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"ANTIBACTERIAL ACTIVITY OF PROTEIN 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 Protein by flower using mortal piston. Paper disk and Agar well methods was employed to determine the antibacterial activity against some pathogenic bacteria species like *Bacillus, Enterobacter, Enterococci, E.coli, Pseudomonas, Proteus, Klebsiella, Achromobacter, CoNS* and *Staphylococcus aureus*. So, in present studies, Protein extracts was showed better response against *E.coli and Enterococci*. where zone of inhibition showed high. These Extracts were showed significant Antibacterial Activity against pathogenic bacteria species.

KEYWORDS: Calotropis gigantea, Antibacterial Activity, and Protein extracts.

INTRODUCTION
The various pharmacological activities of whole plant or plant parts including latex of *Calotropis procera* (Ait.) R. Brown (family: Asclepiadaceae, nature: shrub, synonym: *Calotropis gigantea*) have been documented. Reported activities included analgesic and antihelmintic activities in the flowers **(Pathak and Arhal, 2007; Iqbal et al., 2005).** It is called as "Ruvi" in Marathi and "Madar" in Hindi. Flowers are regular, bisexual, arranged in simple or rarely compound cymes corymbs. It has been reported traditionally for antifertility, alexipharmic, antihelmintic, purgative and abortifacient activities. The plant has enormous medicinal properties to cures leprosy, leucoderma, ulcers, tumours, piles, diseases of the spleen, liver and abdomen **(Nadkarni KM. 1976)**. 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 **(D.J. Newman, G.M. Cragg and K.M. Snader, 2003 M. Butler**, **2004).**

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, Enterobactor, Pseudomonas, Klebsiella, Enterococci, Achromobacter, CONS (Coagulus Negative Staphylococcus).*

B) Collection of flower Sample
The flowers of Healthy disease free plant *Calotropis gigantea* used in this study were obtained from the local area of Palod village, District- Raipur (Chhattisgarh), India. And were identified based on its physical characteristics. The flowers were dried and crushed to small pieces using pestle and mortar and powered in an electric grinder.

C) Preparation of Protein Extraction by Flower
1) Protein Extract of Fresh and Dried flower
1gm of fresh and dried weight of the flower was dissolved into 2 mL of phosphate buffer and mixed nicely. Centrifuged at 10,000 rpm for 15 minute at 4 ºC, supernatant was taken. Further, the equal volume of acetone was added and centrifuged at 10,000 rpm for 15 min at 4 ºC. The pellet was taken and dissolved in phosphate buffer. This was taken as pure sample. The process was preceded in the same way as above for the extraction of crude sample which was dividing of acetone **(Sambrook J, Fritsch EF, Maniatis T., 1989).**

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 as described by **Omenka and Osuoha (2000).**

1) Paper Disc Technique

Sterile filter paper discs (6.0 mm diameter) were soaked with the test extracts and dried at 40ºC 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 37ºC 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 37ºC for 48 hours. The zones of inhibition were measured and recorded.

RESULTS AND DISCUSSION

The Flower of *Calotropis gigantea* plant used for present studies of Antibacterial activity test. In the study of antibacterial activity prepared protein by fresh and dried flower, for using Antibacterial activity in protein extract of *Calotropis gigantea* against human pathogenic bacteria's, and each Antibacterial activity test made in triplicate form.

- The observed results of Antibacterial activity of **protein Extract** using **paper dics method** against pathogenic bacteria's are given below:

Bacillus- The Antibacterial activity of protein extract against *Bacillus* on the extract of fresh and dried flower. The higher zones of inhibition showed (11±0.33) in fresh extract, but dried extract didn't show significant result. So it is unmarkable. **(fig. no.1 (a), Table no.1 (a).**

Table.1). Antibacterial activity of *Calotropis gigantea* Protein extract using Paper dics method against Human pathogenic bacteria.

