

# Antibacterial Activities of In Vitro Grown Chrysanthemum Morifolium Leaf Extract Against Human Pathogenic Bacteria

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**Abstract-** The main objective of this work is to study the possibility of antibacterial effect of *Chrysanthemum morifolium* plant's leaf extracts against some known pathogenic bacteria, which can be used to discover bioactive natural products. This may serve as feed for the development of new pharmaceuticals compounds. Studies on the antibacterial properties of leaf extracts of *Chrysanthemum morifolium* was evaluated by using zone of inhibition studies and by taking minimum inhibitory concentration. Microorganisms used in this study includes *Escherichia coli*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus pyogenes* which are grown as lawn culture on Muller-Hinton medium. Penicillium-G was used as standard antibiotic controls for all test bacterial strains. The different extracts of leaf exhibit antibacterial activity against all these experimental strains. Inhibition zones are revealed by aqueous extract is comparatively more than the other organic solvent extracts. The aqueous extract shows antibacterial activity against all the test organisms. The extract shows maximum antibacterial activity against *Enterococcus faecalis* followed by *Escherichia coli* and *Staphylococcus aureus*.

**Keywords-** *Chrysanthemum morifolium*, Antibacterial activity, Human pathogenic bacteria, Leaf extract, Inhibition zone.

## I. INTRODUCTION

*Chrysanthemum morifolium* is one of the non-toxic, biocompatible and eco-friendly herbs. It has great potential use as phytomedicine as they have antibacterial properties. So these plants can be used to discover bioactive natural products that may serve as feeds for the development of new pharmaceuticals compounds. It is one of the best choices for researchers to develop antibacterial drugs from its leaf extracts.

The flowering plants of the genus *Chrysanthemum* belongs to family Asteraceae. They are native to Asia and

northeastern Europe. Most of the species of this genus originate from East Asia and the center of diversity is in China.

The genus *Chrysanthemum* was named by Carolus Linnaeus from two Greek words, 'Chrys', which means golden (the color of the original flowers), and 'anthemon', meaning flowers. *Chrysanthemums* are one of the prettiest varieties of perennial herbs that start blooming early in the autumn seasons. This plant is also known to as favorite flower for the month of November. The flowers of this plant bloom in various forms, shape and sizes. The flowers may be daisy-like, decorative, pompons or buttons and are available in a wide array of colours - from white, to yellow and gold, pink, orange, bronze, deep red, maroon, violet and purple. Some *Chrysanthemums* are having a mix of two and even more colours but yellow is the most common color among all. Some species has stronger fragrance and aroma than others; the best species is said smells like the *Dryobalanops aromatica*. Till today, the *Chrysanthemum* flower already became a famous ornamental flower with most varieties and species.

### A. Botanical Description of *Chrysanthemum morifolium*:

*Chrysanthemums* originally came from China, India and Korea. The Chinese varieties are the tallest, reaching heights of 0.2~2.0m. Indian or pompon varieties have smallest flowers. *Chrysanthemums* are perennial herb, height 50 to 140 cm, all densely white hair, slightly woody stem base, slightly purple, young branches with a little edge.

Leaves alternate, ovate or ovate-lanceolate, long 3.5 to 5 cm wide and 3 to 4 cm, apex obtuse, base cordate or broadly cuneate, margin usually pinnatifid, coarsely toothed lobes or doubly serrate, both surfaces densely white villous; petiole shallow grooves. Capitulum generate axillary, diameter of 2.5 to 5 cm; involucre hemispherical, bracts 3-4 layers, green, hair, margin membranous transparent, light brown, outer bracts smaller, ovate or ovate lanceolate, bracts

broadly ovate second layer, inner bracts oblong; small receptacle, projecting, hemispherical; ray florets female, on the edge of the tongues linear-oblong, up to 3 cm, apex obtuse, white, yellow, pink or lilac, no stamen, pistil 1, style short, stigma 2-lobed; tubular flowers bisexual, located in the central, yellow, each with 1 flower outer membranous scales ovate, corolla tube ca. 4 mm, apex 5-lobed, lobes triangular-ovate, stamens 5, poly drug, filaments very short, isolated, pistil 1, ovary inferior, oblong, linear style, stigma 2-lobed. Achenes oblong, with 4-sided, flat-topped cut, smooth and hairless.

