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Phytochemical Characterization and Antibacterial Activity of *Cyathocline purpurea* (Asteraceae) Against Some Dental Bacteria

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Abstract

Present paper deals with the study of *Cyathocline purpurea* species of family Asteraceae from chittorgarh district of Rajasthan. Phytochemical characterization was done of plant leaves methanolic extract. Plants leaves extract of 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml was applied against four bacterial species *Micrococcus luteus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli* isolated from dental patients. Zone of inhibitions of above concentrations against isolated bacterial species were recorded to study the antibacterial activity of extract. All concentractions of extract showed antibacterial properties by zone of inhibition against dental bacteria. Maximum zone of inhibition was recorded in 100 mg/ml extract concentration against all mentioned bacteria.

Keywords: *Cyathocline Purpurea*, Dental Bacteria, Zone of Inhibition.

Introduction

Rajasthan is one of the largest states of India, covering 3, 42,239 sq km. and 11% of the total area of the country. It has a natural region, located in the western part of the India. It is known to have unique floral diversity in the Asia. Rajasthan is rich in biodiversity due to its variable climatic and geographical conditions. In which Chittorgarh district lies between latitudes 24°14'24" and 25°04'06" north and longitudes 74°02'10" and 75°46'12"east in the south eastern part of the state. This district faces several problems of dental diseases in human being. Keeping in mind present study is carried out to control the bacterial activity with live bioresources of *Cyathocline purpurea* of family Asteraceae.

Review of Literature

Plants are natural and traditional sources of medication in large parts of the world. Herbs have been used since ancient time by physician and also by layman to treat a great variety of human diseases. The wide varieties of herbs in single form or in mixture have been extensively investigated in basic biological sciences to evaluate their chief as well as supplementary, complementary and synergistic action in health and diseases (Rajeshwari and Andulla, 2012).The world Health Organization (WHO) estimates that approximately 80% of the world population relies primarily on traditional medicines as a source of primary health care (WHO, 1996).Plant continue to serve as possible sources for new drugs and chemicals derived from medicinal plants have proven to be an abundant source of biologically active compounds many of which have been the basis for the development of new lead chemicals for pharmaceuticals (Srivastav *et al*.,2011).

The species *Cyathocline purpurea* (Buch-Ham ex D.Don) Kuntze is commonly known as false daisy, It is also known as bhandhaniya.It is herbaceous in nature and completes its life cycle in annual or biennial. It is strongly aromatic, glandular-hairy and upto 60 cm in height. Its stems usually purplish or purple tinged, branched from base. Leaves are pinnatisect and lower obovate but upper all pinnatifid of 2.5-15 cm long and its segments are toothed to lobed, glabrescent or thinly hairy. Heads are purple or rose purple of 5 mm across and terminal in position, rounded panicled corymbs. Involucral bracts 2- seriate, pilose, linear- lanceolate and acute. Plants corolla is of marginal florets with 1.5 mm and of disc florets with 2 mm long. Achenes are minute and pappi are absent (Hajra *et al*, 1995).

Cyathocline purpurea in traditional Chinese medicines is used as an herbal remedy for human Tuberculosis, Malaria and Bleeding. (Yu *et al*., 1993).The pharmaceutical properties of aromatic plants are partially attributed to essential oils. Essential oils are natural, complex, multi-component systems composed mainly of terpenes (Edris, 2007). The essential oil and their isolates present in medicinal plant Cyathocline purpurea have been found to exhibit strong antimicrobial activity and used against different diseases including toothache, caries and gingivitis (Joshi, 2012).

It is widely used indigenously as germicide and appetizer and used as antimicrobial, antifungal, antiviral, antifertility and pharmacological activities. (Gupta *et.al.,*2013).Guainolide present in *Cyathocline purpurea* shows anti-inflammatory and anti oxidative potential (Tambewagh *et. al.,*2017).

