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Research Article

PHARMACOGNOSTIC, PHYTOCHEMICAL PROPERTIES AND ANTIBACTERIAL ACTIVITY OF *CALLISTEMON CITRINUS* VIMINALIS LEAVES AND STEMS

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ABSTRACT

Callistemon citrinus Viminalis is commonly known as 'Bottlebrush' plant of family myrtaceae that has a great medicinal importance. Traditional uses of the aerial parts of *Callistemon citrinus* in ethnic tribal communities are in practice, and very little are known about its importance on scientific grounds. The present study deals with the pharmacognostic evaluation including examinations of morphological and microscopic characters, ash values, powder analysis, extractive values, and moisture content. Phytochemical analysis was carried out for the identification of various plant constituents. The antibacterial potential was examined against *Bacillus subtilis* (NCIM 2079), *Escherichia coli* (NCIM 2065) and *Pseudomonas aeruginosa* (NCIM 2200). The transverse section of leaves showed the presence of polygonal epidermal tissues, unicellular covering trichomes, anomocytic stomata, calcium oxalate crystals, parenchymatous tissues, lignified xylem, phloem (vascular bundle) and stem showed the presensce of medullary rays, oil glands cortex. Phytochemical study confirmed the presence of steroid, saponin, terpenoids, flavonoids, proteins were further characterized by TLC analysis and compared with available literature. Total ash, acid insoluble ash, water soluble ash, water soluble extractive, alcohol soluble extractive and moisture content of leaves were 4.65%, 1%, 2.45%, 11.5%, 14.5% and 3.4% w/w respectively and stems were found 10.5%, 4.5%, 3.6%, 13.5%, 12.5% and 4% w/w . The present study may contribute to the development of standardization parameters of the plant which helps in the botanical identification of *Callistemon citrinus.*

Keywords: *Callistemon citrinus*, Pharmacognostic study, Phytochemical evaluation, Thin layer chromatography, Antibacterial activity.

INTRODUCTION

Callistemon citrinus commonly known as Bottlebrush belongs to a family myrtaceae and comprises over 30 species that has a great medicinal importance [1]. They are woody aromatic trees or shrubs (ca. 0.5 m to 7 m tall) widely distributed in the wet tropics, notably Australia, South America and tropical Asia, but are now spread all over the world [2]. *Callistemon* species have attractive narrow foliage and white papery bark [3]. The 1, 8-cineole and alpha-terpineol have been isolated as major compounds from the leaves and flowers of *Callistemon citrinus* (syn. *Callistemon lanceolatus*) that have anthelmentic activity[4]. Moreover, antistaphylococcal, nematicidal, larvicidal, pupicidal, antithrombotic activities of the genus callistemon, and antioxidant activities have been documented [5,6]. Moreover, traditional uses of the aerial parts of *Callistemon citrinus* in ethnic tribal communities are in practice, and very little are known about its importance on scientific grounds [7]. In China callistemon species, especially *C. viminalis*, are used in Traditional Chinese Medicine pills for treating hemorrhoids [8]. *Callistemon* are also used as weed control [9] and as bioindicators for environmental management [10]. The work was undertaken to study pharmacognostic, phytochemical and antibacterial activity of *Callistemon citrinus*.

MATERIALS AND METHODS

Chemicals

All the chemicals and reagents used were of analytical grade purchased from Sigma Chemical Co. (St Louis,MO, USA) and Merck (Darmstadt, Germany).

Plant material

The *Callistemon citrinus* viminalis was obtained from Nasik district (M.S.) and authenticated by Dr. D. A. Patil, reader and the authorized plant identifier of Department of Botany, SSVPS College, North Maharashtra University, Dhule (M.S); a specimen is preserved in the college herbarium (KBHSS/PCG/2010/105).

