

Antibacterial Activity of Plant Extraction of Leaf Extract of Medicinal Plant *Calotropis* Species

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Abstract

The *calotropis species* is a member of the plant family Asclepiadaceae, a shrub plant. The medicinal plants generally contain number of compounds that may be potential natural Antimicrobial agents which may serve as alternative, effective, cheaper and safe Antimicrobial agents for the treatment of common microbial infections. The plants *Calotropis (gigantea)* were successively extracted with Chloroform, Ethanol, Methanol, Aqueous using mortal piston. Paper disk and Agar well methods was employed to determine the antibacterial activity against some pathogenic bacterial species like *Bacillus*, *Enterobacter*, *Enterococci*, *E.coli*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Achromobacter*, *CoNS* and *Staphylococcus aureus*. So, in present studies, Aqueous fresh leaves extract showed better response to all extracts against *Enterobacter sp.* where zone of inhibition showed high. These all Extracts were showed significant Antibacterial Activity against pathogenic bacterial species.

Keywords: *Calotropis gigantea*, Antibacterial Activity, Ethanolic, Aqueous, Chloroform, and Methanolic.

Introduction

Calotropis species is a member of the plant family Asclepiadaceae, a shrub about 6m high⁽¹⁾. Morphologically the plant is erect, tall, large, much branched and perennial shrub with milky latex or small tree that grows on a height of 5.4m, with milky latex throughout. The secretion from the root bark is traditionally used for the treatment of skin diseases, enlargements of abdominal viscera and intestinal worms⁽²⁾. Ethno medical literature contains a large number of plants including, *Calotropis gigantea* that can be used against diseases, like diabetes, atherosclerosis, ischemic heart disease, disorders induced by free radicals and other reactive oxygen species. India is very rich in natural resources and the knowledge of traditional medicine and the use of plants as source of new drugs is an innate and very important component drug discovery. *Calotropis gigantea* is a xerophytic, erect shrub, growing widely throughout the tropical and subtropical regions of Asia and Africa. Plants contain many biologically active molecules with different medicinal properties⁽³⁾.

The plant, *Calotropis gigantea* L. grows widely throughout the Indian subcontinent. The root bark of this plant is used as medicine in treatment of leprosy, piles, wounds, tumours, parasitic infections and dysentery⁽⁴⁾.

Materials and Methods

A) Bacterial Isolate - The Test bacterial sample was collected from Chhattisgarh Institute of Medical Science (CIMS), Bilaspur (Chhattisgarh). The sample was 2-3 days old in the form of slant. The typed cultures of bacteria and fungi were sub-cultured on Nutrient agar slant at 4°C and subcultured onto nutrient broth using a sterilized wire loop. The bacteria used were *Bacillus*, *Staphylococcus*, *E.coli*, *Proteus*, *Enterobacter*, *Pseudomonas*, *Klebsiella*, *Enterococci*, *Achromobacter*, *CONS* (*Coagulus Negative Staphylococcus*).

B) Collection and preparation of Plant Sample - The Healthy disease free plant *calotropis sp. (Gigantea)* leaves were aseptically collected from Kargi Road Kota, District- Bilaspur (Chhattisgarh), India. The Fresh plant samples were washed thoroughly 2-3 times with running tap water and then sterile water. The fresh leaves are crushed and blended using mortal piston, and the leaves were shade dried for 10 days and blended into powder using a mortal piston. And the leaves were dried in hot air oven at 42°C. After 10 days, and blended into powder using a mortal piston for further analysis.

C) Preparation of leaf and Protein (Flower) Extraction –

1) Ethanolic & Chloroform Extract of Fresh, Shade and Oven Dried leaves - The leave of apical twig of plant *Calotropis sp.* was done with water, ethanol & chloroform 60%. The fresh, Shade and Oven dried leave were crushed in mortar pestle. The Crushed leaf and powders are each weighing 10g and it's dissolved in 100 ml of solvent. The suspended solutions were left to stand for 5 days; the extracts were filtered by Whatmann paper no.1 and stored at 4°C⁽⁵⁾.