Objective of the Study

The present study was carried out with the following objectives

1. Collection of *Cyathocline purpurea* (Buch- Ham ex D. Don) Kuntze, family Asteraceae of its ethnobotanical importance.
2. To study the phytochemicals of methanolic leaf extract of *Cyathocline purpurea*.
3. Quantitative analysis of phenol and flavonoid of plant leaves extract by spectrophotometric method.
4. To test the antibacterial efficacy of the leaf extract with reference to common dental bacterial species *Micrococcus luteus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli.*

Methodology

Collection and Identification of Plant Material

The plant *Cyathocline purpurea* was collected from Ghambhiri river bank of district Chittorgarh during the floristic survey of family Asteraceae in Rajasthan, India during the months of July-August 2017. Plant selection and collection was based on ethno botanical survey, traditional use and literature survey. Plant was selected on the basis of good activity according to traditional medicine for the treatment of diseases. The plant materials were authenticated by the Department of Botany, M. L. V. Government College, Bhilwara, Rajasthan and the voucher specimens were deposited their under accession number 1295. To evaluate the scientific basis of the traditional medicinal use of the plant, the antibacterial activities of extract along with phytochemical properties were evaluated.

Extraction

The fresh leaves of *Cyathocline purpurea* were washed with distilled water and shaded dried at room temperature and pulverized into fine powder in a laboratory grinder. 100 gm of the dried leaves powder was extracted with methanol solvent using Soxhlet.The soxhletion with methanol solvent was due for three days to obtain the extract. After that, the extract was evaporated in water bath at 55^{o}C to obtain crude as semi solid mass for subsequent phytochemical analysis and determination of bioactive compounds and antibacterial susceptibility **(**Gupta 2007).

Phytochemical Analysis

Phytochemical analysis was carried out using protocol suggested by Khandelwal (2010). The qualitative phytochemical tests which were performed are summarized in Table number 1.

Statistical Analysis

The method which was used to perform all the measurement related to experiments were in thrice and was shown as average of three analysis of standard deviation. The significance value for this was $p < 0.05$.

Quantitative Analysis of Phytochemicals

Determination of Phenol Content in Plant Extract

The method which was used for quantitative analysis of phenol concentration was spectrophotometric for analysis of plant extract of 1 mg/ml concentration. In water 2.5 ml 7.5 % $NaHCO_3$ a reaction mixture of 0.5 methanolic solution of leaf extract with 2.5 ml of 10 % Folin-Ciocalteu's reagent was dissolved and 0.5 ml methanol with 2.5 ml 10% Folin –Ciocalteu's reagent was used to make blank solution in water and 2.5 ml of 7.5 % $NaHCO_3$. An incubation time of 90 minute was given to the sample. At maximum wavelength of 760 nm, absorbance was determined by spectrophotometer. For statistical analysis, sample was prepared in triplet form to analyze the mean value of absorbance. Sample was repeated in thrice for statically analysis to know the mean value of absorbance. It was again done for the standard solution of Gallic acid and the calibration line was constructed. Through the measured absorbance the concentration of phenol was read as mg/ml from the calibration line. Gallic acid equivalent (mg of GA/g) extract to express the content of phenol in leaf extract (Katalinic, 2006)

Determination of Flavonoid Content in Plant Extract

The amount of total flavonoid content in extracts was determined by aluminum chloride assay through Spectrophotometric method. A dilute sample solution of 0.5 ml was mixed with 2 ml water with 0.15 ml of 5% $NaHCO_3$ and after 6 minutes 10 % $AlCl_3$ solution of 0.15 ml was mixed after 6 minutes and allowed to stand for 6 minutes and 4% NaOH of 2 ml was added to mixture and water is added immediately for final make up of 5 ml. This mixture was thoroughly mixed and allowed to set for next 15 minutes. Absorbance of the mixture was determined at 510 nm versus prepared water blank. Rutin was used as standard compound for the quantification of total flavonoid. Total flavonoid content was expressed as mg rutin/g dry weight (mg Rutin/g), through the calibration curve of rutin. All the samples were analyzed in three replications (Yousufmalla, 2013).

