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Screening of Preliminary Phytochemicals and *In vitro* Antibacterial Activity of Methanolic Extracts of Stem, Leaf, Root and Leaf Callus of *Corynandra felina* (L.f.) Cochrane & Iltis

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Abstract *Corynandra felina (L.f.) Cochrane & Iltis, an endemic medicinal herb in peninsular India, belongs to the family Cleomaceae.* It is used in traditional medicine for epistaxis, astringent, antihyperlipidemic, antidiabetic, anticancer, and anti-inflammatory therapies. This study was carried out to screen the preliminary phytochemicals in methanolic extracts of stem, leaf, root and leaf callus through chemical methods and reveal the presence of alkaloids, phenols, tannins, saponins, cardiac glycosides, terpenoids and anthraquinones. The methanolic extracts of stem, leaf, root and leaf callus show antibacterial activity against bacterial strains viz. *Bacillus subtilis*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas fluorescens, Escherichia coli.* Zone of inhibition values was used to evaluate the antibacterial activity. Maximum zone of inhibition 20.0 ± 0.2 mm was found in stem extract against *Klebsiella pneumonia*, 15.2± 0.2 mm was found in leaf extract against *Klebsiella pneumonia* and no activity was observed in root and leaf callus extracts. Our study revealed that the phytochemicals of *C. felina* are showing antimicrobial activity and there is a possibility to develop drugs against pathogenic bacterial strains.

Keywords *Cleome felina,* Endemic, Phytochemical, Antibacterial Activity, Zone of Inhibition, *Klebsiella pneumonia*

1. Introduction

Several traditional herbal medicines have evolved contributions in large part to the healing properties of herbs. Active phytochemical components can be found in most herbs. Excess deaths have increased, especially in individuals who have come into contact with antibiotic-resistant microorganisms [1]. Drug resistance has rendered certain antibiotics ineffective [2], which has sparked a quest for novel medications. One potential therapy for multidrug-resistant illnesses brought on by multidrug-resistant bacteria is herbal medicine [3]. To combat their difficulties in the human body, pathogenic bacteria are continually evolving drug resistance to various medications or antibiotics [4]. According to their usage in conventional medicines, bioactive chemicals have been studied in plants from the Cleomaceae family [5, 6]. There are about 350

species of herbaceous plants and shrubs in the Cleomaceae family, most of which are annual but can sometimes be perennial. They are frequently grown in tropical and subtropical areas [7]. The main genus of the Cleomaceae family, Cleome, is well-known for its therapeutic benefits. Some Cleome species (*Cleome felina* L.f., *Cleome viscosa* L., *Cleome chelidonii* L.f.) have recently been placed into the genera *Corynandra* [8]. *Corynandra felina* (L.f.) Cochrane & Iltis belongs to the family Cleomaceae and it is commonly called as cat spider flower. It is endemic to peninsular India [9-12]. In India, it is distributed in Andhra Pradesh, Telangana, Tamil Nadu, Maharashtra, Pondicherry and Karnataka. It is "Annual diffuse herbs 10-35 cm. Stem decumbent, woody, branched, densely strigose-appressed hairs. Leaves are trifoliate, sessile, covered with hairy, oblong or obovate, apex obtuse, and margin ciliate. The inflorescence is racemes or solitary, bisexual, flowers pink or purple, pedicel slender, sepals are 4, linear, free, apex sub acute, scabrid. Petals are four elliptic and pubescent. Stamens are 20-40, purple or violet. Ovary is superior to the bi-carpellary. Fruit is a capsule. It has many seeds that are 14-16 orbicular [13]. It treats epistaxis [14] and astringent [15]. The plant extracts have antihyperlipidemic and anti-diabetic properties [16] and anticancer activity [17]. Whole plants cure anticancer, anti-inflammatory, and antimicrobial activities [18]. The phytochemical analysis and antimicrobial activity of *C. felina* L.f leaf and leaf-derived callus are reported earlier [19]. This research aimed to assess the anti-bacterial capacity and identify any phytochemicals in the leaves, stem, root, and leaf callus of *Corynandra felina* (L.f.) Cochrane & Iltis.