2) Methanolic Extract of Fresh, Shade and Oven dried leaves - The 50gm fresh, Shade and Oven dried leave of apical twig of plant *Calotropis sp.* were crushed in mortar pestle and sequentially extracted by shaking for 2 hours on Wrist Action Shaker after overnight soaking in 150 ml of relevant solvent. After filtration, samples were rinsed with additional 3 x 60 ml portions of the solvent. Combined filtrates were dried at room temperature under electric fan. The extracts were stored in the refrigerator at 4°C until required⁽⁶⁾.

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3) Aqueous Extract of fresh, Shade and Oven dried leaves - 100 grams of fresh, shade and Oven dried apical leaves of *Calotropis sp.* were weighed out and crushed directly by grinder and dipped into 400 ml cold distilled water into a conical flask stoppered with rubber corks and left for 7 days with occasional shaking. Filtered off using sterile filter paper (Whatman no. 1) into a clean conical flask and subjected to water bath evaporation where the aqueous solvent was evaporated at its boiling temperature of 100°C. The standard extracts obtained were then stored in a refrigerator at 4°C for antibacterial activity test⁽⁷⁾.

D) Media preparation- The Muller Hinton Agar medium is used for antibacterial activity test against human pathogenic bacteria.

E) Antibacterial activity Test- The antimicrobial activity of aqueous, chloroform and ethanolic extract was determined by filter paper disc and agar well diffusion method⁽⁸⁾.

1) Paper Disc Technique - Sterile filter paper discs (6.0 mm diameter) were soaked with the test extracts and dried at 40°C for 30 minutes. The prepared culture plates were seeded with each of the test bacteria and the filter paper discs were placed on each plate. The plates were incubated at 37°C for 48 hours. The zones of inhibition were measured and recorded.

2) Agar Well Diffusion -The culture plates seeded with test organisms were allowed to solidify and punched with a sterile cork borer (6.0 mm diameter) to make open wells. The open wells were filled with 0.05 ml of the extract. The plates were incubated at 37°C for 48 hours. The zones of inhibition were measured and recorded.

Results and Discussion

The plant *Calotropis gigantea* was used for present studies of Antibacterial activity test. For the study of antibacterial activity prepared extract of plant leaves. The extract of plants are Ethanolic, Methanolic, Chloroform and Aqueous for leaves extract, for using Antibacterial activity. The pathogenic bacterial species are used for Antibacterial activity of plant Extract. The plant leaves extract against tested bacteria's, each Antibacterial activity test made in triplicate form.

Bacillus- The Antibacterial activity of plant extracts against *Bacillus*. There are three extract of Ethanolic leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (16±0.25) in Fresh leaf, (15±0.40) in Shade dried and (12±0.13) in Oven dried leaf extract. Similarly in Methanolic leaf extract, the zone of inhibition (20±0.12) in Fresh leaf, (13±0.43) in Shade dried and (11±0.07) in Oven dried leaf extract, and also in

Chloroform leaf extract, the zone of inhibition (22±0.11) in Fresh leaf, (20±0.07) in Shade dried and (15±0.03) in Oven dried leaf extract, and in Aqueous leaf extract, the zone of inhibition (20±0.67) in Fresh leaf, (13±0.43) in Shade dried and (15±0.33) in Oven dried leaf extract, are showed by the observation. So the Chloroform leaf extract showed the better result as compared to Ethanolic, Methanolic and Aqueous leaf extract. In the higher zone of inhibition against *Bacillus sp.* is (22±0.11) (**fig. no. 1 (a), Table no.1 (a).**

Table.5). Antibacterial activity of *Calotropis sp.* Leaf Extract using Paper discs method against Human pathogenic bacteria.**Table no. 5 (a)** Antibacterial activity against *Bacillus*.