Pharmacognostic study

Macroscopy and Microscopy of leaves and stem

The shape, size, color, odor, taste, surface texture and fracture characteristics of the leaves and stems were determined. Microscopy of stem and leaves were studied by taking the transverse section (T.S.) using a microtome. The obtained sections were cleared with chloral hydrate solution, for the identification of various regions. Powder characteristic of the dried stem was separately performed. Phloroglucinol- hydrochloric acid (1:1), iodine and glycerin were used as staining agent and aid [11,12].

Physico-Chemical Constants

Total ash, water soluble ash, acid insoluble ash and sulphated ash were determined. Alcohol and water soluble extractive values were determined to find out the amount of water and alcohol soluble components [13,14].

Behavior of leaf powder with different chemicals / reagents

Behavior of leaf and stems powder with different chemical reagents were studied to detect the presence of phytoconstituents with color changes under daylight by reported method [15].

Extraction of plant material

The leaves and Stem of the plant were dried in natural sunlight for 7 days. The plant materials were pulverized and powders were successively treated with petroleum ether, chloroform and methanol by using hot percolation method. The extracts were evaporated to dryness under reduced pressure at 45°C to give solid residue. The residue were weighed and stored in refrigerator for further phytochemical study [16].

Phytochemical Screening

The petroleum ether, chloroform and methanol extracts were screened for phytochemical for the presence of its constituents utilizing standard methods of analyses[17].

Thin layer chromatography

For the TLC fingerprint the petroleum ether extract, chloroform extract and methanolic extract were subjected to thin layer chromatographic analysis, to find the presence of number of chemical constituents to support the chemical test. Analytical TLC plates were prepared by pouring the silica gel G slurry on the glass plates. Drying the thin layer plates, for 30 minutes in air and then in an oven at 110° C for another 30 minutes. For qualitative work, spot was applied in a row along one side of plate, about 2cm from edge,

by using capillary tubes. The range of sample volume was controlled, spreading not more than 0.5cm.The plate was placed in previously saturated TLC chamber with mobile phase. The chromatographic condition were described in table 1. The R_f values are compared with standard drug and colours are recorded[18,19].

Antibacterial activity[20,21,22]

Microorganisms

The following cultures were used: *Bacillus subtilis* (NCIM 2079), *Escherichia coli* (NCIM 2065) and *Pseudomonas aeruginosa* (NCIM 2200). The cultures are obtained from National Collection of Industrial Microorganism (NCIM) Pune, India. The cultures of these bacteria were grown in nutrient broth at 37°C and maintained nutrient agar slants< 12°C.

Chemical

Ciprofloxacin was procured from Ranbaxy research lab., Gurgaon, India. All the chemicals were of analytical grade and used as received.

Preparation of Inoculum

Several colonies of a 48 hr culture of *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* were suspended in sterile saline solution (0.9%). Turbidity was adjusted to an absorbancy of 0.18 to 0.25 at 625nm.

Well plate method

The test solution of petroleum ether, methanolic and chloroform extract of leaves and stems were prepared at a concentration of 25, 50, 75, 100 mg/ml. Ciprofloxacin was taken as standard for antibacterial activity at a conc. 10 µg/ml. Nutrient agar medium was prepared and sterilized by an autoclaving at 15 psi for 15 minutes. In aseptic room the medium was poured into sterile petri dishes to uniform depth and then allowed to cool at room temperature. The inoculums of test organisms were spread on Nutrient agar plates. Wells of 6 mm were punched into the agar medium and filled with test solution and compared with control. The plates were incubated for 48 hours at 37°C. The antibacterial activity was evaluated by measuring the zone of inhibition against test organism.

RESULT AND DISSCUSSION

Morphological study

The leaves of *Callistemon citrinus* was evergreen, lanceolate (ca. 3-6 mm wide and 40-70 mm long) in shape, alternate in arrangement, entire margin, pinnate venation, very aromatic and stem was grey in colour (Fig.1).