Antibacterial Activity of *Cyathocline purpurea* Leaf Extract

Isolation of Dental Bacterial Strains

Antimicrobial activity, 20 samples from different dental micro flora of patients from Bishnoi Dental Hospital, Nimbahera were isolated by using swab method and spread on nutrient agar media plates and then were incubated at 37^{o}C. After culturing, the bacterial isolates were stained by Gram's staining and observed under compound

microscope at 100X. On the basis of morphological and biochemical characterization as per Bergey's manual, different bacterial isolates were tentatively identified at Department of Biotechnology, Mewar Girls College Chittorgarh as *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Micrococcus luteus* (Keer *et al*., 2017)

Disc Diffusion Method

Disc diffusion method to test antibacterial activity of the extract against bacterial isolates. (Kirby Bauer 1966). Broth culture of bacterial strains maintained O.D. at 660 nm (up to 1 x 10^6) was spread on nutrient agar media. Disc of 6 mm size Whatman filter paper of different concentrations of *Cyathocline purpurea* of 25, 50, 75, 100 mg/ml were placed on nutrient agar media. Disc diffused media was incubated at 37^{o}C for 8 hr. After incubation, zone of inhibition was measured using zonal scale of Hi Media.

Discussion and Results

In present study of Cyathocline purpurea plant collection and its methanolic extract phytochemical and antibacterial activity against isolated dental bacteria positive findings are reported which have not been reported by earlier workers as (Edris, 2007; Gupta 2007; Gupta *et.al.*2013; Hajra *et al., 1995*; Joshi, 2012; Katalinic ,2006; Keer et al., 2017; Kirby Bauer 1966; Rajeshwari and Andulla, 2012; Srivastav *et. al*.,2011;Tambewagh *et. al* .2017; Yousufmalla,2013; Yu et al., 1993)

It was found that the resultant percent yield for soxhlet extraction of *Cyathocline purpurea* leaves(solvent – 500 ml) was 5.8%.Qualitative analysis of alkaloids, glycosides, phenols, flavonoids, saponins,carbohydrates, amino acids, terpenoids, steroids,protein,tannin results of extract are shown in table 1.Quantitative results were analysed by spectrophotometric methods.The standard curve equation y = 0.039x – 0.040, R^2 = 0.981 was expressed in total phenolic compound as mg/g Gallic acid equivalent. Second equation y = 0.002x -0.003, R^2 = 0.967 was expressed in total flavonoid compound as mg/g Rutin equivalent. The total phenolic content was 29 ± 0.05 mg GA/g in methanol extract and total flavonoid content was 9.5 ± 0.05 mg Rutin/g in table 2 and 3 respectively. For the concentration range from 25 mg/ml to 100 mg/ml (25mg/ml, 50mg/ml, 75mg/ml, and 100 mg/ml) zone of inhibition was measured to show antibacterial activity against bacterial strains in table 4. Maximum zone of inhibition of 100 mg/ml extract was recorded against *Micrococcus luteus* of 12 mm (Fig. 1), *Bacillus subtilis* of 11 mm (Fig. 2), *Pseudomonas aeruginosa* of 9 mm (Fig. 3) and *Escherichia coli* of 8 mm (Fig.4).

Suggestions

In present study "Phytochemical characterization and antibacterial activity against some dental bacteria of *Cyathocline purpurea* from district chittorgarh of Rajasthan, India." There are following suggestions

1. Traditional botany should be studied with modern approaches.
2. Medicinal plants are rich source of antimicrobial agents and they should be properly investigated with fine techniques.
3. Allopathic drugs cure disease in effective manner but have chances of side effects and if such plants are commonly introduced to our society that is not in notice it would be very beneficial, cheap, and commonly available and have no side effects.
4. Mostly dental problems are common nowadays and it would be very beneficial for dental patients.