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	20±0.67	13±0.43	15±0.33
2	Ethanol	16±0.25	15±0.40	12±0.13
3	Methanol	20±0.12	13±0.43	11±0.07
4	Chloroform	22±0.11	20±0.07	15±0.03

Staphylococcus aureus- The Antibacterial activity of plant extracts against *S.aureus*. There are three extract of Ethanolic leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (11±0.56) in Fresh leaf, (9±0.48) in Shade dried and (8±0.45) in Oven dried leaf extract. Similarly in Methanolic leaf extract, the zone of inhibition (9±0.28) in Fresh leaf, (15±0.55) in Shade dried and (6±0.39) in Oven dried leaf extract, and also in Chloroform leaf extract, the zone of inhibition (10±0.01) in Fresh leaf, (7±0.08) in Shade dried and (9±0.35) in Oven dried leaf extract, and in Aqueous leaf extract, the zone of inhibition (15±0.67) in Fresh leaf, (14±0.83) in Shade dried and (11±0.35) in Oven dried leaf extract, are showed by the observation. So the Aqueous leaf extract showed the better result as compared to Ethanolic, Methanolic and Chloroform leaf extract. In the higher zone of inhibition against *Staphylococcus aureus* is (15±0.67) (**fig. no. 1 (b), Table no.1 (b). Table. 5 (b).**

Antibacterial activity against *Staphylococcus aureus*:

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	15±0.67	14±0.83	11±0.35
2	Ethanol	11±0.56	9±0.48	8±0.45
3	Methanol	9±0.28	15±0.55	6±0.39
4	Chloroform	10±0.01	7±0.08	9±0.35

Table.5 (c). Antibacterial activity against *E.coli*.

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	12±0.28	22±0.35	15±0.17
2	Ethanol	12±0.32	13±0.56	12±0.13
3	Methanol	6±0.43	19±0.67	12±0.58
4	Chloroform	Nil	19±0.03	11±0.48

Proteus- The Antibacterial activity of plant extracts against *Proteus*. There are three extract of Ethanolic leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (21±0.45) in Fresh leaf, (17±0.14) in Shade dried and (11±0.48) in Oven dried leaf extract. Similarly in Methanolic leaf extract, the zone of inhibition (16±0.13) in Fresh leaf, (15±0.45) in Shade dried and (13±0.03) in Oven dried leaf extract, and also in Chloroform leaf extract, the zone of inhibition (15±0.22) in Fresh leaf, (18±0.65) in Shade dried and (19±0.07) in Oven dried leaf extract, and in Aqueous leaf extract, the zone of inhibition (25±0.37) in Fresh leaf, (28±0.44) in Shade dried and (21±0.45) in Oven dried leaf extract, are showed by the observation. So the Aqueous leaf extract showed the better result as compared to Ethanolic, Methanolic and Chloroform leaf extract. In the higher zone of inhibition against *Proteus sp.* is (28±0.44) (**fig. no.1 (d), Table no.1 (d).**)

Table.5 (d). Antibacterial activity against *Proteus*.

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	25±0.37	28±0.44	21±0.45
2	Ethanol	21±0.45	17±0.14	11±0.48
3	Methanol	16±0.13	15±0.45	13±0.03
4	Chloroform	15±0.22	18±0.65	19±0.07

Enterobacter- The Antibacterial activity of plant extract against *Enterobacter*. There are three extract of Ethanolic leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (30±0.43) in Fresh leaf, (20±0.63) in Shade dried and (14±0.33) in Oven dried leaf extract. Similarly in Methanolic leaf extract, the zone of inhibition (26±0.67) in Fresh leaf, (20±0.15) in Shade dried and Nil in Oven dried leaf extract, and also in Chloroform leaf extract, the zone of inhibition (25±0.03) in Fresh leaf, (20±0.48) in Shade dried and (15±0.06) in Oven dried leaf extract, and in Aqueous leaf extract, the zone of inhibition (32±0.08) in Fresh leaf, (23±0.25) in Shade dried and (25±0.11) in Oven dried leaf extract, are showed by the observation. So the Aqueous leaf extract showed the better result as compared to Ethanolic, Methanolic and Chloroform leaf extract. In the higher

zone of inhibition against *Enterobacter sp.* is (32±0.08) (**fig. no.1 (e), Table no.1 (e)**)

Table.5 (e). Antibacterial activity against *Enterobacter*.