Microscopical Evaluation

The leaf surface shows the anomocytic stomata which is characteristics of myrtaceae family. Transverse section shows the epidermal layer followed by cuticle layer and vascular bundles (xylem and phloem), pericyclic fibers, Collenchyma, unicellular trichomes etc. The transverse section of stem shows epidermal layer, 2-3 layer of cork tissue, 7-8 layer of cortex tissue, Medullary rays, endodermis, xylem vessels, oil glands, sclerides in stellar region and pith at center region(figure 2, 3) .Observation and result pertaining to micro chemical tests and behavior of specific reagent towards plant tissue were represented in Table 2**.**

The powder microscopy of leaf shows the fragments of unicellular covering trichomes, xylem vessels, parenchymatous cells and stems shows Medullary rays, starch grains, xylem vessels, cork, cortex tissue (Figure. 4).

Physiochemical study

Many physiochemical parameters were evaluated and result was depicted in table 3. Behavior of powder drug towards different chemical reagent were present in table 4.

Phytochemical screening

Phytochemical investigation of petroleum ether, chloroform and methanolic extract of leaves and stem showed presence of phenolic compounds, saponins, terpenoids, fatty acids, flavonoids and carbohydrates (Table 5).

Thin Layer Chromatography

The plate was developed in respective mobile phase and sprayed with respective spraying reagent (table 1).pertroleum ether extract showed pink – violet spot for triterpenes and steroids. Chloroform extract showed light blue and dark blue color for saponin glycoside. Methanolic extract showed yindicate terpenoids and steroid and yellow fluroscence under UV light (fig.5 & table 6).

Antimicrobial activity

Antimicrobial activity of various extracts of leaves and stems were studied by measuring the zone of inhibition formed around the agar well and the results are given in Table7, 8. All the extracts showed good activity against *P. auregenosa, E. coli* and *B. subtilis.* All extracts failed to show any activity against any of the fungi used. Thus the plant shows antimicrobial activity and can be a potent ingredient for herbal products.

Table 1: Shows chromatographic conditions for different extracts

Extract	Plate number	Mobile phase	Spraying reagents
Petroleum ether extract of laves and stem	Plate 1	Tolune: Ethyl acetate	Libermann-burched
Chloroform extract of leaves and stems	Plate 2	Acetone:n-propanol: Water (35:35:5)	Anisaldehyde- sulphuric acid and heat at 100°C
Methanolic extract of leaves and stems	Plate 3	Ethyl acetate: Methanol: Water: Toluene (10:1.5:1.3:2)	Uv at 254nm

Fig. 1: shows the morphology of leaves and stems of *callistemon citrinus* viminalis