The Chrysanthemum flowers normally we are seen, actually is the flower head (capitulum) composed of many real Chrysanthemum flowers. There are two forms of flowers grow on the flower head: one is tubular flower, normally called "flower centre", its corollas connected together cylindrical shape, is hermaphrodite flower, gynoecium grow in the centre, 2 stigmas, around it grows 5 stamens; another is ligulate flower, grow on the edge of anthotaxy, normally called "petals", the stamen of it degraded, 1 gynoecium, ligulate flower mostly big shape and colorful. Achene (normally called seeds) length 1~3mm, width 0.9~1.2mm, color brown, contains a seed inside it.



Figure-1 *Chrysanthemum morifolium* Plant

## B. Health Benefits and Therapeutic Uses of Chrysanthemum

- Chrysanthemum can help the body fight off a range of infections including Streptococcal and Staphylococcal infections.
- The traditional Chinese have used the tea to treat influenza, fevers, inflammations and even heatstroke due to its cooling effect. The herb also helps to correct imbalances that may affect the liver and also helps in dealing with kidney function, thus helping with their treatment.
- A rinse made from the flower can be helpful to treat skin infections.
- Chrysanthemum tea is a great aid to digestion, helping the body to digest greasy food more easily. The tea is also helpful in relieving nasal and head congestion. It is often used to treat obesity and as an aid to lose weight, Because of its zero calorie content.
- It is also said to improve vision and hearing and is given in cases of dizziness, blurred vision and spots in front of the eyes. It may also be helpful in cases of night blindness and to treat conjunctivitis.
- New research has shown that the falconoid acacetin that is present in Chrysanthemum has the ability to inhibit malignant cell growth in the prostate region. This may make it a useful weapon in the battle against prostate and other forms of cancer.
- It is believed to be good for the heart and has been known to lower blood pressure levels. It may also be able to increase blood flow to the heart.
- Traditional Chinese medicine has used these properties of the flower to treat cases of hypertension and angina.

## C. A Cautionary Note:

Some people may be allergic to Chrysanthemum and can experience adverse reactions on consuming or handling it. Physical handling of the flower may result in skin irritation and consumption can cause stomach upset. People having allergies to daisies and asters should avoid Chrysanthemum. Consult your physician before taking Chrysanthemum, especially if you are on other medication that may adversely react with it.

## II. MATERIALS AND METHODS

### A. Collection of plant

The tissue culture plants of *Chrysanthemum morifolium* was developed and leaves were collected from the Nursery, BTC centre, OUAT, Bhubaneswar, Odisha, India. Collected leaves were carefully examined and then followed by sterile procedure.

### B. Extraction of plant material

Various extracts of study plant was prepared according the methodology of Indian pharmacopoeia (Anonymous, 1966). The leaves were washed and dried in shade condition at room temperature. Then the dried leaves were subjected to pulverization to get coarse powder. The coarse powder material was subjected to Soxhlet extraction separately and successively with acetone, ethanol, and methanol, aqueous and n-hexane. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50°C). All the extracts were put in airtight containers and stored in refrigerator. All the extracts were involved in microbiological investigations.

### C. Microbial strain and media used:

The present investigation has been undertaken to find out the effectiveness of the acetone, ethanol, and methanol, aqueous and n-hexane extracts of *Chrysanthemum morifolium* were against five human pathogenic bacterial strains such as *Escherichia coli* (ATCC8739), *Staphylococcus aureus* (ATCC 6538), *Streptococcus pyogenes* (ATCC 19615), *Enterococcus faecalis* (ATCC51299) and *Klebsiella pneumonia* (ATCC13883)

Muller Hinton Media (HT-Media) 36 g was mixed with distilled water and then sterilized in autoclave at 15 minutes. The sterilized media were poured in the Petri dishes. The solidified plates were poured with 5 mm diameter of media. Well formed plates were used for the study of antibacterial studies.