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	32±0.08	23±0.25	25±0.11
2	Ethanol	30±0.43	20±0.63	14±0.33
3	Methanol	26±0.67	20±0.15	Nil
4	Chloroform	25±0.03	20±0.48	15±0.11

Enterococci- The Antibacterial activity of plant extract against *Enterococci*. There are three extract of Ethanolic leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (12±0.09) in Fresh leaf, (27±0.67) in Shade dried and (25±0.09) in Oven dried leaf extract. Similarly in Methanolic leaf extract, the zone of inhibition is Nil in Fresh leaf, (26±0.33) in Shade dried and (24±0.25) in Oven dried leaf extract, and also in Chloroform leaf extract, the zone of inhibition (21±0.18) in Fresh leaf, (23±0.48) in Shade dried and (20±0.15) in Oven dried leaf extract, and in Aqueous leaf extract, the zone of inhibition (20±0.52) in Fresh leaf, (30±0.40) in Shade dried and (22±0.11) in Oven dried leaf extract, are showed by the observation. So the Aqueous leaf extract showed the better result as compared to Ethanolic, Methanolic and Chloroform leaf extract. In the higher zone of inhibition against *Enterococci sp.* is (30±0.40) (**fig. no.1 (f), Table no.1 (f).**)

Table.5 (f). Antibacterial activity against *Enterococci*.

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	20±0.52	30±0.40	22±0.11
2	Ethanol	12±0.09	27±0.67	25±0.09
3	Methanol	Nil	26±0.33	24±0.25
4	Chloroform	21±0.18	23±0.48	20±0.15

Pseudomonas- The Antibacterial activity of plant extracts against *Pseudomonas*. There are three extract of Ethanolic leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (13±0.33) in Fresh leaf, (30±0.08) in Shade dried and (27±0.03) in Oven dried leaf extract. Similarly in Methanolic leaf extract, the zone of inhibition is Nil in Fresh leaf, (24±0.67) in Shade dried and (17±0.40) in Oven dried leaf extract, and also in Chloroform leaf extract, the zone of inhibition is Nil in Fresh leaf, (20±0.13) in Shade dried and (11±0.44) in Oven dried leaf extract, and in Aqueous leaf extract, the zone of inhibition (23±0.67) in Fresh leaf, (27±0.22) in Shade dried and (25±0.17) in Oven dried leaf extract, are showed by the observation. So the Aqueous leaf extract

showed the better result as compared to Ethanolic, Methanolic and Chloroform leaf extract. In the higher zone of inhibition against *Pseudomonas sp.* is (27±0.22) (**fig. no.1 (g), Table no.1 (g).**)

Table.5 (g). Antibacterial activity against *Pseudomonas*.

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	23±0.67	27±0.22	25±0.17
2	Ethanol	13±0.33	30±0.08	27±0.03
3	Methanol	Nil	24±0.67	17±0.40
4	Chloroform	Nil	20±0.13	11±0.44

Klebsiella- The Antibacterial activity of plant extracts against *Klebsiella*. There are three extract of Ethanolic leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (15±0.04) in Fresh leaf, (18±0.35) in Shade dried and (16±0.11) in Oven dried leaf extract. Similarly in Methanolic leaf extract, the zone of inhibition (7±0.03) in Fresh leaf, (15±0.05) in Shade dried and (20±0.43) in Oven dried leaf extract, and also in Chloroform leaf extract, the zone of inhibition (10±0.01) in Fresh leaf, (24±0.12) in Shade dried and (14±0.23) in Oven dried leaf extract, and in Aqueous leaf extract, the zone of inhibition (19±0.67) in Fresh leaf, (23±0.55) in Shade dried and (20±0.32) in Oven dried leaf extract, are showed by the observation. So the Aqueous leaf extract showed the better result as compared to Ethanolic, Methanolic and Chloroform leaf extract. In the higher zone of inhibition against *Klebsiella sp.* is (23±0.55) (**fig. no.1 (h), Table no.1 (h).**)

Table.5 (h). Antibacterial activity against *Klebsiella*.

