

## Antimicrobial activity of *Crinum defixum* Ker - Gawler Leaves

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### ABSTRACT

*Crinum defixum* Ker - Gawler is a bulbous herb which has widely used in indigenous medicine for many diseases; mainly leaf and bulb extracts are of medicinal importance. Leaf extract is used as a treatment for pimples, body-ache, dropsy, carbuncle, paronychia, leprosy, fever and diarrhoea. The bulbs are crushed and applied on to piles and itching. The present study to determine the possible phytochemicals and antimicrobial activity of various leaf extracts of *Crinum defixum* Ker - Gawler plant. The phytochemical screening demonstrated the presence different types compound like flavonoids, tannins, phenolic compounds, saponins and glycosides. The ethanol, methanol and crude extracts of *Crinum defixum* Ker - Gawler showed significant antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *pseudomonas aeruginosa*, *proteus vulgaris* and antifungal activity against *Candida albicans*, *Aspergillus flavus* when compare to different concentrations ( $\mu\text{g/mL}$ ). The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disk in millimetres. Thus, the present findings revealed the medicinal potential of *Crinum defixum* Ker - Gawler to develop a drug against various human ailments.

### 1. INTRODUCTION

Plants are major source of medicine, have been playing important roles in the human health around the globe [1]. Plants remain the most common source of antimicrobial agents. The usage of natural medicine have minimum the side effects [2,3]. In rural areas peoples depends herbal medicines for the treatment of different types of diseases. In recent years government and pharmaceutical side spent a lot of moneys to developing natural medicines extracted from herbals. It indicates that the importance of the natural drugs and human health care system.

A wide range of medicinal plant parts are used in extract form, and they varied from plant properties. The different parts of the plants are leaf, root, stem, flower, fruit, and seeds and modified plant organs. While some of these are raw drugs are collected in small quantities by the local folk healers and local communities. Many other raw drugs are collected in large quantities and are trade marketed in many herbal industries [4]. *Crinum defixum* Ker - Gawler (Amaryllidaceae) is a bulbous herb and is commonly known as Bon – naharu (meaning wild garlic) in Assam [5]. This has widely distributed in tropical and sub-tropical regions of the world. The *Crinum* species grow to a height of about 1m, depending on the species which have a long neck up to 30 cm height. Leaves spirally arranged, sessile, linear, and strap-shaped with smooth edges. The flowers are directly attached to the bulb and pleasant odour. The bulb of this plant is fusiform, stoloniferous base and it has cylindrical neck [6].

The *Crinum* species have highly medicinal importance. The leaves were used to treating pimples, body-ache, leprosy, paronychia, carbuncles, diarrhoea and leucorrhoea. The bulb is nauseant, emetic, emollient and diaphoretic. It is also used in treatment of burns, itching's, whitlow and carbuncle [7]. The literature survey of *Crinum defixum* Ker - Gawler has shown the presence of caranine, crinamine, crinine, galanthamine, galanthine, haemanthamine and hippetrine [8]. In the recent years a new alkaloid 5  $\alpha$ -hydroxyhomolycorine has also been isolated from *Crinum*

*defixum* Ker – Gawler [9]. Ethanolic extract of dried leaves of *Crinum defixum* Ker - Gawler has been reported to exhibit a free radical scavenging [10], analgesic, anti-inflammatory properties [11]. Hence the present study was focused to evaluation of antimicrobial potential of the plant.

## 2. MATERIALS AND METHODS

### Collection of plant materials

The leaves of *Crinum defixum* Ker - Gawler were collected from Poondi village, Thanjavur District, Tamilnadu. The botanical identity of the plant of was confirmed by Dr.S.John Britto, Rapinat Herbarium, St. Joseph's College, Tiruchirappalli.

### Preparation of Extracts

The fine powder (5 Kg) was extracted with 95% ethanol at room temperature for ten days. The extract was filtered and concentrated under reduced pressure in a rotary evaporator and extracted for various solvents in increasing order of polarity from n-Hexane, Chloroform, Ethyl acetate, Acetone, Butanol, Ethanol and Methanol. The yields of fractions are dried and weighed. The dried fractions of ethanol, methanol and crude extracts were subjected to screening of phytochemical and antimicrobial activity.