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Table 1 Photochemical Screening of *Cyathocline purpurea* Leaf Extract

Sr. No.	Chemical Tests		Result
1	**Alkaloids**	Mayer's reagent	Present
		Dragendorff's reagent	Present
2	**Phenols**	Ferric chloride	Present
3	**Glycosides**	Keller killayani test	Present
4	**Saponins**	Foam test	Present
5	**Flavonoids**	$FeCl_3$ test	Present
6	**Amino acids**	Millons Test	Absent
7	**Carbohydrates**	Fehling's solution test	Absent
8	**Steroids**	Lieberman Buchard Test	Present
9	**Terpenoids**	Salkowski test	Present
10	**Tannin**	Folin-Denis test	Present
11	**Protein**	Biuret test	Absent

Table 2. Total Phenolic Content of Methanol Extract in mg/g equiv. to Gallic Acid

Sr. No.	Absorbance of Extract	Concentration of Extract	Total Phenolic Content mg/g equiv. to Gallic Acid
1	.362	1mg/ml	29.0
2	.364	1mg/ml	30.0
3	.360	1mg/ml	28.0
	Mean ± SD		29±.05

Table 3. Total Flavonoid Content of Methanol Extract in mg/g equiv. to Rutin

S. No.	Absorbance of Extract	Concentration of Extract	Total Flavonoid Content mg/g equiv. to Rutin
1	.128	1mg/ml	10.0
2	.126	1mg/ml	9.5
3	.124	1mg/ml	9.0
	Mean ± SD		9.5±.05

Table 2. The Zone of Inhibition for Different Concentrations of Plant Extracts

Sr. No.	Bacterial Isolates	Concentration of Extract			
		25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
1	***E.coli***	06 ± 0.27	07 ± 0.35	7.5 ± 0.45	08 ± 0.50
2	***P.aeruginosa***	06 ± 0.35	07 ± 0.45	08 ± 0.50	09± 0.40
3	***B.subtilis***	08 ± 0.30	09 ± 0.32	10.5 ± 0.37	11 ± 0.42
4	***M.luteus***	9.5 ± 0.35	10 ± 0.37	11 ± 0.39	12 ± 0.36