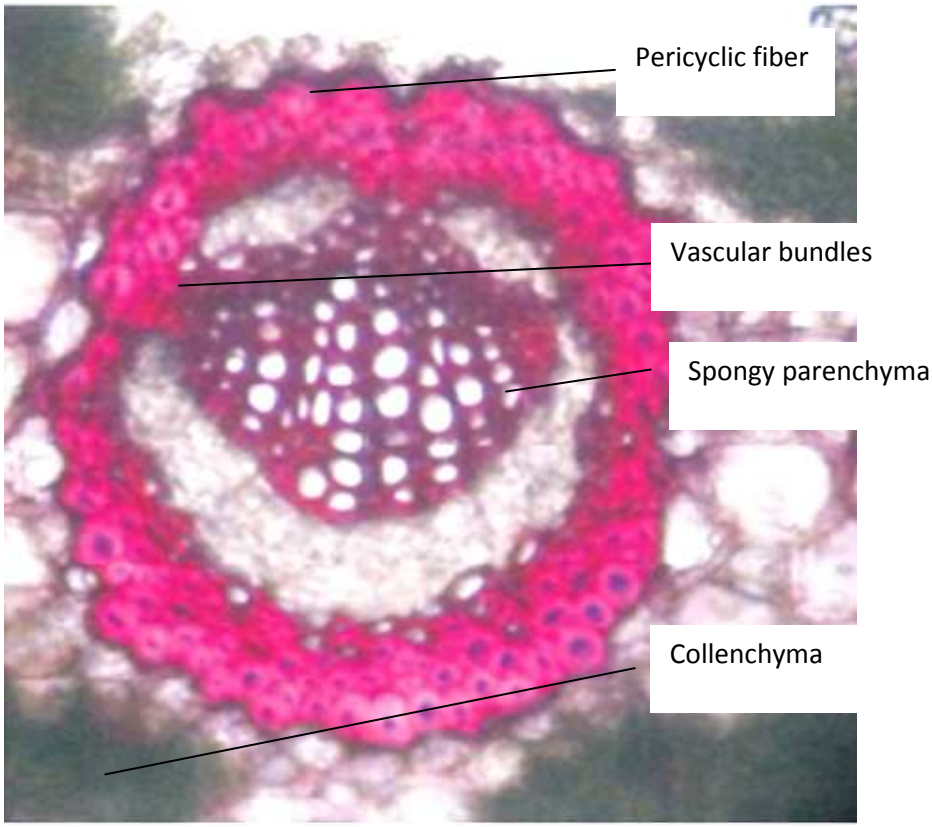


Fig. 2: Shows transverse section of leaves treated with phloroglucinol: hydrochloric acid(1:1)