2. Materials and Procedures

2.1. Plant Material

Fresh plants of *Corynandra felina* (L.f) Cochrane & Iltis were collected at Gopalpuram, Hanamakonda district, Telangana state, India, in August 2021. Dr Md Mustafa, a plant taxonomist from Kakatiya University in Warangal and Telangana State, India, identified and confirmed the plant.

2.2. Preparation of Samples

The plants were separated into stems, leaves, roots, and *In vitro* callus sections. Each portion was pounded separately to generate a fine powder sample, then sieved through with a sieve with a mesh size of 2 mm. The fine powder sample was kept apart and in clean, dry sample containers for extraction.

2.3. Callus Induction

Murashige and Skoog's [20] media were supplemented with different concentrations of 2, 4-D, NAA, and IAA (1.0 - 5.0 mg/L), either alone or in combination with BAP (0.5 mg/L) or Kn (0.5 mg/L), to induce leaf callus. The leaf explants placed with Ms + NAA (2.0 mg/L) + BAP (0.5 mg/L) showed the highest mean fresh and dry weights (mg) of callus (428.5 ± 0.59 and 40.9 ± 0.59, respectively) (Table 1, Fig. 1). The dry powder callouses were used in the tests with phytoconstituents.

Table 1. Induction of Callus from Leaf, explant *Corynandra felina* was cultured on MS media supplemented with NAA (1.0 - 5.0 mg/L) and combination with BAP (0.5-3.0 mg/L) and KN (0.5-3.0 mg/L) after four weeks of culture

Hormone & Conc.			Leaf	
NAA	BAP	KN	Callus Fresh weight(mg) Mean ± S.E	Callus Dry weight(mg) Mean ± S.E
1.0	0.5	---	216.3 ±0.48	20.8 ±0.47
2.0	0.5	---	428.5 ±0.59	40.9 ±0.59
3.0	0.5	---	383.6 ±0.53	37.6 ±0.43
4.0	0.5	---	332.8 ±0.67	32.6 ±0.39
5.0	0.5	---	276.5 ±0.84	25.4 ±0.78
1.0	---	0.5	---	---
2.0	---	0.5	276.7 ±0.46	26.5 ±0.78
3.0	---	0.5	346.8 ±0.68	32.7 ±0.69
4.0	---	0.5	298.2 ±0.76	28.6 ±0.57
5.0	---	0.5	208.6 ±0.85	19.3 ±0.68

Data represent average of 10 cultures. Each experiment was repeated thrice.

2.4. Preparation of the Plant Extracts

A "Soxhlet extractor" was used to do methanol solvent extraction. A thimble containing the sample is put into the Soxhlet extractor's main chamber. On top of a flask containing the extraction solvent, the Soxhlet extractor is put. Ten grammes of dried plant extract powder were extracted using around 100 millilitres of solvent. On a heating mantle, the solvent extraction procedure was carried out. These extracts were concentrated by mixing them at 120 rpm for 24 hours at room temperature. They were labelled, maintained in sterile, airtight bottles, and utilized for phytochemical analysis, and the filtrate was used for combined antimicrobial activity and phytochemical analysis.

2.5. Qualitative Phytochemical Screening

Qualitative tests for alkaloids, flavonoids, Phenols, tannins, saponins, cardiac glycosides, terponoids, and anthraquinones were carried out with their plant extracts using the chemical methods [21] given below.

