

# Evaluation of Pharmacological Activity of Selected Flowers Used in Bathukamma - State Festival of Telangana

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## ABSTRACT

**Objective:** The present study was aimed at evaluating the antimicrobial and antioxidant activity of ten different flowers used in floral festival called Bathukamma.

**Methods:** As most of the flowers used in this festival are known to possess medicinal values, their methanol extracts were tested to detect various pharmacological activities. Well diffusion method for antimicrobial activity, DPPH assay for antioxidant activity and TLC were employed.

**Results:** Of the flowers used, *Crossandra*, Hibiscus, *Nerium*, *Tagetes*, *Celosia argentea* and *Cassia auriculata* exhibited high antibacterial activity. Others with moderate activity. High antifungal activity was showed by *Tagetes*, *Chrysanthemum*, *Celosia cristata*, *Celosia argentea* and *Cucurbita maxima*. Of the extracts used, *Nerium*, *Tagetes*, *Chrysanthemum*, *Celosia argentea* and *Nelumbo* exhibited very high radical scavenging activity. TLC separation achieved several visible and UV detected components in extracts of Hibiscus, *Chrysanthemum*, *Celosia argentea*, *Cassia auriculata* and *Cucurbita*.

**Conclusion:** These results support the tradition of releasing these flowers in water during festival which significantly enhances the antimicrobial activity of water bodies there by aids in their self purification process.

**Keywords:** Antimicrobial, Antioxidant, *Crossandra*, *Nerium*, *Tagetes*, *Celosia cristata*, *Celosia argentea*, *Nerium*, *Nelumbo*, *Cassia auriculata*.

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## INTRODUCTION

India is known for its traditions and celebrations of festivals. Every festival has a scientific reason to support its celebration. In this regard, present study is planned to evaluate pharmacological activity of flowers used in Bathukamma festival which has been recently declared as state festival of newly formed Telangana state<sup>1</sup> It is a floral festival in which everyday various colored flowers are arranged row after row in a brass plate, called as Bathukamma, (figure 1) placed in front of Diety and daily worshipped for a week. In the evening it is carried to nearby pond or any water body and released in it.<sup>2</sup> (figure 2).

*Celosia argentea* is most important flower used in this festival. It is commonly known as silver cock's comb, safaid murga, (Gunugu in Telugu), belongs to family Amaranthaceae. It is a common weed throughout India and used in traditional medicine for Diarrhoea, dysentery, abdominal pain etc. It is known to possess antibacterial activity against several burn and wound pathogenic bacteria.<sup>3</sup> *Cassia auriculata* of family Caesalpinaceae is the second most important flower, commonly known as Senna (Tangedu in Telugu). It is common plant in Asia, often used in Ayurvedic medicine as a remedy for Diabetes, Ulcers, Leprosy, Conjunctivitis, skin problems etc. It is known to possess antibacterial and antioxidant activity.<sup>4</sup> *Tagetes erectus* of family Asteraceae is most often used in Bathukamma has great medicinal value and used in ulcers, laxation, eye diseases, Kidney troubles, muscular pain, Rheumatism, Bronchitis etc. It also exhibits antibacterial activity.<sup>5</sup> Other flowers like *Chrysanthemum indicum* of family Asteraceae, *Nerium oleander* of family Apocyanaceae, *Cucurbita maxima* of family Cucurbitaceae, *Nelumbo nucifera* of Nelumbonaceae, *Hibiscus rosa sinensis* of Malvaceae, *Crossandra infundibuliformis* of

Acanthaceae and *Celosia cristata* of family Amaranthaceae are all ornamental and colourful. They also possess antibacterial and antioxidant activities.<sup>6-12</sup>

These plants have been shown to possess therapeutic values due to the presence of antioxidants, which reduce tissue injuries caused by free radicals.<sup>12</sup> Some of the flowers are even consumed like *Nelumbo*, *Hibiscus* etc which are high in antioxidant components. Though usually free radicals are detoxified by antioxidants in the body, if overproduction of Reactive Oxygen Species (ROS) occurs, oxidative damage to lipids, proteins and DNA may lead to chronic diseases in humans. The presence of antioxidants aid in reducing ROS. There is always increasing interest in finding natural sources of antioxidants.<sup>13</sup> To support the significance of immersing these flowers in water reservoirs, their antibacterial, antifungal and antioxidant potential was studied in the present investigation.