### Phytochemical screening

The ethanol, methanol and crude leaf extract of *Crinum defixum* Ker - Gawler plant revealed the following phytochemicals (Table.1).

### *In-vitro* antimicrobial activity (Disc diffusion method)

The ethanol, methanol and crude extracts were prepared in various concentrations such as 50, 100, 150 µg/mL respectively and used for antimicrobial activity.

### Test microorganisms

Pure cultures of *Escherichia coli*, *Staphylococcus aureus* (Gram positive bacteria), *Pseudomonas aeruginosa*, *Proteus vulgaris* (Gram negative bacteria) specie of bacteria's and *Candida albicans*, *Aspergillus flavus* specie, of fungi's were procured from Rontgen Laboratory, Thanjavur. These microorganisms were identified and confirmed by Microbiologists, Department of Microbiology, Thanjavur Medical College, Thanjavur.

### Preparation of 24 hours pure culture

A loop full of each of the microorganisms was suspended in about 10 mL of physiological saline in a Roux bottle. Each of these was streaked onto the appropriate culture slants and was incubated at 37°C for 24 hours except for fungal which was incubated at 25°C for 24 - 48 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8°C until use.

### Preparation of plant extracts solutions for the experiment

The dried *Crinum defixum* Ker - Gawler plant extract was weighed (10mg/mL) and dissolved in sterile distilled to prepare appropriate dilution to get required concentrations of about 50µL (50µg), 100µL (100µg) and 150µL (150µg). Control was used as a respective solvent (Aqueous). They were kept under refrigerated condition unless they were used for the experiment. Standard solution as Chloramphenicol for bacteria and fluconazole (25mg/mL distilled water-

30 $\mu$ L) for fungi used to compare the test solution. They were kept under refrigerated condition unless they were used for the experiment.

### Preparation of dried filter paper discs

Whattmann filter paper (No:1) was used to prepare discs approximately 6 mm in diameter, which are placed in hot air for sterilization. After sterilization, the discs were loaded with different concentrations of prepared plant extract solutions and again kept under refrigeration for 24 hours.

### Application of discs to inoculated agar plates

Previously prepared paper discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down firmly to ensure complete contact with the agar surface. The discs were placed on the medium suitably apart and the plates were incubated at 5°C for 1 hour to permit good diffusion and then transferred to incubator at 37°C for 24 hours. After completion of 24hours, the plates were inverted and placed in an incubator set to respective temperature for 24 hours.

### Antimicrobial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka et al., 2007) [12] using plant extracts. Petri plates were prepared by pouring 30 mL of NA/PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 minutes. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *E.coli*, *S.aureus* (Gram positive bacteria), *P.aeruginosa*, *P.vulgaris*(Gram negative bacteria) were spread on Nutrient agar plates for bacteria and *C.albicans*, *A.flavus* were spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50 $\mu$ l, 100  $\mu$ l and 150  $\mu$ l) were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 hours for the bacteria and 48 hours for fungus at room temperature (30 $\pm$ 1) for 24 - 48 hour for yeasts strains. Each sample was tested in triplicate.

**Table.1. Preliminary phytochemical constituents of *Crinum defixum* Ker – Gawler leaves.**

S.N	Phytochemicals	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Acetone Extract	Ethanol Extract	Butanol Extract	Methanol Extract
1.	Alkaloids	-	Present	Present	Present	Present	-	-
2.	Flavonoids	-	-	-	-	-	-	-
3.	Terpenoids	Present	Present	Present	-	-	-	-
4.	Glycosides	-	-	-	-	-	-	-
5.	Saponins	-	Present	Present	Present	Present	-	-
6.	Steroids	-	-	-	-	-	-	-
7.	Carbohydrates	-	-	-	-	-	-	-
8.	Phenolic compounds	Present	Present	Present	Present	Present	Present	-
9.	Tannins	-	-	-	-	-	-	-
10.	Amino acids	Present	-	-	Present	Present	Present	Present