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	19±0.67	23±0.55	20±0.32
2	Ethanol	15±0.04	18±0.35	16±0.11
3	Methanol	7±0.03	15±0.05	20±0.43
4	Chloroform	10±0.01	24±0.12	14±0.23

Achromobacter- The Antibacterial activity of plant extracts against *Achromobacter*. There are three extract of Ethanolic leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (30±0.09) in Fresh leaf, (22±0.45) in Shade dried and (25±0.34) in Oven dried leaf extract. Similarly in Methanolic leaf extract, the zone of inhibition (16±0.33) in Fresh leaf, (19±0.34) in Shade dried and (15±0.03) in Oven dried leaf extract, and also in Chloroform leaf extract, the zone of inhibition (11±0.17) in Fresh leaf, (22±0.07) in Shade dried and (15±0.15) in Oven dried leaf extract, and in Aqueous leaf extract, the zone of inhibition (24±0.13) in Fresh leaf,

(23±0.08) in Shade dried and (17±0.54) in Oven dried leaf extract, are showed by the observation. So the Ethanolic leaf extract showed the better result as compared to Aqueous, Methanolic and Chloroform leaf extract. In the higher zone of inhibition against *Achromobacter sp.* is (30±0.09) (**fig. no.1 (i), Table no.1 (i).**)

Table.5 (i). Antibacterial activity *Achromobacter*.

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	24±0.13	23±0.08	17±0.54
2	Ethanol	30±0.09	22±0.45	25±0.34
3	Methanol	16±0.33	19±0.34	15±0.03
4	Chloroform	11±0.17	22±0.07	15±0.15

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	15±0.28	7±0.05	6±0.35
2	Ethanol	13±0.11	10±0.09	Nil
3	Methanol	11±0.44	6±0.33	Nil
4	Chloroform	9±0.06	6±0.45	Nil

- The observed results of Antibacterial activity of leaf Extract using Agar well diffusion method against pathogenic bacteria's are given below:

Bacillus- The Antibacterial activity of plant extracts against *Bacillus*. There are three extract of Ethanolic leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (10±0.08) in Fresh leaf, (17±0.06) in Shade dried and (16±0.38) in Oven dried leaf extract. Similarly in Methanolic leaf extract, the zone of inhibition (19±0.15) in Fresh leaf, (17±0.11) in Shade dried and (16±0.33) in Oven dried leaf extract, and also in Chloroform leaf extract, the zone of inhibition (15±0.07) in Fresh leaf, (11±0.45) in Shade dried and Nil in Oven dried leaf extract, and in Aqueous leaf extract, the zone of inhibition (16±0.67) in Fresh leaf, (10±0.15) in Shade dried and (8±0.09) in Oven dried leaf extract, are showed by the observation. So the Methanolic leaf extract showed the better result as compared to Ethanolic, Aqueous and Chloroform leaf extract. In the higher zone of inhibition against *Bacillus sp.* is (19±0.15) (**fig. no.2 (a), Table no.2 (a)**)

Table.2). Antibacterial activity of *Calotropis sp.* Leaf Extract using Agar well method against Human pathogenic bacteria.

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	16±0.67	10±0.15	8±0.09
2	Ethanol	10±0.08	17±0.06	16±0.38
3	Methanol	19±0.15	17±0.11	16±0.33
4	Chloroform	15±0.07	11±0.45	Nil

Table.2 (a). Antibacterial activity against *Bacillus*.

