In most of the cases the presence of different phytochemical constituents were more in the leaf extract with ethanol indicating the suitability of ethanol extractant for extraction of various phytochemical constituents. The similar results were also reported by Edeoga et. al. (2005).[16] Kumar et. al. (2007)[17] noticed the most important bioactive constituents, i.e. alkaloids, tannins, flavanoids and phenols, while Yadav and Agarawal (2011)[18]recorded the presence of proteins, carbohydrates, phenols, tannins, flavanoids and saponins in all the plants under study.

CONCLUSION

The results revealed that clone No. 413 was found to be more suitable in respect of giving a higher percent yield of crude extracts as compared to all other clones. The chemoprofiling exhibited the presence of Tannins, Alkaloids and Phenols in crude extracts of all the clones of *Eucalyptus tereticornis* by using methanol and ethanol solvents. It was noticed that ethanol appeared to be more suitable extractant for extraction of various phytochemical constituents present in the crude extract of different clones of *Eucalyptus tereticornis*.

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