**Table.2. Antibacterial activity of *Crinum defixum* Ker - Gawler plant leaves against *Escherichia coli* bacteria at different concentrations**

Samples	50 $\mu$ l (mm)	100 $\mu$ l (mm)	150 $\mu$ l (mm)	Standard (Chloromphenical for bacteria) (mm)	Control (solvent)
Crude extract	8 $\pm$ 0.56	10 $\pm$ 0.70	12 $\pm$ 0.84	15 $\pm$ 1.05	0
Ethanolic extract	5 $\pm$ 0.35	7 $\pm$ 0.49	9 $\pm$ 0.63	11 $\pm$ 0.77	0
Methanolic extract	6 $\pm$ 0.42	9 $\pm$ 0.63	10 $\pm$ 0.70	13 $\pm$ 0.91	0

Values are expressed as Mean  $\pm$  SD for triplicate.

**Table.3. Antibacterial activity of *Crinum defixum* Ker - Gawler plant leaves against *Staphylococcus aureus* bacteria at different concentrations**

Samples	50 $\mu$ l (mm)	100 $\mu$ l (mm)	150 $\mu$ l (mm)	Standard (Chloromphenical for bacteria) (mm)	Control (solvent)
Crude extract	8 $\pm$ 0.56	9 $\pm$ 0.63	10 $\pm$ 0.70	15 $\pm$ 1.05	0
Ethanolic extract	5 $\pm$ 0.35	7 $\pm$ 0.49	9 $\pm$ 0.63	11 $\pm$ 0.77	0
Methanolic extract	6 $\pm$ 0.42	8 $\pm$ 0.56	10 $\pm$ 0.70	13 $\pm$ 0.91	0

Values are expressed as Mean  $\pm$  SD for triplicate.

**Table.4. Antibacterial activity of *Crinum defixum* Ker - Gawler plant leaves against *pseudomonas aeruginosa* bacteria at different concentrations**

Samples	50 $\mu$ l (mm)	100 $\mu$ l (mm)	150 $\mu$ l (mm)	Standard (Chloromphenical for bacteria) (mm)	Control (solvent)
Crude extract	8 $\pm$ 0.56	9 $\pm$ 0.63	10 $\pm$ 0.70	15 $\pm$ 1.05	0
Ethanolic extract	7 $\pm$ 0.49	8 $\pm$ 0.56	7 $\pm$ 0.49	11 $\pm$ 0.77	0
Methanolic extract	5 $\pm$ 0.35	6 $\pm$ 0.42	8 $\pm$ 0.56	13 $\pm$ 0.91	0

Values are expressed as Mean  $\pm$  SD for triplicate.

**Table.5. Antibacterial activity of *Crinum defixum* Ker - Gawler plant leaves against *proteus vulgaris* bacteria at different concentrations**

Samples	50 $\mu$ l (mm)	100 $\mu$ l (mm)	150 $\mu$ l (mm)	Standard (Chloromphenical for bacteria) (mm)	Control (solvent)
Crude extract	9 $\pm$ 0.63	10 $\pm$ 0.70	12 $\pm$ 0.84	15 $\pm$ 1.05	0
Ethanolic extract	8 $\pm$ 0.56	12 $\pm$ 0.84	15 $\pm$ 1.05	11 $\pm$ 0.77	0
Methanolic extract	7 $\pm$ 0.49	10 $\pm$ 0.70	12 $\pm$ 0.84	13 $\pm$ 0.91	0

Values are expressed as Mean  $\pm$  SD for triplicate.

**Table.6. Anti – fungal activity of *Crinum defixum* Ker - Gawler plant leaves against *Candida albicans* fungus at different concentrations**