E.coli- The Antibacterial activity of plant extracts against *E.coli*. There are three extract of Ethanolic leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (13±0.33) in Fresh leaf, (9±0.08) in Shade dried and (11±0.23) in Oven dried leaf extract. Similarly in Methanolic leaf extract, the zone of inhibition (15±0.09) in Fresh leaf, but Nil in Shade dried and Oven dried leaf extract, and also in Chloroform leaf extract, the zone of inhibition (14±0.13) in Fresh leaf, (10±0.11) in Shade dried and (9±0.32) in Oven dried leaf extract, and in Aqueous leaf extract, the zone of inhibition (16±0.67) in Fresh leaf, (11±0.17) in Shade dried and (9±0.02) in Oven dried leaf extract, are showed by the observation. So the Aqueous Fresh leaf extract showed the better result as compared to Ethanolic, Methanolic and Chloroform leaf extract. In the higher zone of inhibition against *E.coli sp.* is (16±0.67) (**fig. no.2 (b), Table no.2 (b)**).

Table.2 (b). Antibacterial activity against *E.coli*.

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	16±0.67	11±0.17	9±0.02
2	Ethanol	13±0.33	9±0.08	11±0.23
3	Methanol	15±0.09	Nil	Nil
4	Chloroform	14±0.13	10±0.11	9±0.32

Enterococci- The Antibacterial activity of plant extract against *Enterococci*. There are three extract of Ethanolic leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (23±0.33) in Fresh leaf, (22±0.09) in Shade dried and (20±0.19) in Oven dried leaf extract. Similarly in Methanolic leaf extract, the zone of inhibition (17±0.07) in Fresh leaf, (16±0.23) in Shade dried and (15±0.12) in Oven dried leaf extract, and also in Chloroform leaf extract, the zone of inhibition (16±0.45) in Fresh leaf, (14±0.11) in Shade dried and (11±0.9) in Oven dried leaf extract, and in Aqueous leaf extract, the zone of inhibition (24±0.45) in Fresh leaf, (21±0.17) in Shade dried and (20±0.23) in Oven dried leaf extract, are showed by the observation. So the Aqueous Fresh leaf extract showed the better result as compared to Ethanolic, Methanolic and Chloroform leaf extract. In the higher

zone of inhibition against *Enterococci sp.* is (24±18.45)

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	24±0.45	21±0.17	20±0.23
2	Ethanol	23±0.33	22±0.09	20±0.19
3	Methanol	17±0.07	16±0.23	15±0.12
4	Chloroform	16±0.45	14±0.11	11±0.9

(fig. no.2 (c), Table no.2 (c).**Table.6 (c).** Antibacterial activity against *Enterococci*.

Klebsiella- The Antibacterial activity of plant extract against *Klebsiella*. There are three extract of Ethanolic leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (16±0.12) in Fresh leaf, (15±0.42) in Shade dried and (12±0.33) in Oven dried leaf extract. Similarly in Methanolic leaf extract, the zone of inhibition (20±0.54) in Fresh leaf, (13±0.09) in Shade dried and (11±0.07) in Oven dried leaf extract, and also in Chloroform leaf extract, the zone of inhibition (22±0.12) in Fresh leaf, (20±0.43) in Shade dried and (15±0.67) in Oven dried leaf extract, and in Aqueous leaf extract, the zone of inhibition (20±0.18) in Fresh leaf, (13±0.45) in Shade dried and (15±0.35) in Oven dried leaf extract, are showed by the observation. So the Methanolic leaf extract showed the better result as compared to Ethanolic, Aqueous and Chloroform leaf extract. In the higher zone of inhibition against *Klebsiella sp.* is (20±0.54) (**fig. no.2 (d), Table no.2 (d)**).

Table.6 (d). Antibacterial activity against *Klebsiella*.

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	20±0.18	13±0.45	15±0.35
2	Ethanol	16±0.12	15±0.42	12±0.33
3	Methanol	20±0.54	13±0.09	11±0.07
4	Chloroform	22±0.12	20±0.43	15±0.67

CoNS- The Antibacterial activity of plant extract against *CoNS*. There are three extract of Ethanolic leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (14±0.33) in Fresh leaf, (9±0.21) in Shade dried and (7±0.67) in Oven dried leaf extract. Similarly in Methanolic leaf extract, the zone of inhibition (18±0.38) in Fresh leaf, (6±0.33) in Shade dried and (7±0.17) in Oven dried leaf extract, and also in Chloroform leaf extract such as Fresh leaf, Shade and Oven dried leaf. In the zone of inhibition (11±0.03) in Fresh leaf, (9±0.08) in Shade dried and (7±0.02) in Oven dried leaf extract, and in Aqueous leaf extract, the zone of inhibition (18±0.13) in Fresh leaf, (10±0.15) in Shade dried and (6±0.01) in Oven dried leaf extract, are showed by the observation. So the Aqueous Fresh leaf extract showed the better

result as compared to Ethanolic, Methanolic and Chloroform leaf extract. In the higher zone of inhibition against *CoNS sp.* is (18±0.13) (fig. no.2 (e), Table no.2 (e)).

