

Screening of *Dahlia pinnata* for its Antimicrobial Activity**Authors:**

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ABSTRACT:

The demand for more and more drugs from plant sources is continuously increasing. The present study deals with the antibacterial activity of different plant part (Root, stem, leaf and flowers) extracts of *Dahlia pinnata*. The antibacterial activity of both fresh and dried plant parts were determined in aqueous, alcohol, chloroform and petroleum ether extracts using agar disc diffusion method against *E.coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Agrobacterium tumefaciens*. *Dahlia pinnata* possessed highest antibacterial activity in its chloroform extract of dried leaves against *Enterobacter aerogenes*.

Keywords:

Dahlia pinnata, Antibacterial activity, *E. coli*, *S. typhi*.

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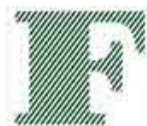
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INTRODUCTION

In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. According to National Health Experts, 2000 different plants are used for medicinal preparations for both internal and external use in India alone. *Dahlia pinnata* belongs to the family asteraceae and is a perennial growing to 1m. It is in flower from June to October. The flowers are hermaphrodite (has both male and female organs) and are pollinated by Insects. The flower petals are used in salads. Root - cooked and used as a vegetable. A bitter flavour. A sweet extract of the tuber, called 'dacopa', is used as a beverage or as a flavouring. It is mixed with hot or cold water and sprinkled on ice cream. Its naturally sweet mellow taste is said to combine the characteristics of coffee, tea and chocolate. The root is rich in the starch inulin. Whilst not absorbed by the body, this starch can be converted into fructose, a sweetening substance suitable for diabetics to use. An orange dye is obtained from the flowers and seed heads. The aim of the present study is to determine the antibacterial activity of various extracts of *Dahlia pinnata* against some pathogenic bacteria.

MATERIALS AND METHODS

Collection of Plant Material:

Fresh plant parts were collected from many residential gardens, local nurseries and farm houses, at different localities, in Jodhpur, during their growing seasons. Their identity was confirmed by Botanical Survey of India, Jodhpur, from the literature available on exotic plants and also from literature available in Department of Botany, J.N.V,University, Jodhpur. The voucher specimens were deposited in herbaria of the Department of Botany, J.N.V.University, Jodhpur(Raj.), India.

Preparation of Plant Extracts:

From Fresh Plant Parts:

25 g of fresh plant parts, viz. Stem, Leaves, roots and flowers were washed for 3-4 times with tap water and distilled water, then surface sterilized with 90% alcohol. Subsequently, the plant materials were grounded in 100 ml of distilled water, ethanol, chloroform and petroleum ether separately for aqueous, alcoholic extracts, chloroform extracts and petroleum ether extracts, respectively. The macerates were kept for 24 hours at room temperature to evaporate the solvents. The

macerates were squeezed through double layered muslin cloth and filtered through filter paper. After filtration, aliquot was centrifuged at 10,000 rpm for 20 minutes. The supernatants were filtered through Whatman No. 1 filter paper and then sterilized by passing through 0.2 micron disposable filters. The extracts were diluted to get a concentration of 50 mg per ml and were used for the *in vitro* studies.

From Dried Plant Parts:

The selected plants were thoroughly washed and then dried under shade at $28 \pm 2^{\circ}\text{C}$ for about 10 days. The dried plant samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50–150mm. The plant powder was stored in air sealed polythene bags at room temperature before extraction. 25g of dried plant powder was packed in a Whatmann filter paper no.1 and was extracted in a soxhlet apparatus using 100ml of solvent. Solvents used for extraction were Petroleum ether (60°C – 80°C), Chloroform (61°C), Ethanol (78.5°C) and Aqueous (80°C) as solvents and the extracts were dried. The dried extracts were stored in a refrigerator at 4°C . Finally, concentration of 5 mg per disc was loaded on each disc.

Preparation of Inoculum:

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of Nutrient Agar Medium and were incubated without agitation for 24 hrs at 37°C . The cultures were diluted with fresh Nutrient Agar broth to achieve optical densities corresponding to $2.0 \cdot 10^6$ colony forming units (CFU/ml) for bacteria.

Antimicrobial Susceptibility Test:

All the plant extracts were screened against five pathogenic bacterial strains. The tested organisms were *E.coli* (MTCC No. 729), *Salmonella typhi* (MTCC No.734), *Klebsiella pneumoniae* (MTCC No.109), *Enterobacter aerogenes* (MTCC No. 111) and *Agrobacterium tumefaciens* (MTCC No. 431), obtained from IMTECH, Chandigarh, India. The disc diffusion method (Bauer *et al*, 1966) was used to test the antimicrobial activity of the plant extracts. 20ml of sterilized nutrient agar medium for *E.coli*, *S.typhi*, *K.pneumoniae*, *E.aerogenes* and *A.tumefaciens* were poured into each sterile petridish. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly. The entire agar surface of each plate was inoculated



with this swab, first in the horizontal direction and then in a vertical direction, which ensure the even distribution of organism over the agar surface. The filter paper discs (5mm in diameter) soaked in 0.1 ml of the plant extract (In case of fresh extract) are loaded with 5 mg/ disc, of dry extract and were placed on the surface of the bacteria seeded agar plates and the compound was allowed to diffuse for 5 minutes and then the plates were incubated at 37°C for 24h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate.