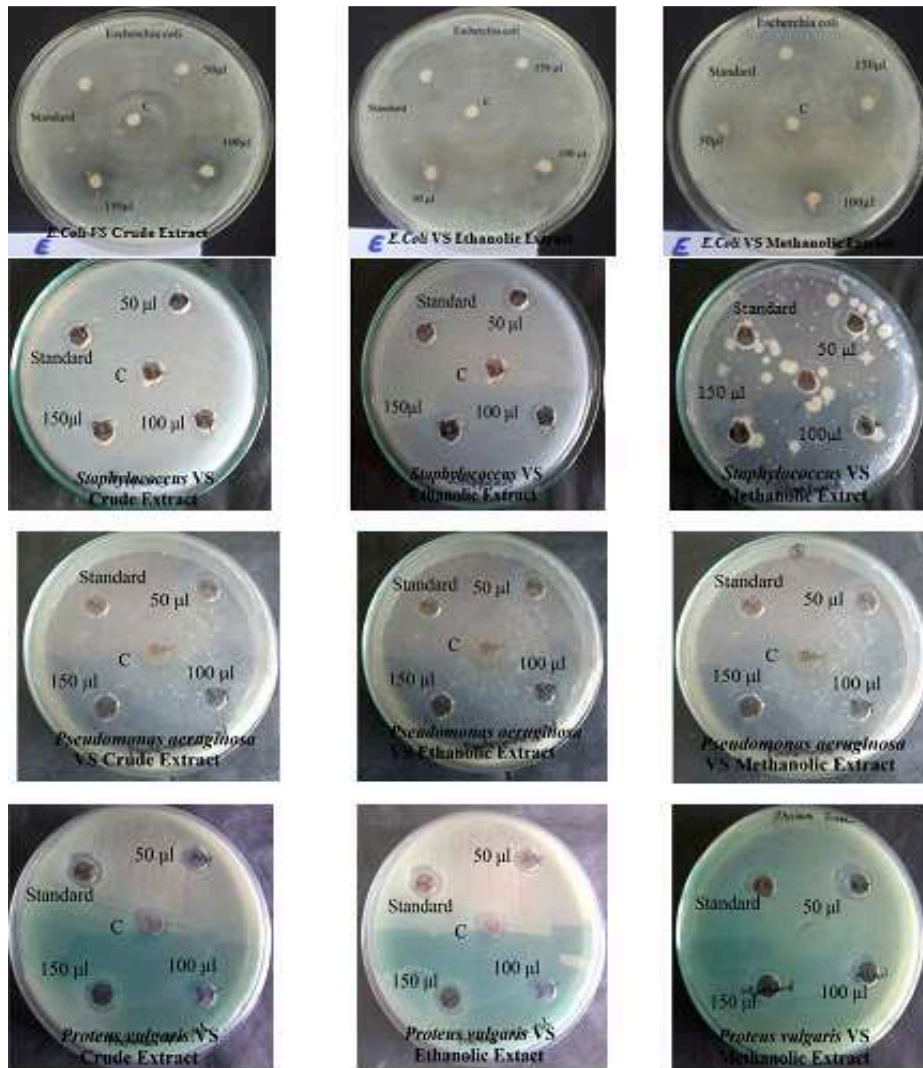
Samples	50 $\mu$ l (mm)	100 $\mu$ l (mm)	150 $\mu$ l (mm)	Standard (Nystatin for fungi) (mm)	Control (solvent)
Crude extract	6 $\pm$ 0.42	8 $\pm$ 0.56	9 $\pm$ 0.63	13 $\pm$ 0.91	0
Ethanollic extract	7 $\pm$ 0.49	8 $\pm$ 0.56	10 $\pm$ 0.70	14 $\pm$ 0.98	0
Methanolic extract	5 $\pm$ 0.35	7 $\pm$ 0.49	8 $\pm$ 0.56	11 $\pm$ 0.77	0

Values are expressed as Mean  $\pm$  SD for triplicate.