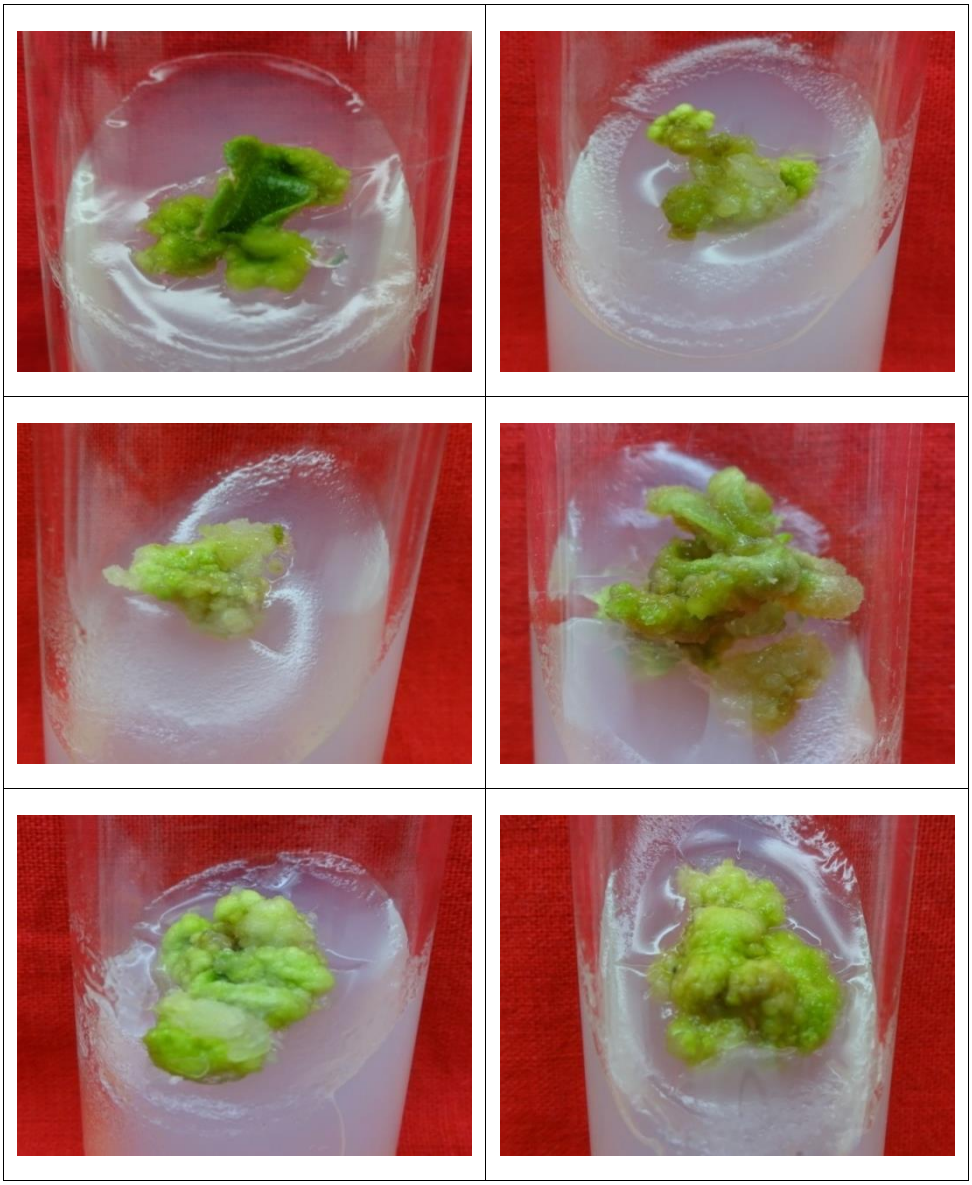

Figure 1. Induction of Callus from Leaf, explant of *Corynandra felina* plant

2.5.1. Test for alkaloids (Wagner's Test)

5 ml of dilute hydrochloric acid was mixed with 50 mg of solvent-free extracts after filtering. Alkaloids were checked for in the filtrate. Wagner's reagent and a small quantity of filtrate were added at the tube's end. The reddish-brown solution verifies the presence of alkaloids.

2.5.2. Test for Flavonoids (Shinodas Test)

In the solution, mix zinc dust and concentrated hydrochloric acid. After a while, it turns magenta, indicating the existing flavonoids.

2.5.3. Test for Phenols (Ferric Chloride Test)

Five millilitres of filtered water and a few drops of a neutral, 5% ferric chloride solution serve to adjust the extract. Deep green colours indicate the possible presence of phenols.

2.5.4. Test for Tannins (Ferric Chloride Test)

Tannins were identified by the extract's blue or green colour after they received a ferric chloride solution heating process.

2.5.5. Test for Saponins (Foam Test)

To make 20 ml, 50 mg of extract was diluted with water. A two-cm layer of foam is formed on top of the suspension after 15 minutes of shaking, indicating the presence of saponins.

2.5.6. Test for Cardiac glycosides (Kellar - Kiliani Test)

Two millilitres of the filtrate were added to one millilitre each of concentrated sulfuric acid, ferric chloride, and glacial acetic acid. The solution became green-blue, suggesting the presence of cardiac glycosides.