## MATERIALS AND METHODS

### Collection of flowers and preparation of flower extracts

The fresh flowers were collected, washed and shade dried.<sup>14</sup> They were powdered and subjected to cold extraction using methanol in the shaking condition. 10g of flowers were extracted with 100ml of methanol.(figure3) Later this was filtered using Whatman no 1 filter paper and dried in desiccator. The extract was weighed and yield of extract was calculated, stored at 4<sup>0</sup>C till use.

$$\% \text{ yield} = \frac{\text{weight of the extract}}{\text{weight of the original sample}} \times 100$$

### Antibacterial activity

Antibacterial activity of extracts were tested using well diffusion method.<sup>15-17</sup>

The test organisms were supplied by Department of Microbiology KMC, Warangal. Two Gram positive Bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) and four Gram negative bacteria (*E. Coli*, *Proteus vulgaris*, *Pseudomonas aureginosa*, *Klebsiella Pneumoniae*) were used for the study. The organisms were sub cultured on Nutrient Agar slants and stored at 4°C in refrigeration and used for inoculation into broth for antibacterial activity.

For well diffusion method, the ( $5 \times 10^5$  cells (cfu) of the test bacterial strain) culture (0.1ml) was swabbed on top of the solidified media and allowed to dry for 15 min. Wells were punched in the Nutrient agar plates using sterile cork borer. 100µl of crude extract in DMSO was added into the wells. Plates were incubated at 37°C for 24 hrs. Zone of inhibition was measured with Himedia antibiotic scale and experiment was repeated three replicates.

#### Antifungal activity

For antifungal activity<sup>18</sup> four fungal strains (*Aspergillus niger*, *Fusarium oxysporum*, *Penicillium notatum*, *Rhizopus nigricus*) are used. Fungal spore suspension was swabbed on the surface of the solidified Asthana Hawkens agar medium. Now 200µl of each extract was added into the wells and plates were incubated at 28°C for 6 days. Inhibition zones were measured and tabulated.

#### In vitro Antioxidant activity

Free radical scavenging activity was performed for all the flower extracts.<sup>19,20</sup> The flower extracts were tested for their ability to bleach DPPH (2,2 Diphenyl, 2-picryl-hydrazyl) radical. Crude extracts of flowers were prepared at various concentrations (200, 400, 600, 800, 1000µg/ml) in methanol and analysed against 4ml of 4% DPPH. Briefly, to 1ml of

the flower extract 1ml of methanol and 4ml of DPPH was added and incubated in dark for 20 min. Control tube was maintained with only methanol and DPPH. Quercetin was used as standard antioxidant. The reduction of DPPH radical was measured at 517nm using spectrophotometer. DPPH FRSA was detected using following formula,

$$\% \text{ of FRSA} = \frac{\text{controlAb} - \text{TestAb}}{\text{controlAb}} \times 100$$

#### TLC Analysis

Thin layer chromatography was done to separate components in extracts.<sup>21-24</sup> The analysis was carried out on precoated TLC sheets. Five different mobile phases (table-5) were selected to establish the Rf values of different components separated. TLC sheets were developed in a vertical separating chamber previously saturated with the approximate mobile phase, at room temperature. After drying sheets were visualised with iodine vapours and UV light and photographed. The Rf values were calculated using following formula.

$$R_f = \frac{\text{Distance travelled by the solute (cm)}}{\text{Distance travelled by the solvent (cm)}}$$

#### Statistical analysis

The results were analysed by standard deviation (SD) statistical method. Values expressed were obtained from three independent experiments and averaged.

## RESULTS AND DISCUSSION

All the flowers employed gave out sticky, dark brown to chocolate colored extracts after drying in the desiccators. They exhibited different extractive values. (Table-1). Two flower extracts *Tagetes* and *Celosia cristata* with higher extractive values than others.

Of crude flower extracts tested, *Crossandra*, *Hibiscus*, *Nerium*, *Tagetes*, *Celosia argentea* and *Cassia* have shown very good activity compared to others.

(Table- 2) Most of them with inhibitory zones greater than 10 mms. Others have shown moderate inhibitory action. Gram positive bacteria employed were more inhibited than Gram negative bacteria. (figure 4) *E.coli* and *Proteus* were least inhibited by all extracts. *Staphylococcus* was very efficiently inhibited by all extracts used.

Greater antifungal action was exhibited by *Tagetes*, *Chrysanthemum*, *Celosia cristata*, *Celosia argentea* and *cucurbita* extracts. All the filamentous fungi used for the study were inhibited. (Table- 3) *Rhizopus* was more effectively inhibited than others. All the fungi used cause spoilage of most human food and even contaminate water. All the flower extracts showed antifungal action though moderately against some fungi.