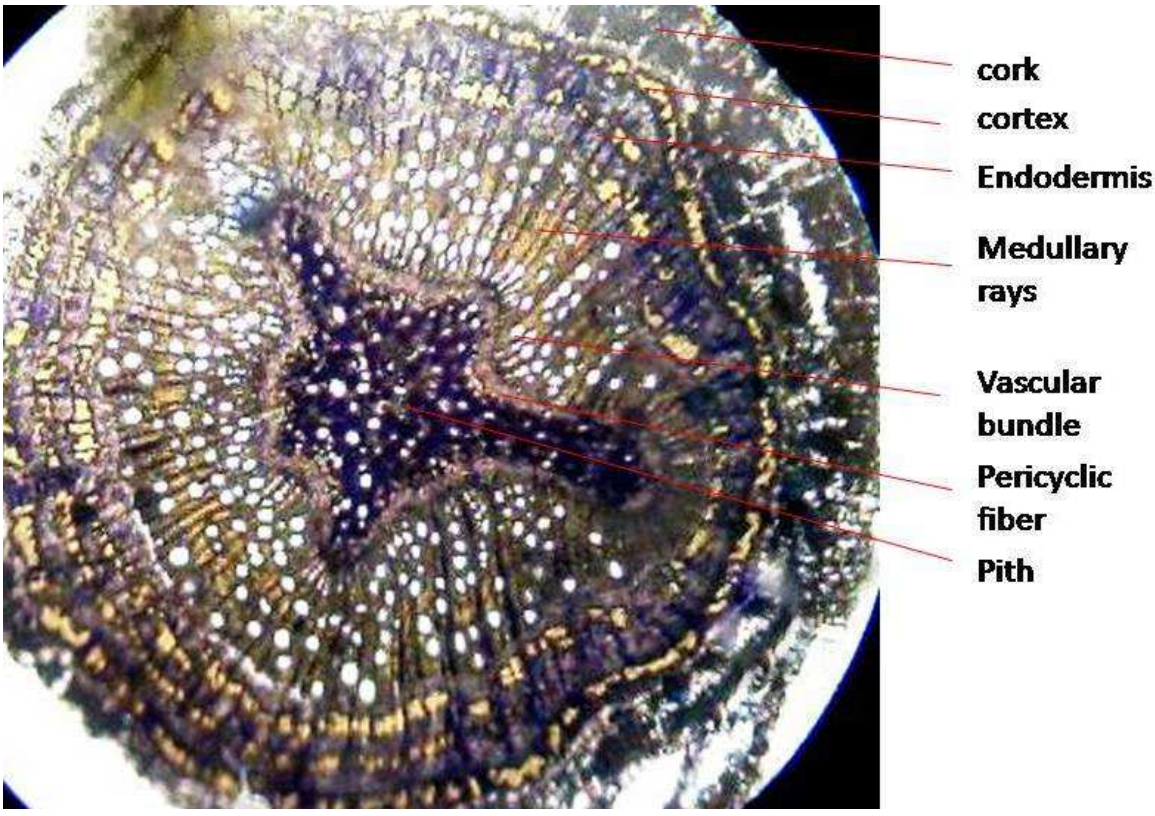


Fig. 3A: Shows transverse section of *callistemom citrinus* stem

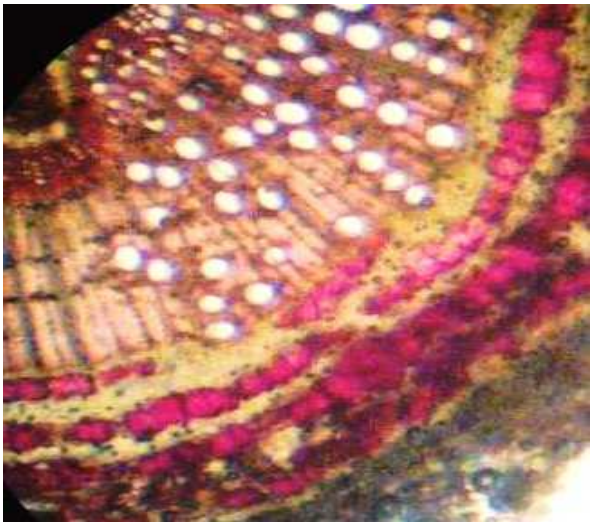


Fig. 3B: Medullary rays

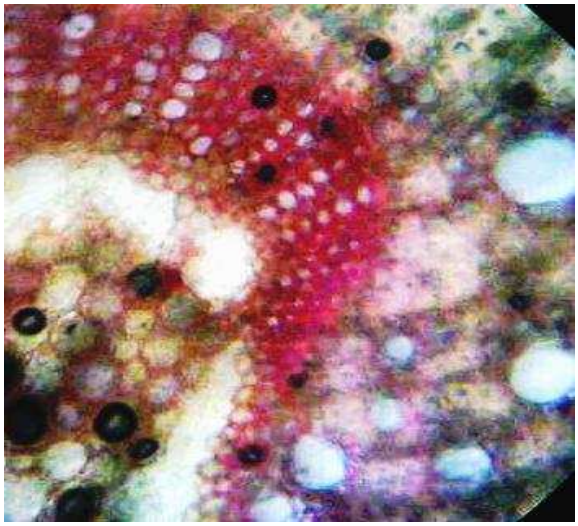
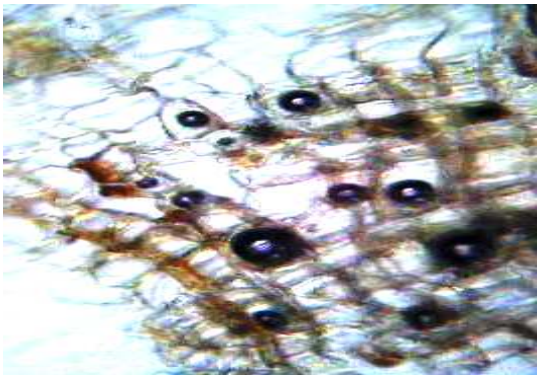