2.5.7. Test for Terpenoids (Liber Mann – Buchard's Test)

The crude extract was mixed with a few drops of acetic anhydride, boiled, and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration or the formation of a deep red colour in the lower layer would represent a positive test for triterpenoids.

2.5.8. Test for Anthraquinones (Borntrager's Test)

The test solution was treated for 5 minutes with 5 ml of 10% sulfuric acid, filtered even while warm, and mixed with an equivalent amount of benzene. In the interest of separating the benzene layer, it was separated in half and treated with a 10% ammonia solution; the rose-pink colour of the ammonia layer showed the presence of anthraquinones.

2.6. Test Organisms for Anti-Bacterial Activity

The antibacterial analysis of the extracts was carried out against gram-negative bacteria *Escherichia coli* (MTCC 424), *Klebsiella pneumoniae* (MTCC 2727), *Pseudomonas fluorescens* (MTCC 9768) and gram-positive bacteria *Bacillus subtilis* (MTCC 3053), *Staphylococcus aureus* (MTCC 96). These bacterial strains were acquired from Chandigarh, Punjab, India's Microbial Type Culture Collection (MTCC). The bacterial strain was grown in nutrient broth and kept at 4 ℃ until usage on nutrient agar (Hi-Media). The paper disc diffusion method was used to test the anti-bacterial properties. [22].

2.7. Antimicrobial Susceptibility Testing

Nutrient agar plates were prepared, and pathogenic bacteria were spread on the agar plates. The activated samples were placed on discs [23] and incubated at 30 ± 2 for 24 hours. A distinct zone of bacterial inhibition was seen surrounding the sample after 24 hours of incubation, and the zone's measurement was determined. The degree of inhibition was presented in terms of the diameter of the inhibition zone as measured in millimetres with a transparent ruler [24]. The effect of the extract on bacteria was compared with that of a standard antibiotic, ampicillin. All experiments were performed in triplicate.

2.8. Statistical Analysis

Three separate tests were run, and the findings were presented as mean and standard deviation (S.D.).

3. Results

Methanolic extracts of stem, leaf, root and callus revealed the presence of phytochemicals and were reported in (Table 2, Fig. 2). *C. felina*, contains a wide range of phytochemicals like alkaloids, phenols, tannins, saponins, cardiaglycosides, terpenoids, and anthraquinones which were found in the leaf, stem, root and callus. Out of eight phytochemical tests, the methanol extract of the stem showed the presence of alkaloids, phenols, tannins, saponins, and terpenoids. Negative results were recorded for flavonoids, cardiac glycosides and anthraquinones. Alkaloids were present in all four parts of the extractions i.e., leaf, stem, root and callus. Flavonoids were not found in any of the part. Only anthraquinones were found in the root extract, and cardiac glycosides were found in the callus extract only. Phenols and tannins were present in leaf and stem extracts. Terpenoids were found in stem and root extracts. Saponins were identified in leaf, stem and callus extracts.

The antibacterial activity of a methanol extract of the stem, leaf, root and leaf callus of *C. felina* was assessed by their zone of inhibition values (Table 3, Fig. 3,). Present study revealed that the stem extract showed significant antibacterial activity against *Klebsiella pneumonia* (20.0 ± 0.2 mm), followed by *Staphylococcus aureus* (11.0 ± 0.1 mm), *Escherichia coli* (10.0 ± 0.2 mm), *Bacillus subtillis* (9.0 ± 0.1 mm) and *Pseudomonas fluorescens* (7.0 ± 0.2 mm). Leaf extract noticed significant antibacterial activity against *Klebsiella pneumonia* (15.0 ± 0.2mm), followed by *E. coli* (12.0 ± 0.1mm), *Pseudomonas fluorescens* (11.0 ± 0.1mm), *Staphylococcus aureus* (10.0 ± 0.2mm) and *Bacillus subtillis* (8.0 ± 0.2mm).