**Figure-2.** Leaf Powder for extraction



**Figure-3.** Solvents with leaf extracts



**Figure-4.** Microbial strain in culture media

### D. Antimicrobial assay

10% (W/V) test solution of each extract was prepared by dissolving 1 gm of each extract separately in 10 ml of sterile dimethyl formide (DMF). 0.1% W/V solution of penicillium-G in 10 ml of sterile water was used as standard. Antimicrobial activity was tested by agar diffusion method employing 24 hours cultures of microorganisms. Required volume of chosen concentration was mixed with the medium and poured aseptically into the sterilized petridishes. After solidification of the medium, bacterial strains were inoculated and the petridishes are incubated at 37°C. After 24 hours of time interval the growth of culture were observed. Normal control without any antibiotic and test compound was maintained to check error (Bauer *et al.* 1966)

### E. Replicates

For all the study plant extracts for each concentration three replicates were maintained. Totally 15 Petri plates were maintained for 10% (1gm/10ml) concentration of extract of each solvent.

### F. Statistical analysis

Mean and standard deviations were calculated to facilitate the comparison of the data for various concentrations of plant extracts in all samples (Sails Bose, 1982). Limits of mean can be calculated by relating the standard deviation of the sample mean to the probability of deviations from the population mean by confidence interval.

## III. RESULT AND DISCUSSION

In this study, different concentration of acetone, ethanol, methanol, aqueous and N-hexane extracts of *Chrysanthemum morifolium* were carried out against five known human bacterial strains. The different solvent extract of the leaf were taken in same concentration (10%) for antimicrobial study by disc diffusion assay (using sterile disc) against some human pathogenic bacterial strains grown as lawn culture on Muller-Hinton medium. Penicillium-G was used as standard antibiotic controls for all test bacterial strains.

Antibacterial activities of leaf extracts of *Chrysanthemum morifolium* were evaluated in five human pathogenic bacterial strains. The different leaf extracts of *Chrysanthemum morifolium* exhibited promising results against these bacterial strains (Table-1, Figure 5).

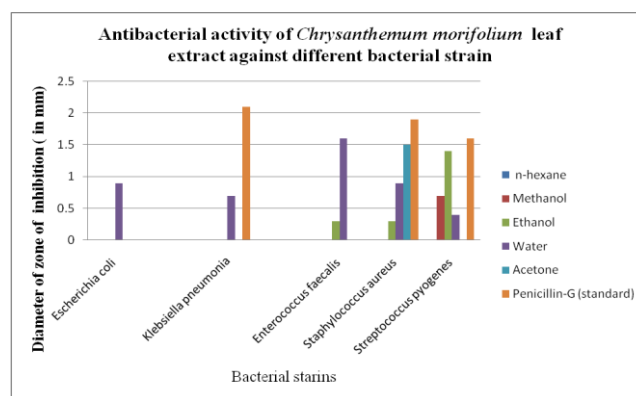
High frequency of antibacterial activity was observed in the case of *Staphylococcus aureus* and *Streptococcus pyogenes*. Moderate rate of antibacterial activity was observed in case of *Klebsiella pneumonia* and *Enterococcus faecalis*. Minimum antibacterial activity was observed in case of *Escherichia coli*. The aqueous extracts shows antibacterial activity against all the test organisms. Both ethanolic extracts and standard antibiotics exhibit moderate activity against *Klebsiella pneumonia*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus pyogenes*. Methanolic extracts shows antibacterial activity only against *Streptococcus pyogenes*.