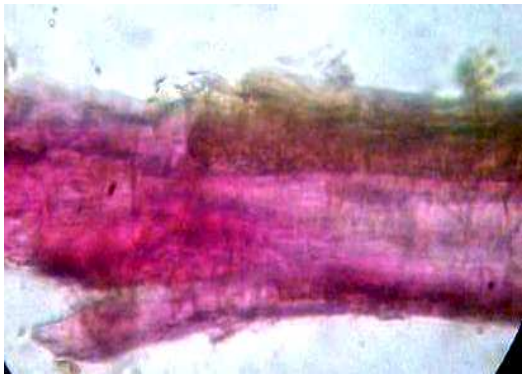
Fig. 3C: Vascular bundle

Table 2: Shows Histochemical reaction of leaves and stems

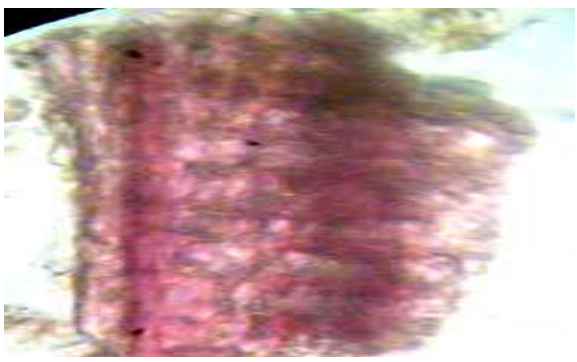
Reagents	Constituents	Colors	Histological zones
Aniline So_4 + H_2SO_4	Lignin	Yellow	Cortex
Phloroglucinol + HCl	Lignin	Pink	Xylem vessels, medullary rays
Weak Iodine solution	Starch	blue	Cortex
Sudan red II	Volatile oil	Red	Stellar region of coterx, mesophyll
Millons reagent	Proteins	White	pith
H_2SO_4	Ca. Oxalate	Needles/prismatic	coterx



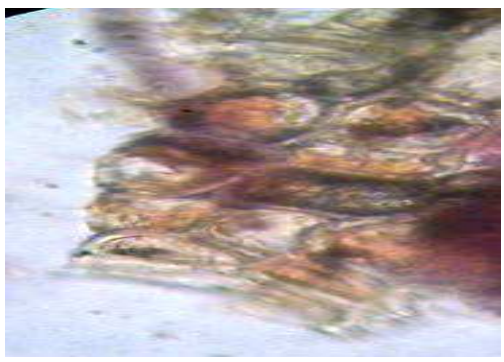
A: Cortex



B: Sclerenchymatous fibres

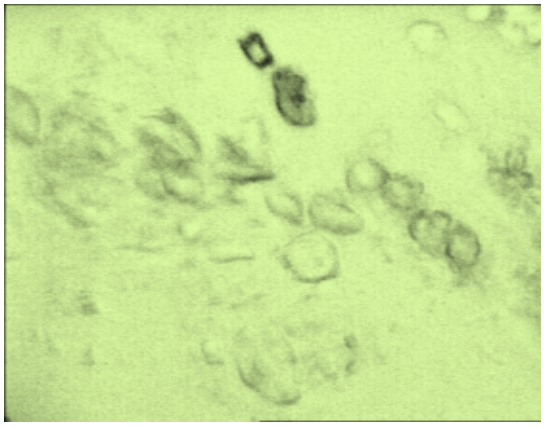


C: Medullary rays



D: Volatile oil gland

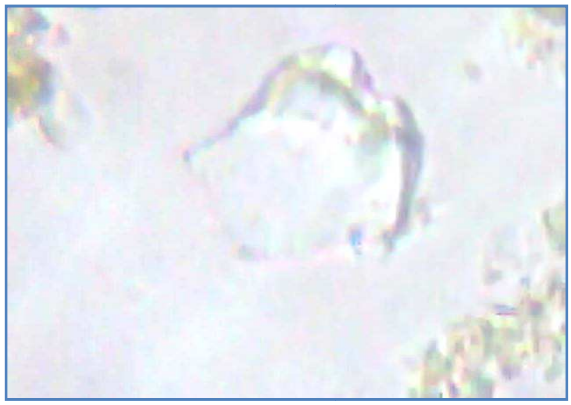
Fig. 4: Shows powder characteristics of *C. Citrinus* stem



A: oil glands

B: Xylem fibres

C: covering trichomes



D: Calcium oxalate crystals

Fig. 5: Shows powder characteristics of *C. Citrinus* leaves

Table 3: Shows physiochemical parameter

S. No.	Parameters	% w/w (leaf)	%w/w (stem)
1.	Ash value		
	i. Total ash	4.65	10.5
	ii. Acid insoluble	2.5	4.5
	ii. Water soluble	2.45	3.6
2.	Extractive values		
	i. Water soluble	11.5	13.4
	ii. Alcohol soluble	14.4	12.5
3.	Moisture content (LOD)	3.4	4

Behavior of powder drug towards different chemical reagent was present in table 4.

