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Preliminary phytochemical content and antibacterial activity of Ukshi (*Calycopteris floribunda* Lam.) leaves

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Abstract
Calycopteris floribunda Lam. is a large diffuse or scandent, climbing shrub belongs to the family Combretaceae. In this study, various solvent extracts (petroleum ether, chloroform, ethyl acetate, methanol and aqueous) of *Calycopteris floribunda* leaves were investigated for phytochemical and antibacterial activities. Among the other solvent extracts, ethyl acetate extract of leaves was showed the effective results for alkaloids, tannins, flavanoids, resins, cardiac glycosides, phenols, terpinoids, sterols and saponins. The leaves of *Calycopteris floribunda* showed the presence of terpinoids, sterols, alkaloids and flavanoids. The methanolic leaf extract was showed the better antibacterial activity against the four pathogenic bacteria strains such as *Bacillus subtilus, Pseudomonas arginoses, Actinomycetes sp.* and *Seratia sp.* than the other solvent extracts. Among the other bacteria, Bacillus strain was highly affected by the leaf extract.

Keywords: *Calycopteris floribunda*, Ukshi, Pullani, Combretaceae, Phytochemical compounds and Antibacterial activity.

1. Introduction
Calycopteris floribunda Lam. is an evergreen climbing shrub, locally known as Ukshi or Pullani. It is commonly referred as a lifesaver by the forest dwellers. During summer period the vine stores water which people often use to quench their thirst. The plant parts especially leaves being used medicinally for various complications such as intestinal worms, colic, leprosy, malarial fever, dysentery, ulcers and vomiting. The fruits are useful in jaundice, ulcers, pruritus and skin diseases. A number of phenolic and non-phenolic flavonoids including cytotoxic, anthelmintic and antiviral properties have been isolated from the plant. The tender copper coloured leaves ground into paste or dry powders administered for the expulsion of bacteria, free radicals and round worms [4, 7]. According to Nadkarni, the extract of leaves exhibits the colour reactions of santonin. Sreekanth et.al, (2007) done their studies on leaf extracts of *C. floribunda* revealed that the extracts were toxic to calf, rabbits and rats [9]. Nowadays, Microorganisms were developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases [2]. This situation forced scientists to search for new antibacterial substances from various sources, such as medicinal plants. Among the different plant derivatives, secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity [6]. So, there is a continuing need for new antibacterial and antifungal agents since none of the available drugs is free from adverse effects and limitation. In this present study, the phytochemical and antibacterial properties of leaf extracts were studied.

2. Materials and methods
The plant materials were collected from Muthanga forest, Wayanad, Kerala. The collected fresh leaves were cleaned and dried in shade for a week. Finally sample was grinded to fine powder mechanically.
The powdered sample in which 10 g was kept steeped for 72 h in the solvents like petroleum ether, chloroform, ethyl acetate, methanol and distilled water. The extracts were filtered and concentrated by evaporation. In the phytochemical study, the presence of alkaloids was tested by Mayer's and Wagner's methods. The presence of flavonoids was determined by Shinoda, sulphuric acid and ferric chloride tests. The presence of terpenoids, cardiac glycosides,

saponins, tannins, resins and phenols were studied respectively by Salkowaski test, Keller-Killani test, Salkowaski test, Foam test, Braemer's test, sulphuric acid test and ferric chloride test. The presence of sterols was estimated by Salkowaski and Liberman-Burchard's tests. The results were tabulated (Table No.1). Antibacterial activity was studied by agar diffusion method. The nutrient agar plates were swabbed with each of the microbial strains such as Bacillus, Pseudomonas, Actinomycetes and Seratia. A sterile swab was dipped into the broth and expressed any excess moisture by pressing the swab against the side of the tube. The filter paper disks were impregnated with the solvent extracts of the leaves of *C. floribunda.* Each free disc was placed into the agar with the sterileforceps and incubated at 37 °C for 3 to 4 days. The zone of inhibition was measured separately for each bacterial strain using a scale. The results were collected and tabulated (Table No 2).

Fig 1: Habit of *Calycopteris floribunda* Lam.

3. Result and discussion

In the present study, the leaf extracts were tested for the preliminary phytochemical analysis and antimicrobial activity.

Table 1: The presence of secondary metabolites in the leaves of *Calycopteris floribunda*

S. No.	Tests for the presence of secondary metabolites	Phytochemical solvents				
		Petroleum ether	Chloroform	Ethyl acetate	Methanol	Aqueous
1	Mayer's test for alkaloids	+	-	+	-	-
2	Wagner's test for alkaloids	+	-	++	++	-
3	Shinoda test for flavonoids	+	-	+	++	+
4	Sulphuric acid test for flavonoids	++	-	+	+	+
5	Ferric chloride test for flavonoids	+	+	++	+	+
6	Salkowaski test for terpenoids	++	+	+++	+++	++
7	Keller-Killani test for cardiac glycosides	+	-	+	-	+
8	Salkowaski test for sterols	++	+	++	+++	++
9	Liberman-Burchard's test for sterols	-	-	+	++	-
10	Foam test for saponins	+	+	++	-	-
11	Braemer's test for tannins	+	++	+	+	+
12	Sulphuric acid test for resins	-	-	+	-	-
13	Ferric chloride test for phenols	-	-	+	+	-

(- = absent, + = present, ++ = moderately present, +++ = strongly present)

Among the other solvent extracts, phytochemical compounds like alkaloids, flavonoids, glycosides, steroids, terpenoids, phenols, cardiac glycosides and tannins are present in ethyl acetate extract of leaves (Table No.1). the leaf extract showed that the strong presence of terpinoids and moderate presence of alkaloids, flavonoids and sterols.

Table 2: Antibacterial activity of *Calycopteris floribunda* leaves

Sl. No.	Solvent extracts	Bacterial strains with zone of inhibition in mm			
		Bacillus subtilus	*Pseudomonas arginoses*	*Actinomycetes sp.*	*Seratia sp.*
1.	Petroleum ether	6	5	1	2
2.	Chloroform	4	2	2	1
3.	Ethyl acetate	6	4	4	1
4.	Methanol	8	6	4	2

The antibacterial activity of the leaves of *Calycopteris floribunda* extract has been evaluated against the pathogenic microbes such as *Bacillus subtilus, Pseudomonas arginoses, Actinomycetes sp.* and *Seratia sp.* Among the other microbes, Bacillus strain shows maximum inhibition zone (8 mm) in leaf extract (Table.No.2). Methanolic extract of the plant leaves showed the maximum inhibition zone compared with the other solvents. The antibacterial activity of this plant is similar to antibacterial activity of the other plant extracts has been reported previously [1, 3]. The results are more or less similar to phytochemical and antibacterial studies of some other medicinal plants like as *Holarrhena antidysenterica* and *Piper chaba* [5, 6, 8].

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