Table 2. Preliminary phytochemical screening of methanolic extract of *Corynandra felina*

S.No	Phytochemical	Test	Leaf	Stem	Root	Callus
1.	Alkaloids	Wagner's test	+	+	+	+
2.	Flavonoids	Shinodas test	-	-	-	-
3.	Phenols	Ferric chloride test	+	+	-	-
4.	Tannins	Ferric chloride test	+	+	-	-
5.	Saponins	Foam test	+	+	-	+
6.	Cardiac glycosides	Kellar - Kiliani test	-	-	-	+
7.	Terpenoids	Liber Mann – Buchard's test	-	+	+	-
8.	Anthraquinones	Borntrager 's test	-	-	+	-

Indication of sign (+) present and (-) absent

Table 3. The bacterial growth inhibition zones of methanolic extracts of *Corynandra felina*

Extract	Test organisms with their zone of inhibition (mm)				
	Bacillus subtilis	*Klebsiella pneumonia*	*Pseudomonas fluorescens*	*Escherichia coli*	*Staphylococcus aureus*
Leaf	8.0 ±0.2	15.0 ±0.2	11.0 ±0.1	12.0 ±0.1	10.0 ±0.2
Stem	9.0 ±0.1	20.0 ±0.2	7.0 ±0.2	10.0 ±0.2	11.0 ±0.1
Callus	00 ±00	00 ±00	00 ±00	00 ±00	00 ±00
Root	00 ±00	00 ±00	00 ±00	00 ±00	00 ±00
Control (Ampicillin)	33.0 ±0.2	32.0 ±0.1	36.0 ±0.2	36.0 ±0.2	31.0 ±0.1

Values are means of three independent replicates and ± indicates standard deviation.