Standard antibiotics shows highest zone of inhibition against *Klebsiella pneumonia* (2.1 mm), *Staphylococcus aureus* (1.9 mm) and *Streptococcus pyogenes* (1.6 mm). In case of aqueous extracts the diameter of inhibition zone ranges between 0.4 to 1.6 mm. Highest zone of inhibition found in *Enterococcus faecalis* (1.6 mm) followed by *Escherichia coli* and *Staphylococcus aureus* (0.9 mm) whereas in *Klebsiella*

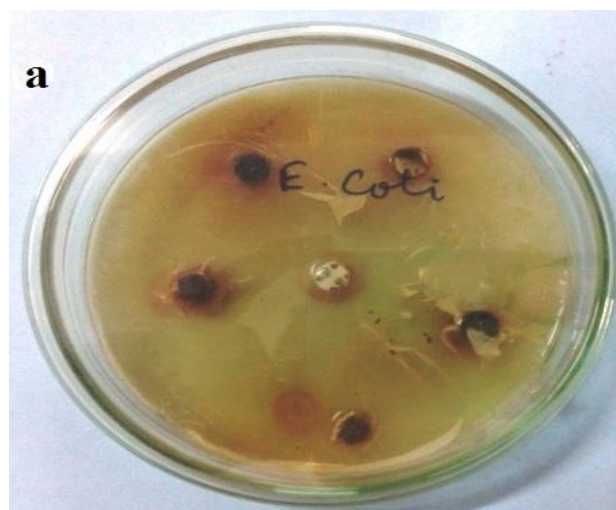
*pneumonia* and *Streptococcus pyogene* least zone of inhibition was observed i.e 0.7 and 0.4 mm respectively. In ethanol extract maximum zone of inhibition was found against *Streptococcus* (1.4 mm) and Minimum zone of inhibition were found against both *Enterococcus faecalis* and *Staphylococcus aureus* (0.3mm). Methanolic extracts only produce antimicrobial activity against *Streptococcus pyogene* (0.7mm) and acetone shows against *Staphylococcus aureus* (1.5mm). The n-hexane extract doesn't show any effect on any test microorganisms.

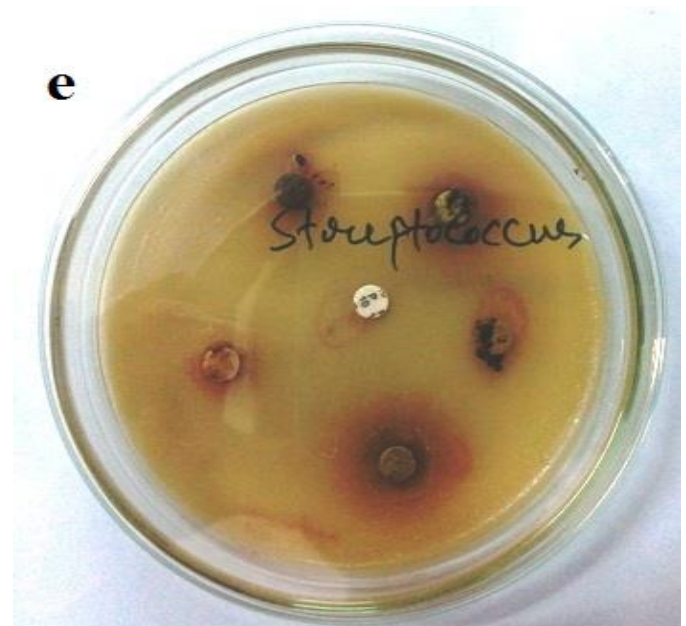
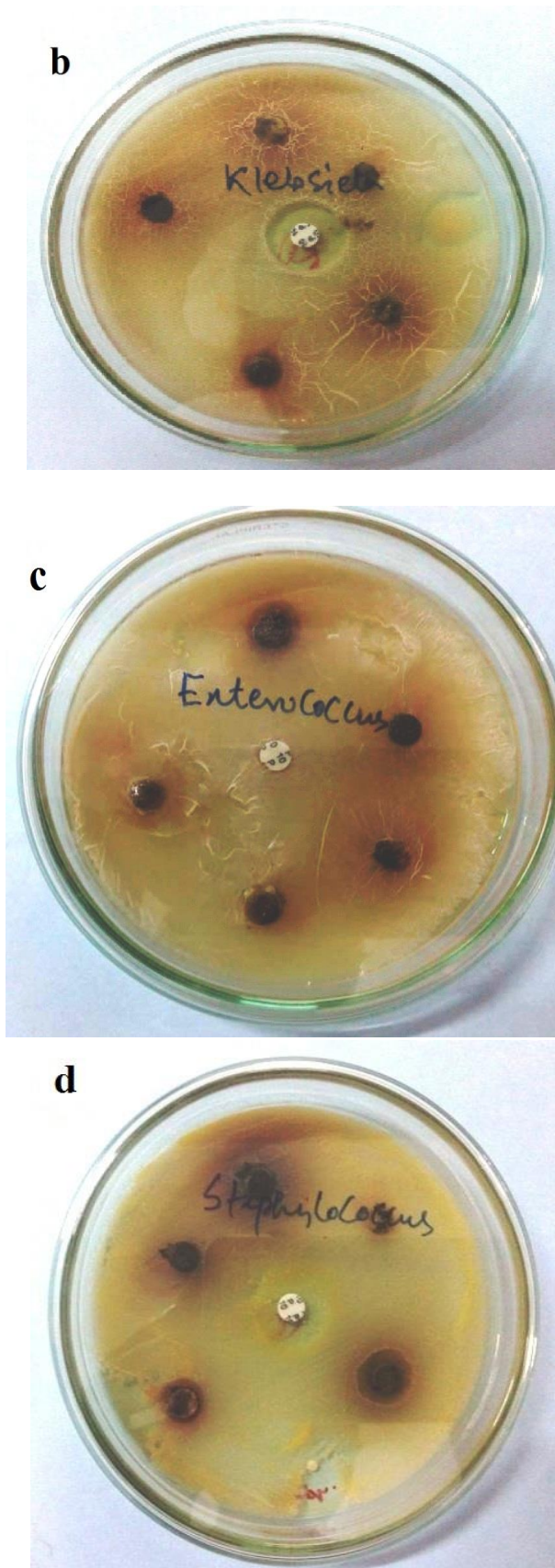
**Table-1 Antibacterial activity of *Chrysanthemum morifolium* against 5 test bacteria**