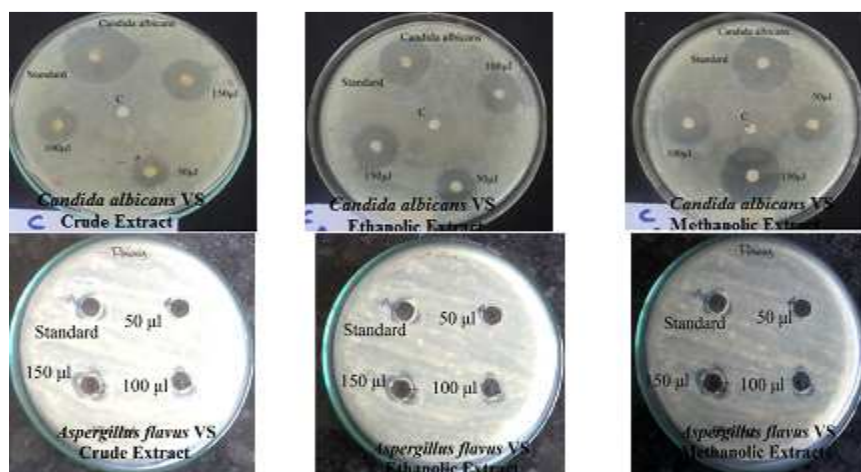
**Table.7. Anti – fungal activity of *Crinum defixum* Ker - Gawler plant leaves against *Aspergillus flavus* fungus at different concentrations**

Samples	50 $\mu$ l (mm)	100 $\mu$ l (mm)	150 $\mu$ l (mm)	Standard (Nystatin for fungi) (mm)	Control (solvent)
Crude extract	5 $\pm$ 0.35	6 $\pm$ 0.42	9 $\pm$ 0.63	13 $\pm$ 0.91	0
Ethanollic extract	5 $\pm$ 0.35	7 $\pm$ 0.49	8 $\pm$ 0.56	14 $\pm$ 0.98	0
Methanolic extract	6 $\pm$ 0.42	9 $\pm$ 0.63	10 $\pm$ 0.70	11 $\pm$ 0.77	0

Values are expressed as Mean  $\pm$  SD for triplicate.



**Fig.1. Zone inhibitions of Antibacterial activity of *Crinum defixum* Ker - Gawler plant leaves against different pathogens at different concentrations**



**Fig.2. Zone inhibitions of Anti – fungal activity of *Crinum defixum* Ker - Gawler plant leaves against different pathogens at different concentrations**

### 3. RESULT

Result obtained in the present study the antimicrobial activity of the *Crinum defixum* Ker - Gawler plant leaves extracts are shown table 2 to 7. The result shows *Crinum defixum* Ker - Gawler plant extract were effective against both antibacterial and anti-fungal activities. For antibacterial activity the highest activity was recorded as the crude extract of *Crinum defixum* Ker - Gawler against *E.coli* at diameter of zone inhibition by 12 mm at 150  $\mu$ L, for *Crinum defixum* against *S.aureus* the highest zone of inhibition was observed by 10 mm at 150  $\mu$ L for methanol and crude extracts, and *Crinum defixum* against *P.aeruginosa* the highest zone of inhibition was observed by 10 mm at 150  $\mu$ L for crude extract, and *Crinum defixum* against *P.vulgaris* the highest zone of inhibition was demonstrated by 15 mm at 150  $\mu$ L for ethanolic extract, when compared with chloramphenicol as standard.

For anti-fungal activity the highest activity was demonstrated by the ethanolic extract of *Crinum defixum* Ker - Gawler against *C.albicans* at diameter zone of inhibition 10 mm at 150  $\mu$ L, and *Crinum defixum* against *A.flavus* the highest activity was observed by 10 mm at 150  $\mu$ L for methanolic extract respectively, when compared with nystatin as standard.

### 4. DISCUSSION

Plants are main source for the development of new chemotherapeutic agents. The first step towards this goal is the *in-vitro* antibacterial activity assay [12]. Most of the thesis is available on the antibacterial, anti -fungal, anti-inflammatory, antiviral and anthelmintic properties of plants [13,14]. Some of these conclusions are clearly indicates that the identifying the active principle responsible for such activities and in the developing drugs for the therapeutic agents. In the present study, the ethanol, methanol and crude leaf extract of *Crinum defixum* Ker - Gawler was showed the activity against *E.coli*, *S.aureus* (Gram positive bacteria), *P.aeruginosa*, *P.vulgaris*(Gram negative bacteria) and *A.flavus*, *C.albicans* fungus and plant based products have been effectively proven for their utilization as source for antimicrobial compounds.

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**Antimicrobial Activity of *Crinum defixum* Ker - Gawler Leaves**

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