Table.2 (e). Antibacterial activity against *CoNS*.

S.No.	Name of Extract	Zone of Inhibition in (mm)		
		Fresh	Shade dried	Oven dried
1	Aqueous	18±0.13	10±0.15	6±0.01
2	Ethanol	14±0.33	9±0.21	7±0.67
3	Methanol	18±0.38	6±0.33	7±0.02
4	Chloroform	11±0.11	9±0.15	7±0.01

According to them, the aqueous extract didn't show any activity against human pathogenic bacteria's.⁽⁹⁾ Thus Ethanolic and methanolic leaf extract show better activity against, pathogenic bacteria's. Both extract more effective against, *Proteus* and *Pseudomonas sp.*⁽¹⁰⁾, but in present studies, Aqueous leaves extract showed better response against, *Enterobacter* and in Ethanolic leaf extracts show better response against, *Achromobacter* and *Enterococci* and in Methanolic leaf extract showed few response against, *Bacillus* and *Klebsiella*. *Calotropis gigantea* plant extracts like leaves extract are showing few responses in all tested pathogenic bacterial sp. So these Extracts were showed effective and significant Antibacterial Activity against pathogenic bacterial species.

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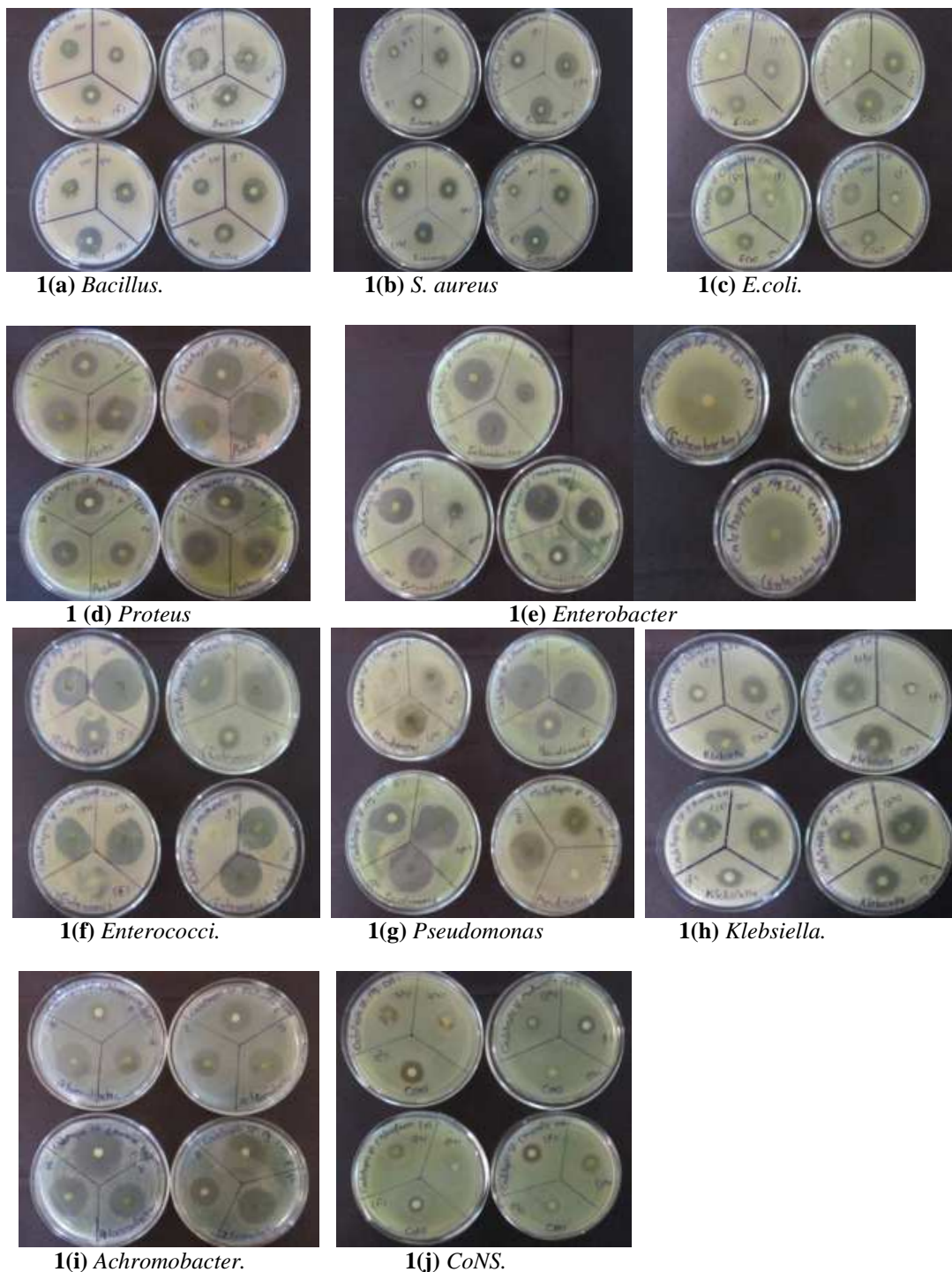