RESULTS

The present study was aimed to screen both the fresh and dry plant part extracts of *Dahlia pinnata* against five pathogenic bacteria. **Table 1** illustrates antibacterial activity of fresh plant part extracts (Root, Stem, Leaves and Flowers) and **Table 2** illustrates antibacterial activity of dried plant part extracts (Leaves and Flowers). The plants extracts responded to bacteria as follows:

E.coli:

Fresh root extract was found to be effective against this bacterium. Fresh leaf extracts showed some antibacterial activity with maximum in chloroform extract. Dried leaves and flower extracts showed significant antimicrobial activity. Chloroform and petroleum ether extract of dried

leaves exhibited inhibition zones of 7mm and 8mm respectively. Dried flower also showed inhibition zone of 5mm in aqueous extract and 6mm in alcoholic extract.

S.typhi:

No significant antibacterial activity was recorded in fresh plant part extracts against *S.typhi*. Fresh leaves inhibited the growth of bacteria to some extent in aqueous, alcoholic and petroleum ether extracts. Again in dried plant parts, there was no significant activity observed, except inhibition zone of 6mm in the chloroform extract of dried leaves.

K.pneumoniae:

Fresh plant part extracts were not able to inhibit the growth of tested bacteria. Maximum zone of inhibition was recorded in the petroleum ether extract of flower (5mm). Dried leaves extract exhibited antibacterial activity in alcoholic and petroleum ether solvents. Dried flower inhibited bacteria in chloroform and petroleum ether extracts.

E.aerogenes:

Fresh root and stem extracts exhibited moderate antibacterial activity against *E.aerogenes*, Similar results were recorded in fresh flower extracts. Dried leaves inhibited the growth of bacteria to significant extent as revealed by the inhibition zones of 6mm, 7mm, 11mm and 9mm in aqueous, alcoholic, chloroform and petroleum ether extract respectively.

Table 1. Antibacterial activity of fresh plant part extract of *Dahlia pinnata*

Plant Part	Plant Extracts	Zone of Inhibition (mm)				
		<i>E. coli</i>	<i>Salmonella typhi</i>	<i>Klebsiella pneumoniae</i>	<i>Enterobacter aerogenes</i>	<i>Agrobacterium tumefaciens</i>
Root	Aqueous	3	-	-	-	-
	Alcoholic	2	-	-	2	-
	Chloroform	-	-	-	4	3
	Pet. ether	4	-	-	3	4
Stem	Aqueous	-	2	-	3	-
	Alcoholic	-	-	-	2	3
	Chloroform	4	-	-	3	-
	Pet. ether	6	4	3	5	3
Leaves	Aqueous	-	4	-	-	6
	Alcoholic	3	3	2	-	4
	Chloroform	7	-	-	2	2
	Pet. ether	4	6	4	3	9
Flower	Aqueous	2	-	2	-	3
	Alcoholic	3	-	3	2	5
	Chloroform	-	3	-	5	4
	Pet. ether	-	4	5	6	5

Table 2. Antibacterial activity of dried plant part extract of *Dahlia pinnata*

Plant Part	Plant Extracts	Zone of Inhibition (mm)				
		<i>E.coli</i>	<i>Salmonella typhi</i>	<i>Klebsiella pneumoniae</i>	<i>Enterobacter aerogenes</i>	<i>Agrobacterium tumefaciens</i>
Leaves	Aqueous	-	-	-	6	-
	Alcoholic	-	2	4	7	-
	Chloroform	7	6	-	11	4
	Pet. ether	8	4	6	9	7
Flower	Aqueous	5	-	-	-	-
	Alcoholic	6	2	-	-	4
	Chloroform	5	4	3	-	5
	Pet. ether	4	4	5	4	7

A.tumefaciens:

Fresh root extract was found to be effective against this bacterium. Fresh leaves extract exhibited significant antibacterial activity with inhibition zones of 6mm in aqueous extract and 9mm in petroleum ether extract. Similarly, fresh flower extract also proved to be toxic to *A.tumefaciens*. In case of dried plant parts, dried leaves showed inhibitory in chloroform and petroleum ether extracts. In dried stem, inhibition zones of 4mm, 5mm and 7mm were exhibited by alcoholic, chloroform and petroleum ether extracts respectively.

DISCUSSION

The quest for plants with medicinal properties continues to receive attention as scientists survey plants, particularly of ethnobotanical significance, for a complete range of biological activities, which range from antibiotic to antitumor. Thus far, plants have provided western medicine with an abundance of drugs and treatments for a variety of health problems (Lewis & Elvin-Lewis, 1977; Bruneton, 1999). Whitley (1985) studied the medicinal and nutritional properties of *Dahlia* species and reported antibiotic compounds concentrated in the skin of tubers. Rai and Acharya (1999) reported the antimycotic property of *Dahlia pinnata* against *Fusarium oxysporum*. Rai and Acharya (2000) investigated the fugitoxic potential in essential oils of *Dahlia pinnata*. In the present work antibacterial activity of *Dahlia pinnata* was also tested against some pathogenic bacteria. Fresh root extract inhibited the growth of *E.coli* and *E.aerogenes*. Fresh stem extracts were effective against *E.aerogenes* whereas fresh leaf extract was found to inhibit the growth of *A.tumefaciens*. In the same way fresh flower extracts also reduced the growth of *A.tumefaciens*.

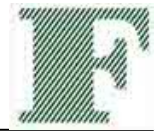
In case of dried plant parts, leaves extract was effective against *E.aerogenes*. Dried flower extracts exhibited antimicrobial activity against *E.coli* and *A.tumefaciens*. Plants belonging to Asteraceae family have been shown to possess high antibacterial as well as antifungal properties (Kasim et al, 2011, Wijaya et al, 2011 and Thorat et al, 2010).

CONCLUSION

The present study reveals that plant parts of *Dahlia pinnata* possess significant antibacterial activity and can be explored for novel antimicrobial agents. Disease control by herbal drugs do not have side effects on body, consequently great efforts have been exerted on the identification of herbal drugs to suppress various diseases. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms.

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Bissa et al.,2011

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