S.No.	Name of the organisms	Zone of Inhibition (in mm)					
		n-hexane	Methanol	Ethanol	Water	Acetone	Penicillin-G (standard)
1	<i>Escherichia coli</i>	-	-	-	0.9	-	-
2	<i>Klebsiella pneumonia</i>	-	-	-	0.7	-	2.1
3	<i>Enterococcus faecalis</i>	-	-	0.3	1.6	-	-
4	<i>Staphylococcus aureus</i>	-	-	0.3	0.9	1.5	1.9
5	<i>Streptococcus pyogenes</i>	-	0.7	1.4	0.4	-	1.6



**Figure-5.** Antibacterial activities of different leaf extracts of *Chrysanthemum morifolium*





**Figure-6.** Inhibitory zones shown by leaf extracts of *chrysanthemum morifolium* against  
**a.** *Escherichia coli* **b.** *Klebsiella pneumonia* **c.** *Enterococcus faecalis* **d.** *Staphylococcus aureus*  
**e.** *Streptococcus pyogenes*

#### IV. CONCLUSION

From the present work it was found that the different leaf extracts of *Chrysanthemum morifolium* except the n-hexane extract exhibit promising results against all these bacterial strains. Among all the extracts, aqueous extract shows the antibacterial activity against all the strain and also shows better result than other extracts.

Thus *Chrysanthemum morifolium* have great potential use and it can be used to discover bioactive natural products that may serve as feeds for the development of new pharmaceuticals compounds. This work presents an overview of presence of phytochemicals in *Chrysanthemum morifolium* and its antibacterial properties. In order to develop more effective drugs in future for various pathogens one should recognizes phytochemicals and their performances. Many research works have been done on presence of phytochemicals in flowers and leafs of *Chrysanthemum morifolium* plants and its action on microbes but more innovative research work still required finding new which lead to develop new pharmaceutical compounds which can act on the common disease caused by bacteria.

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