All the flower extracts exhibited DPPH FRSA activity. (Table -4). Of them *Crysanthemum* and *Nelumbo* extracts were with high inhibition(90%). Others like *Crossandra*, *Hibiscus*, *Nerium*, *Tagetes*, *Celosia cristata* and *Celosia argentea* exhibited fairly good activity with 80% inhibition. This free radical activity is correlated with their antimicrobial activity.

Of the flower extracts evaluated, *Hibiscus*, *Chrysanthemum* and *Celosia argentea* exhibited good number of components in all the solvent mixtures used. (Table-5). *Cassia* and *Cucurbita* responded moderately and showed separation of few components. *Crossandra*, *Nerium*, *Tagetes*, *Celosia cristata* and *Nelumbo* gave out very few components in all the mobile phases employed for the study. In *Celosia argentea* and *Cassia* flower extracts UV light detected spots were observed (figure 5).

Since ancient period, medical practitioners have been using herbal medicine from indigenous plants to treat many diseases. Medicinal plants in their different forms are exploited to combat

several multidrug resistant bacteria. It has been estimated by World Health Organisation that 80% of world's population use traditional medicine. In India, even in several festivals plants and flowers play vital role during worship of God or Goddess. Flowers which are used in almost every festival not only to beautify the environment but also to protect it. Several *Cassia* flowers are used in this festival of which *Cassia fistula* has been proved to be an ethanomedicinal plant with antimicrobial activity.<sup>25</sup> Several species of *Cassia* are medicinally important. *Cassia auriculata* flower extracts in solvents like ethanol, methanol and acetone have been shown to possess antibacterial activity against many pathogenic Gram positive and Gram positive bacteria.<sup>26</sup>

Herbal flowers used in the study possess several secondary metabolites which are pharmacologically important. There are reports on the presence of flavonoids, tannins(anthocyanidins) in the aqueous and ethanolic extracts of *Hibiscus* and *Cassia* flowers due to which they possess antibacterial and antioxidant activities.<sup>27</sup> Studies reported that presence of flavonoids and saponins in *Crossandra*, flavonoids, saponins and tannins in *Celosia argentea* and *Hibiscus* flower aqueous extracts and they also exhibit good antioxidant activity.<sup>28</sup> Studies revealed that ethanolic seed extracts of *Hibiscus cannabinus* exhibited good analgesic and anti-inflammatory activity.<sup>29</sup> *Celosia argentea* is also known as “shokoyokota” among yorubas, which means “make husbands fat” which is used as anti inflammatory, antioxidant and antibacterial agent.<sup>30</sup> *Celosia cristata* is often used flower in this festival. Its leaves are edible and flowers are used as astringent in India. In china its leaves are used in treating dysentery, menstrual bleeding and inflammation. There are reports that this plant has antioxidant and anthelmintic

activity.<sup>31</sup> *Tagetes erecta* is an important flower used in several festivals. This plant as a whole has medicinal properties. There are phytochemicals like Tagetones and ocimenones in this plant. *Tagetes* is also a good source of phytotoxin alpha terthienyl which is used against mosquitoes. The presence of aromatic components makes this plant a good candidate with insecticidal properties.<sup>32</sup> There are reports on the presence of phenols, flavonoids in these flowers which have been detected in their methanol, chloroform, n-butanol and even in aqueous extracts.<sup>33</sup> *Chrysanthemum indicum* flowers have been used in Chinese medicine for the treatment of inflammation, hypertension and analgesic purposes. Flowers are best sources of anthocyanins and possess antioxidant agents.<sup>34</sup> *Nelumbo* also known as sacred Indian lotus is an important Chinese herb and used as astringent and diuretic. In Korea, lotus liquor is prepared from flowers which have high antioxidant activities. Antifungal and antibacterial activity of various parts of *Nelumbo* has been reported against several human pathogens.<sup>35</sup>

Plants produce an array of active phytochemicals which include phenols, flavonoids, alkaloids, anthraquinones, saponins, tannins, glycosides etc. The flowers of such plants do possess most of these which can be detected in TLC. Through TLC they have analysed the presence of many phytochemicals in *Senna siamea*.<sup>36</sup> Methanolic extracts of *Nerium indicum* flowers were used in separation and identification of phenolics and flavonoids. A HPLC method was developed to detect two phenolic acids and three different flavonoid glycosides from the *Nerium* flowers.<sup>37</sup>