Fig. 1. Zone of Inhibition of Human pathogenic bacteria by Paper disc method.

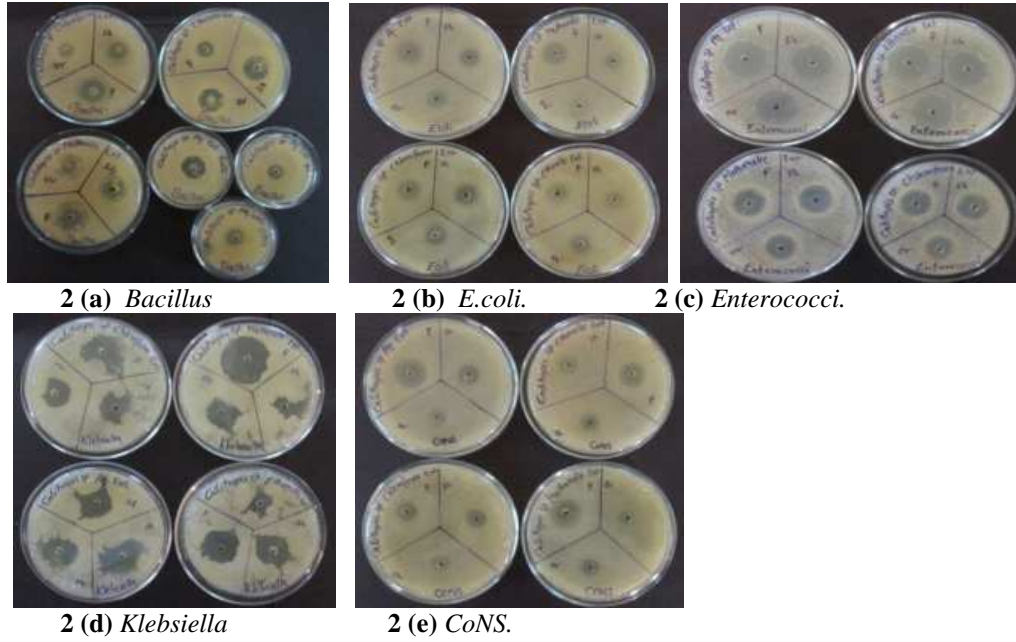


Fig. (2). Zone of Inhibition of Human pathogenic bacteria by Agar well method.