Table 4: Shows Behaviour Of Powder Drug Towards Different Chemical Reagents

Regents	Color/ppt	Constituents
Picric acid	Slight ppt.	Alkaloids present
Conc. H_2SO_4	Reddish brown	Steroids/triterpenoids present
Aq. $Fecl_3$	Bluish black ppt	Tannins present
Iodine solution	Blue	Starch present
Ammonia	No change	Anthroquinone glycosides absent
Spot test	Stains observed	Fixed oils present
Aq. $AgNo_3$	Precipitation	Proteins present
Aq. NaoH	Yellow	Flavonoids present
Mg – Hcl	Magenta	Flavonoids present
Aq. Lead acetate	White ppt	Tannins present
Liberman Burchardt's test	Reddish green	Steroids and tannins are present

Table 5: Phytochemical screening of extracts

S. No.	Chemical test	PEL	PES	CEL	CES	MEL	MES
1.	Alkaloids						
	i.Dragendorff's	-	-	+	+	+	-
	ii.Wagners	-	-	-	+	+	+
2.	Amino acids and proteins						
	Xanthoproetic test	+	+	-	-	++	++
3.	Carbohydrates						
	Molish test	+	+	+	+	++	++
4.	Flavonoids						
	i. Shinoda test	-	-	-	-	++	++
	ii. Lead acetate test	-	-	-	-	++	++
5.	Glycoside						
	i. saponin	-	-	+	+	-	-
6.	Tannins						
	i. Lead acetate test	-	-	+	+	++	++
	ii. Ferric chloride test	-	-	+	+	++	++
7.	Phytosterol						
	i. Salkowski test	++	++	+	+	-	-
	ii. Liebermann's Burchard reaction	++	++	+	+	-	-

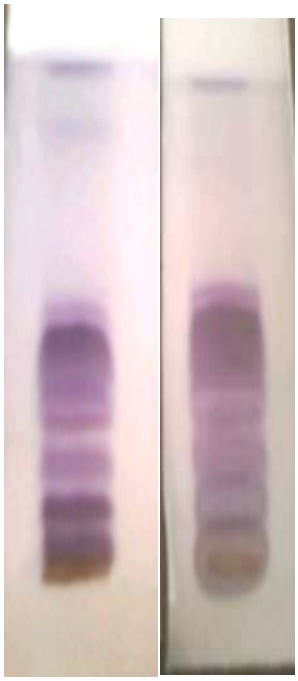


Fig. 6A: TLC of PEL & PES

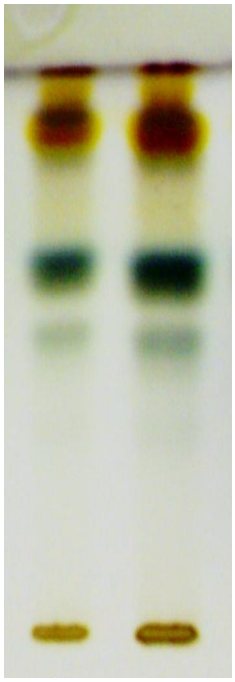


Fig. 6B: TLC pattern of CEL&CES

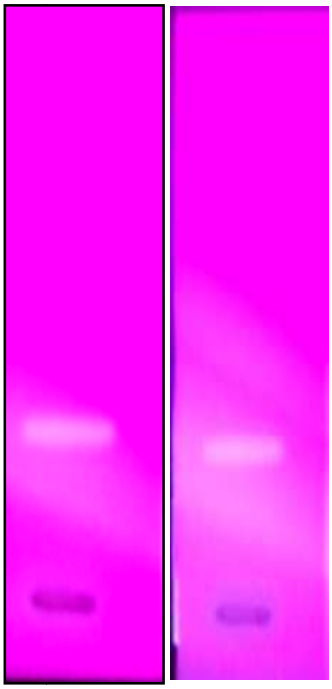


Fig. 6C: TLC pattern of MEL & MES

Fig. 6: Shows TLC patterns of various extracts of leaves and stem of *C. citrinus*