All the flowers tested for antimicrobial and antioxidant activity are used in the Bathukamma festival. This celebration depicts basically preservation of nature's harmony. Antioxidant activity is

determined with DPPH radical which is one of the routinely used rapid and simple assay. Higher radical activity of the flower extracts might be attributed to the presence of high phenolics, flavonoids or tannins in the test extracts. TLC has detected multiple organic components in crude extracts of the flowers which may possess remarkable toxic activity against aquatic bacteria and fungi, which ultimately helps in purification of water. Due to their medicinal properties these flowers become powerful purifying agents. When these flowers are daily released into water ponds for nine days during Dusshera, it aids in cleansing of water and is an eco-friendly approach. Pathogenic and harmful microorganisms in the water will be killed and antioxidants may detoxify harmful chemicals. Thus in total, celebration of this floral festival is environmental friendly and provides support to self-purification of water bodies. This study on antibacterial, antifungal and antioxidant activities of flower extracts provides scientific evidence for the celebration of this festival.

## CONCLUSION

Bathukamma is a floral festival celebrated in Telangana state in India during Dusshera, has scientific support due to the medicinal values of flowers used. The current study of antimicrobial and antioxidant studies of the flowers aids in understanding how they purify water bodies and make the environment much better.

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### Conflict of interest

We declare that we have no conflict of interest.

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**Table 1.** Percentage of Yield of Methanol Extracts of Flower Extracts

Flower used	Extractive value(%)
<i>Crossandra infundibuliformis</i>	3.5
<i>Hibiscus rosa sinensis</i>	3.5
<i>Nerium oleander</i>	3.0
<i>Tagetes erecta</i>	4.4
<i>Chrysanthemum indicum</i>	3.3
<i>Celosia cristata</i>	4.2
<i>Celosia argentea</i>	3.8
<i>Cassia auriculata</i>	3.1
<i>Cucurbita maxima</i>	3.7
<i>Nelumbo nucifera</i>	3.2

**Table 2.** Inhibition Zones (mm) Showed by Flower Extracts (1mg/ml) Against Gram Positive and Gram Negative Bacteria

Flower extracts used	Bacterial strains					
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aurigenosa</i>	<i>Proteus vulgaris</i>
<i>Crossandra infundibuliformis</i>	18±0.5	17±0.5	10±0.4	12±0.2	10±0.3	11±0.5
<i>Hibiscus rosa sinensis</i>	15±0.8	20±0.7	9±0.2	13±0.3	14±0.2	12±0.2
<i>Nerium oleander</i>	14±0.5	18±0.6	10±0.3	10±0.4	10±0.5	9±0.3
<i>Tagetes erecta</i>	13±0.4	18±0.8	14±0.3	12±0.2	12±0.5	11±0.2
<i>Chrysanthemum indicum</i>	10±0.2	10±0.5	8±0.5	6±0.5	7±0.4	8±0.6
<i>Celosia cristata</i>	9±0.5	8±0.6	5±0.6	9±0.4	7±0.6	6±0.8
<i>Celosia argentea</i>	18±0.6	14±0.8	7±0.4	13±0.6	12±0.4	11±0.2
<i>Cassia auriculata</i>	19±0.8	16±0.6	15±0.3	15±0.3	9±0.4	12±0.2
<i>Cucurbita maxima</i>	6±0.3	10±0.5	5±0.3	8±0.8	6±0.4	6±0.4
<i>Nelumbo nucifera</i>	6±0.5	10±0.8	8±0.6	6±0.3	8±0.4	8±0.3
STD Chloramphenicol	10±0.9	9.3±0.9	17±0.2	9±0.9	10±0.8	6±0.8

**Table 3.** Inhibition Zones (mm) Showed by Flower Extracts (2mg/ml) Against Filamentous Fungi

Flower extracts used	Fungal strains			
	<i>Aspergillus</i>	<i>Penicillium</i>	<i>Fusarium</i>	<i>Rhizopus</i>
<i>Crossandra infundibuliformis</i>	15±0.4	9±0.3	10±0.2	10±0.5
<i>Hibiscus rosa sinensis</i>	17±0.5	10±0.4	18±0.4	12±0.6
<i>Nerium oleander</i>	14±0.6	11±0.2	10±0.3	11±0.6
<i>Tagetes erecta</i>	17±0.5	13±0.2	21±0.8	19±0.8
<i>Chrysanthemum indicum</i>	13±0.6	13±0.6	16±0.7	25±0.9
<i>Celosia cristata</i>	12±0.6	19±0.5	14±0.7	23±0.9
<i>Celosia argentea</i>	16±0.4	12±0.4	19±0.6	25±0.6
<i>Cassia auriculata</i>	8±0.8	9±0.7	9±0.5	12±0.8
<i>Cucurbita maxima</i>	13±0.4	23±0.4	22±0.5	17±0.6
<i>Nelumbo nucifera</i>	8±0.2	10±0.3	14±0.6	15±0.4
STD Fluconazole	1.53±0.15	1.36±0.15	0.96±0.057	0.36±0.32