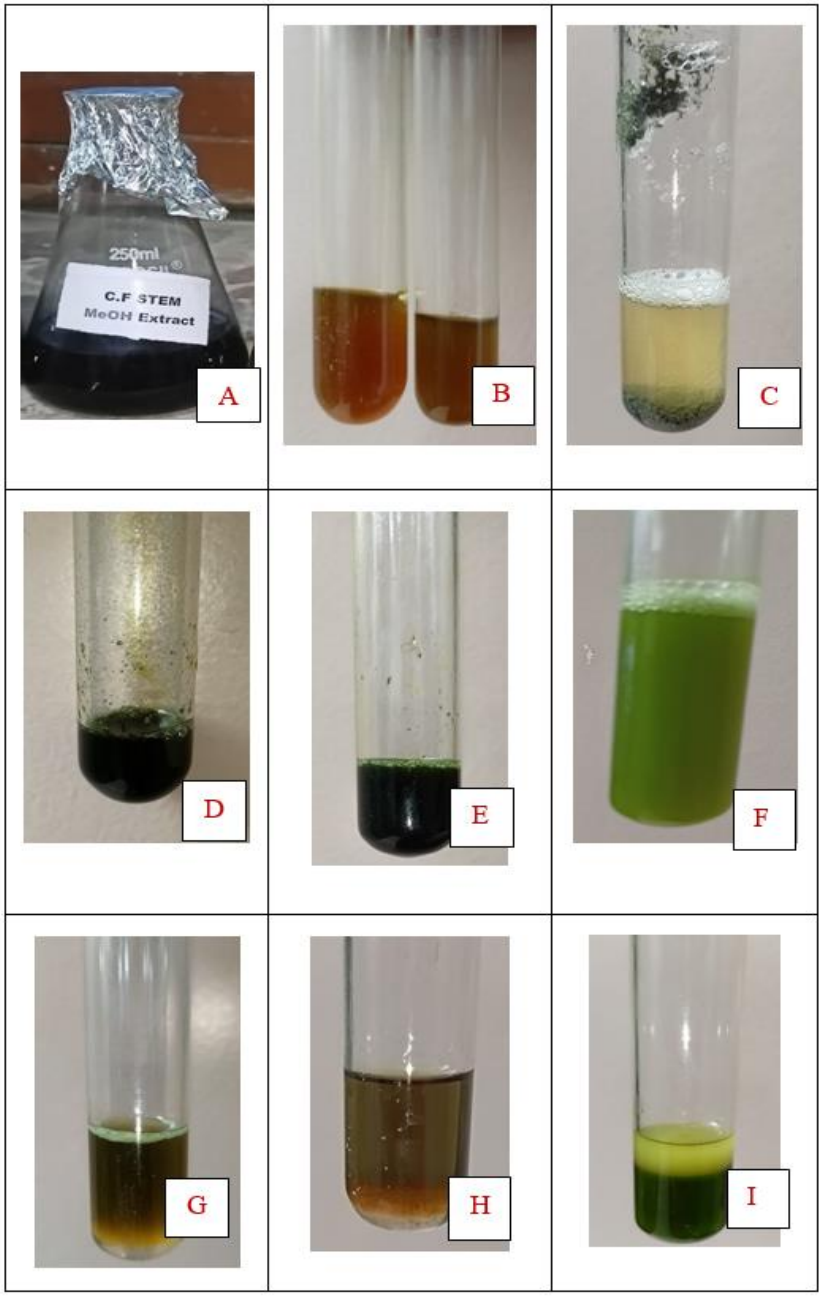

Figure 2. Preliminary phytochemicals screening in stem extracts of *Corynandra felina* in methanol solvent extracts. (A). Methanol stem extract (B). Detection of alkaloids (C). Detection of flavonoids (D). Detection of phenols (E). Detection of tannins (F). Detection of saponins (G). Detection of cardiac glycosides (H). Detection of terpenoids (I). Detection of anthraquinones