Whereas, PEL: Petroleum ether extract of leaves; PES: Petroleum ether extract of stems; CEL: Chloroform extract of leaves; CES: Chloroform extract of stems; MEL: Methanolic extract of leaves; MES: Methanolic extract of stems

Table 6: Thin layer chromatography of leaves and stem extract of *Callistemom Citrinus*

S. No.	Plate no.	Solvent system	Spraying reagent	No. and colour of spot	R*f* value
1.	Plate 1 (Peroleum extract)	Tolune: Ethyl acetate	Libermann-burched	7(violet)	0.12, 0.23, 0.25, 0.32, 0.36, 0.48, 0.53 respectively
2.	Plate 2 (chloroform extract)	Acetone:n-propanol: Water (35:35:5)	Anisaldehyde- sulphuric acid and heat at 100°C	4(grey, greenish blue, yellow, violet)	0.58, 0.67, 0.87, 0.92 respectively
3.	Plate 3 (methanolic extract)	Ethyl acetate: Methanol: Water: Toluene (10:1.5:1.3:2)	Uv at 254nm	1 (yellow)	0.48

Table 7: Shows antibacterial activity of *C. Citrinus* leaves extract

Microorganism	Zone of inhibition(mm)												
	Ciproflox acin (µg/ml)	Petroleum ether extract (mg/ml)				Chloroform extract (mg/ml)				Methanolic extract (mg/ml)			
	10	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**	**25**	**50**	**75**	**100**
B. subtilis	24.6±0.66	--	5±0.78	8±0.55	12±0.33	6±0.00	9.2±0.01	12.3±0.66	15.5±0.33	11.4±0.33	14.54±0.66	20.7±0.00	25.09±0.33
E. coli	25.4±0.33	6±0.33	8.03±0.33	11.2.±0.44	14.5±0.00	9.5±0.33	12.6±0.66	15.6±0.66	18.6±0.66	8.5±0.33	13.4±0.33	18.6±0.66	23.21±0.33
P. aeruginosa	28.7± 0.33	8.4±0.66	12.9±0.00	16.5±0.33	19.4±0.44	5.8±0.33	8.9±0.44	12.3±0.66	15.6±0.33	11.34±0.44	16±0.33	192±0.44	24±0.33

Table 8: Shows antibacterial activity of *C. Citrinus* stem extract

Microorganism	Zone of inhibition(mm)												
	Ciproflox acin µg/ml)	Petroleum ether extract (mg/ml)				Chloroform extract (mg/ml)				Methanolic extract (mg/ml)			
	10	25	50	75	100	25	50	75	100	25	50	75	100
B. subtilis	20.6±0.66	4.5±0.44	9.8±0.44	14±0.55	12±0.33	6±0.00	9.2±0.01	12.3±0.66	15.5±0.33	10.3±0.33	14.23±0.66	18.9±0.00	22.14±0.33
E. coli	24.4±0.33	5±0.33	7.03±0.33	9.9±0.66	11.3±0.00	8±0.33	10.3±0.33	12.7±0.66	14.9±0.66	6.4±0.55	10.4±0.33	15.4±0.66	20.21±0.33
P. aeruginosa	25.7± 0.33	5±0.33	7±0.00	9.2±0.33	12.3±0.44	5.03±0.33	7.8±0.33	10.4±0.66	12.9±0.33	10.34±0.44	14±0.33	18.2±0.44	23±0.33

CONCLUSION

It is concluded that the above pharmacognostic and phytochemical parameters are very useful for the identification and authentication of the species. The results of the present study will also be helpful in preparation of monograph. *Callistemom citrinus* exhibit significant and consistent antibacterial activity with relatively lower MIC values indicating to undertake further fractionation analysis to isolate the antibacterial compound of therapeutic importance.

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