Table.1 (a): Antibacterial activity against *Bacillus.*

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	11±0.33
2	Dried	Nil

Staphylococcus aureus

The Antibacterial activity of protein extract against *S. aureus* on the extract of fresh and dried flower. The higher zones of inhibition showed (13±0.23) in fresh extract and (9±0.07) in dried extract. So the better result is showed fresh extract as compared to dried extract. **(fig. no.1 (b), Table no.2 (b)**

Table: 2 (b). Antibacterial activity against *Staphylococcus aureus*

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	13±0.23
2	Dried	9±0.07

E.coli

The Antibacterial activity of protein extract against *E.coli.* on the extract of fresh and dried flower. The higher zones of inhibition showed (21±0.67) in fresh extract and (17±0.12) in dried extract. So the better result is showed fresh extract as compared to dried extract.**(fig. no.1(c), Table no.2(c)**

Table: 2(c). Antibacterial activity against *E.coli.*

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	21±0.67
2	Dried	17±0.12

Proteus- The Antibacterial activity of protein extract against *Proteus* on the extract of fresh and dried flower. The higher zones of inhibition showed (15±0.13) in fresh extract and (12±0.11). in dried extract. So the better result is showed fresh extract as compared to dried extract. **(fig. no. 1 (d), Table no.2 (d)**.

Table: 7(d). Antibacterial activity against *Proteus.*

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	15±0.13
2	Dried	12±0.11

Enterobacter

The Antibacterial activity of protein extract against *Enterobacter* on the extract of fresh and dried flower. The higher zones of inhibition showed (15±0.09) in fresh extract and (17±0.67) in dried extract. So the better result is showed dried extract as compared to fresh extract. **(fig. no.1 (e), Table no. 2 (e)**

Table: 2(e). Antibacterial activity against *Enterobacter.*

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	15±0.09
2	Dried	17±0.67

Enterococci

The Antibacterial activity of protein extract against *Enterococci* on the extract of fresh and dried flower. The higher zones of inhibition showed (16±0.08) in fresh extract and (19±0.11). in dried extract. So the better result is showed dried extract as compared to fresh extract. **(fig. no.1(f), Table no.2(f)**.

Table.2 (f). Antibacterial activity against *Enterococci.*

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	16±0.08
2	Dried	19±0.11

Pseudomonas

The Antibacterial activity of protein extract against *Pseudomonas* on the extract of fresh and dried flower. The higher zones of inhibition showed (19±0.33) in fresh extract and (18±0.12) in dried extract. So the better result is showed fresh extract as compared to dried extract. **(fig. no. 1 (g), Table no.2 (g).**

Table.2(g). Antibacterial activity against *Pseudomonas.*

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	19±0.33
2	Dried	18±0.12

Klebsiella

The Antibacterial activity of protein extract against *Klebsiella* on the extract of fresh and dried flower. The higher zones of inhibition showed (14±0.09) in fresh extract and (7±0.03). in dried extract. So the better result is showed fresh extract as compared to dried extract. **(fig. no.1 (h), Table no.2 (h).**

Table.2(h) Antibacterial activity against *Klebsiella.*

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	14±0.09
2	Dried	7±0.03

Achromobacter

The Antibacterial activity of protein extract against *Achromobacter* on the extract of fresh and dried flower. The higher zones of inhibition showed (17±0.12) in fresh extract and (15±0.07) in dried extract. So the better result is showed fresh extract as compared to dried extract. **(fig. no.1 (h), Table no.2 (h).**

Table.2 (h). Antibacterial activity against *Achromobacter.*

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	17±0.12
2	Dried	15±0.07

CoNS- The Antibacterial activity of protein extract against *CoNS* on fresh and dried flower. The higher zones of inhibition showed (19±0.33) in fresh extract and (18±0.17) in dried extract. So the better result is showed fresh extract as compared to dried extract **(fig. no.1 (i), Table no.2 (i).**

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

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	19±0.33
2	Dried	18±0.17

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

Bacillus

The Antibacterial activity of protein extract against *Bacillus* on the extract of fresh and dried flower. The higher zones of inhibition showed (20±0.07) in fresh extract and (16±0.03) in dried extract. So the better result is showed fresh extract as compared to dried extract. **(fig. no.2 (a), Table no.2 (a)**.