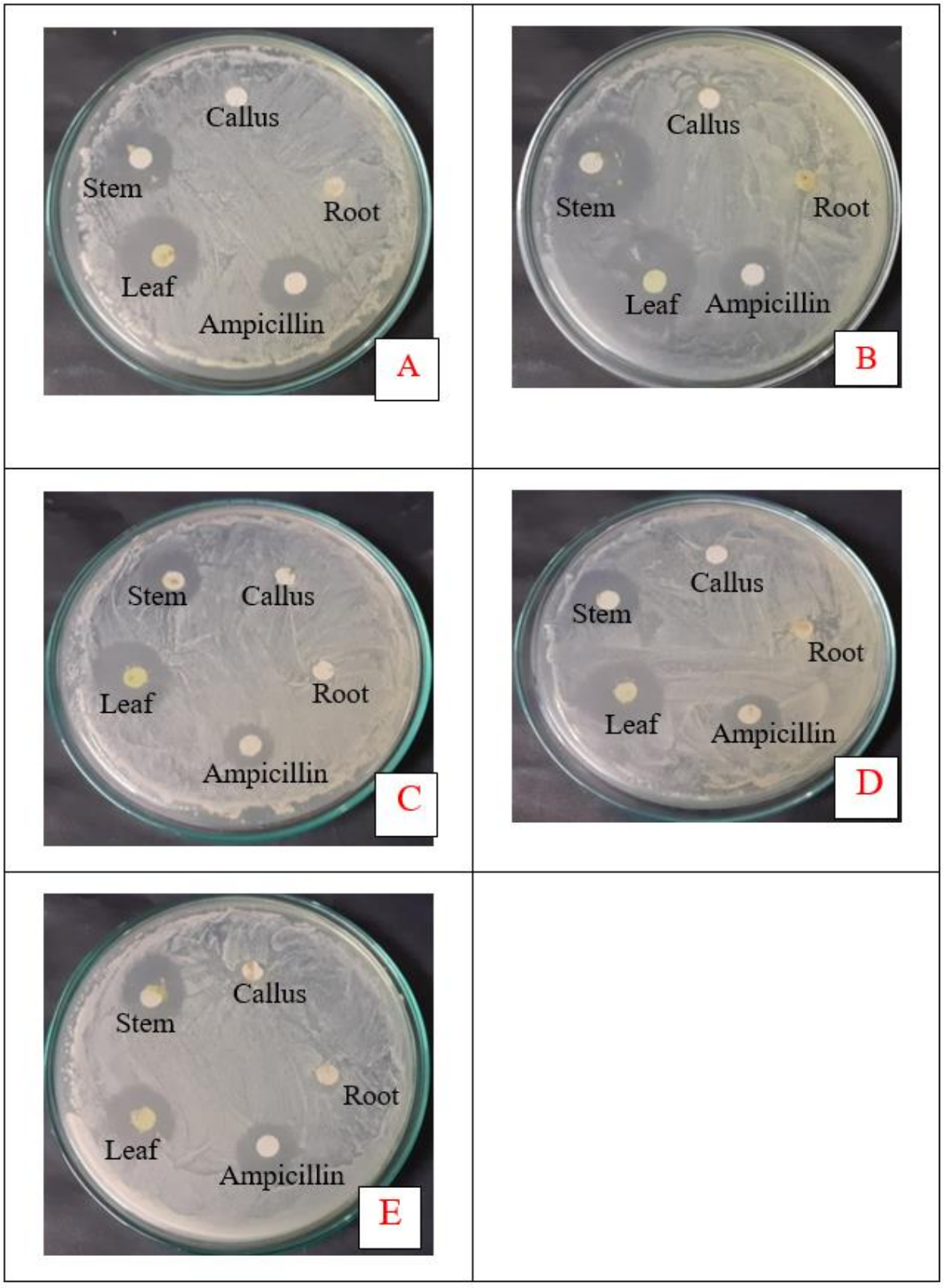

Figure 3. The bacterial growth inhibition zones of a methanolic extract of the leaf, stem, root, and leaf callus of *Corynandra felina* (Ampicillin was used as a control). (A). *Bacillus subtillis* (B). *Klebsiella pneumonia* (C). *Pseudomonas fluorescens* (D). *Escherichia coli* (E). *Staphylococcus aureus*

4. Discussion

Singh [19] evaluated the phytochemistry of a chloroform-methanol leaf extract of *C. felina*. The result was similar to our study. The results showed that the stem has many phytoconstituents compared to other parts like the leaf, root and callus. Many of these compounds have been shown to have various varieties of therapeutic features. The medical and biological effects of the bioactive compounds are diverse. Each phytochemical showed the capacity for a particular biological activity, such as the antioxidant capacity of flavonoids, the anti-bacterial, analgesic, and antispasmodic capacities of alkaloids, and the inflammatory capacity of steroids [25]. Tannins possess astringent, anti-bacterial, and antioxidant properties [26]. Pharmaceutical firms are concentrating their efforts on using medicinal plants as a bioactive source for developing new drugs. Around 25 - 50 % of contemporary medications have a botanical origin. This demonstrates that plants have various curative properties attributable to their chemical makeup. Several medicinal plants were unique in their biological activities but have been employed by different tribes or nations for different ailments. The numerous phytochemical substances found have been known to be helpful in industrial and medical disciplines. According to several studies, alkaloids are said to have antimicrobial studies [27]. Many publications claim that glycosides can reduce blood pressure [28]. It has been discovered that tannin possesses antimicrobial qualities. A few tannins are employed as diuretics and have been shown to stop HIV replication [29]. Biological characteristics, including anticancer and anti-inflammation actions, are present in phenolic compounds [30]. The antioxidant qualities of medicine that are high in phenolic compounds have been discussed in several research [31, 32]. Saponins contained in plants are cardiotonic and are said to have anti-diabetic characteristics. They can reduce coughing, which is particularly helpful in treating upper respiratory tract inflammation [33].

The present study found that stem extract was more effective against the tested pathogens than leaf extract. The root and callus extracts had no anti-bacterial activity. According to Venkatadri et al. [34], Alkaloids, flavonoids, tannins, and phenolic compounds are just a few of the phytochemicals that make up medicinal plants and are what give them their anti-bacterial properties. There have been reports of medicinal plants having antimicrobial qualities from all over the world. The traditional use of herbs or their active constituents has been approved by the World Health Organization today [35]. The most potent and most notable anti-bacterial activity against seven bacterial strains was seen in the methanol extract of *C. viscosa*, as demonstrated [36]. The antimicrobial Test of *C. viscosa's* methanolic extract revealed moderate sensitivity to gram-positive and gram-negative bacteria [37].

5. Conclusions

Phytochemicals found in the stem, leaves, roots, and leaf callus of *Corynandra felina* included alkaloids, phenols, tannins, saponins, terpenoids, and cardiac glycosides. *C. felina* stem and leaf extracts in methanol showed excellent anti-bacterial action against pathogenic bacterial strains. Consequently, the stem and leaves of *C. felina* are a possible source of physiologically active chemical compounds. It is also possible to carry out more studies to learn more about the plant's lead components which are essential for pharmaceutical use.

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