**Table 4.** Antioxidant Activity (DPPPH FRSA) of Flower Extracts

Flower extracts used	Percentage inhibition.(1mg/1ml)
<i>Crossandra infundibuliformis</i>	84.98±0.10
<i>Hibiscus rosa sinensis</i>	80.76±0.21
<i>Nerium oleander</i>	83.98±0.08
<i>Tagetes erecta</i>	80.55±0.11
<i>Chrysanthemum indicum</i>	90.49±0.43
<i>Celosia cristata</i>	87.39±0.12
<i>Celosia argentea</i>	80.85±0.09
<i>Cassia auriculata</i>	73.07±0.84
<i>Cucurbita maxima</i>	72.24±0.32
<i>Nelumbo nucifera</i>	89.79±0.98

**Table 5.** Rf Values of Components Separated with Different Mobile Phases

Mobile phases employed	Flower extracts used									
	<i>Crossandra infundibuliformis</i>	<i>Hibiscus rosa sinensis</i>	<i>Nerium oleander</i>	<i>Tagetes erecta</i>	<i>Chrysanthemum indicum</i>	<i>Celosia cristata</i>	<i>Celosia argentea</i>	<i>Cassia auriculata</i>	<i>Cucurbita maxima</i>	<i>Nelumbo nucifera</i>
Toluene: ethylacetate: acetic acid (3:4:5)	0.5	0.5 0.62 0.82 0.97	0.25	0.22	0.20 0.40 0.87 0.97	0.3	4.47(UV) 0.60(UV) 0.75(UV) 0.87(UV)	0.37 0.50 0.75	0.55 0.97	0.62 0.75
Toluene: ethylacetate: formic acid (3.5:1:1)	0.19 0.85	0.53 0.91	0.48	0.78	0.10 0.40 0.53 0.68	0.51	0.085(UV) 0.31(UV) 0.63(UV)	0.46	0.48 0.85	0.31
Toluene; acetic acid(4:2)	0.75	0.62 0.87	-	0.87	0.37 0.50 0.60	0.62	0.45(UV) 0.62(UV) 0.75(UV) 0.90(UV)	0.45	0.40 0.57	-
Toluene: acetone; formic acid (3:1:1)	0.11	0.11 0.44 0.88	-	-	0.18 0.25 0.52 0.68	-	0.20(UV) 0.40(UV) 0.51(UV) 0.71(UV)	0.12 0.24 0.46 0.55	0.51 0.57	0.61
Carbon tetrachloride: acetone: ferric acid (3:1:1)	0.47	0.42	0.45 0.57	0.47	0.42	0.59	0.47	0.47 (UV)	0.69	0.45 0.71

Uv=Uv detected spots.



**Fig. 1.** Flowers arranged for the bathukamma festival on plates.



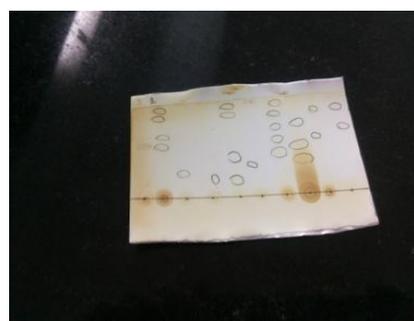
**Fig. 2.** Bathukamma immersed in water reservoir.



**Fig. 3.** Solvent extracts of various flowers.

(a) *Bacillus*(b) *Aspergillus*(c) *Staphylococcus*(d) *Penicillium*(e) *Rhizopus*(f) *Klebsiella*(g) *Aspergillus*(h) *Klebsiella*

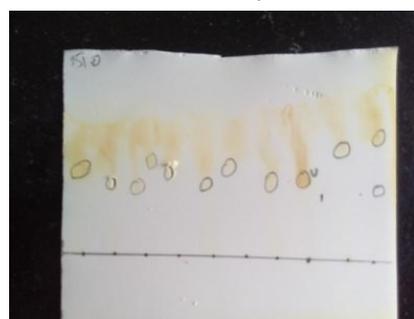
**Fig. 4.** Antibacterial and antifungal activity (Inhibition of organisms *in vitro* by the flower extracts.).



(a) Mobile phase-1



(b) Mobile phase-2



(c) Mobile phase-3



(d) Mobile phase-4

**Fig. 5.** TLC sheets after separation of different components in different mobile phases.