Table.8). Antibacterial activity of *Calotropis sp*. Protein Extract using Agar well method against Human pathogenic bacteria.

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

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	20±0.07
2	Dried	16±0.03

E.coli- The Antibacterial activity of protein extract against *E.coli* on the extract of fresh and dried flower. The higher zones of inhibition showed (15±0.67) in fresh extract and (11±0.09) in dried extract. So the better result is showed fresh extract as compared to dried extract. **(fig. no.2(b), Table no.2(b).**

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

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	15±0.67
2	Dried	11±0.09

Enterococci

The Antibacterial activity of protein extract against *Enterococci* on the extract of fresh and dried flower. The higher zones of inhibition showed (21±0.18) in fresh extract and (19±0.11) in dried extract. So the better result is showed fresh extract as compared to dried extract. **(fig. no.2 (c), Table no.2 (c).**

Table.2 (c). Antibacterial activity against *Enterococci.*

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	21±0.18
2	Dried	19±0.11

Klebsiella

The Antibacterial activity of protein extract against *Klebsiella* on the extract of fresh and dried flower. The higher zones of inhibition showed (14±0.33) in fresh extract and (10±0.09) in dried extract. So the better result is showed fresh extract as compared to dried extract. **(fig. no. 2 (d), Table no.2 (d)**.

Table.2 (d). Antibacterial activity against *Klebsiella.*

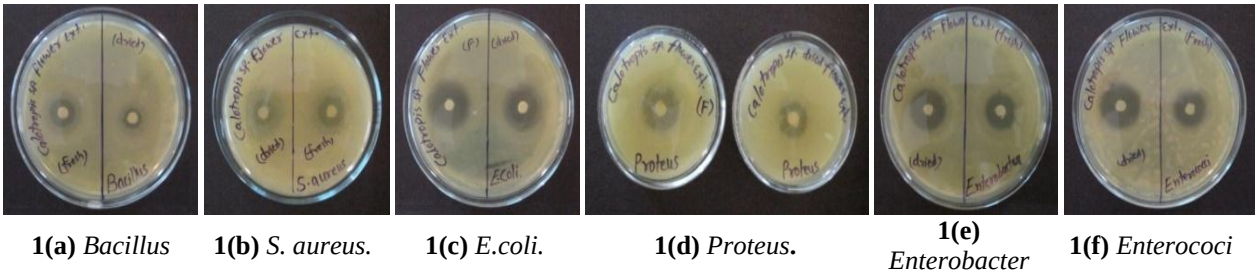
S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	14±0.33
2	Dried	10±0.09

CoNS- The Antibacterial activity of protein extract against *CoNS* on the extract of fresh and dried flower. The higher zone of inhibition on the dried extract in (10±0.05) but fresh extract were didn't show any significant result. So it is unmarkable. **(fig. no.2 (e), Table no.2 (e).**

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

S.No.	Protein Extract	Zone of Inhibition in (mm)
1	Fresh	Nil
2	Dried	10±0.05

According to them, the Antibacterial Activity test against bacterial sp. in protein extract, in which the better response get in *E.coli* **(Ruban et. al; 2012),** In the present studies the Antibacterial Activity in fresh protein extract against bacterial sp., in the present studies, Protein extracts was showed better response against *E.coli and Enterococci*. *Calotropis gigantea* proteins by flowers are showing few responses in all tested pathogenic bacteria sp. So these Extracts were showed effective and significant Antibacterial Activity against pathogenic bacteria species.



1(a) *Bacillus* **1(b)** *S. aureus.* **1(c)** *E.coli.* **1(d)** *Proteus.* **1(e)** *Enterobacter* **1(f)** *Enterococi*

1(g) *Pseudomonas.* **1(h)** *Klebsiella* **1(i)** *Achromobacter.* **1(j)** *CoNS.*

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

2 (a) *Bacillus* **2 (b)** *E.coli.* **2 (c)** *Enterococci.* **2 (d)** *Klebsiella.* **2 (e)** *CoNS.*

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

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