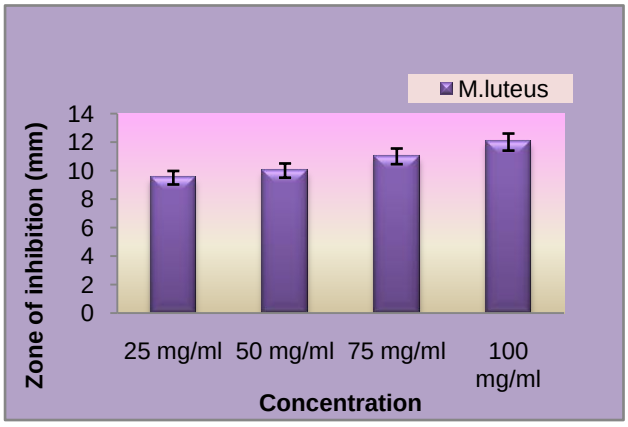

Figure 4. Zone of inhibition against against *M. luteus*

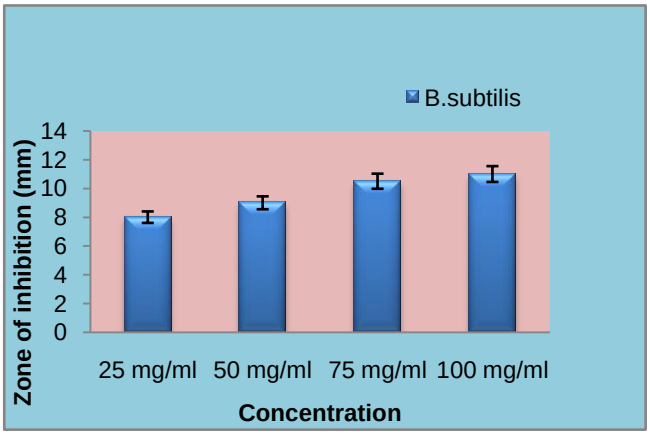

Figure 3. Zone of inhibition against *B. subtilis*

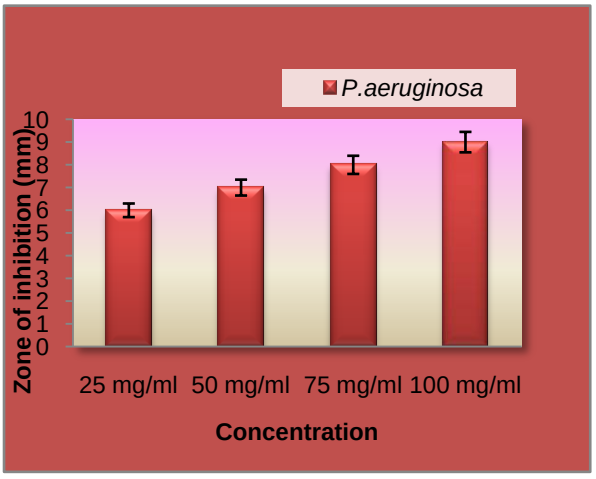

Figure 2. Zone of inhibition against *P. aeruginosa*

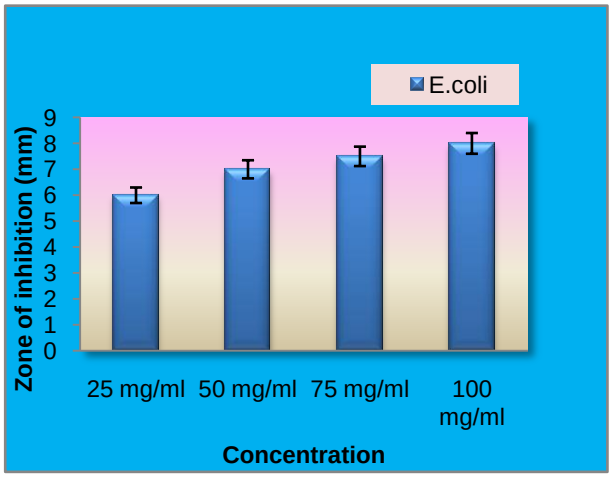

Figure 1. Zone of inhibition against *E.coli*

References

Edris, A.E., (2007). Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytotherapy Research 21, 308–323.

Gupta, M. Mazumder, U.K. and Gomathi, P. (2007). "Antioxidant and antimicrobial properties of Galega purpurea root." AsianJ. Plant Sci., 6: 533-537.

Gupta,A. Joshi,B. Dubey, B. (2013)"Comparative pharmacognostical and phytochemical evaluation of two species of Cyathocline." International Journal of Biomedical Research;04(10):538-545.

Hajra, P. K. Rao, R. R. Singh, D.K.(1995)."Flora of India, Botanical Survey of India, Calcutta":Volume (12),110-113.

Joshi, RK. (2012) "Chemical constituents and antibacterial property of the essential oil of the roots o Cathocline purpurea" Journal of Ethanopharmacology 145(2013) 621-625.

Katalinic, V. Milos, M. Kulisic, T. and Jukic, M. (2006). "Screening 70 medicinal plant extracts for antioxidant capacity and total phenols". Food Chemistry 94: 550-557.

Keer, N.R. Singh, J.and Meena, K.L. (2017). "Phytochemical characterization and antibacterial activity of Eclipta alba(L.) L. (Asteraceae) against some dental micro flora from Mewar region of Rajasthan, India."South Asian Journal of Experimental Biology 7(2): 140-146.

Khandelwal, K.R. Practical Pharmacognosy. 20th ed. Pune: Nirali Prakashan; 2010.

Kirby, W.M. Bauer, A.W. Sherris, J.C. and Turck, M. (1966). "Antibiotic susceptibility testing by a standardized single disc method". American Journal of clinical pathology. 45(4):493-6.

Rajeshwari, C. Andulla, S. (2012)."Antioxidant and antiarthritic potential of coriander (Coriandrum sativum L.) Leaves" SPEN Journal 7:223-228.

Srivastav, S. Singh, P. Mishra, G. Jha, K. Khosha, R. (2011). "Achryanthas aspera an important medicinal plant": a Review Journal of Natural Products Plant Resources 1(1) 1-14.

Tambewagh,U.U.Kandhare, A.D. Honmore, V.M. Bodhankar, S.L.Rajatkar, S.R."Anti-inflammatory and antioxidant potential of Guaianolide isolated from Cyathocline purpurea – role of COX-2 inhibition" International immunopharmacology 11 (52):110-118.

World Health Organization (1996) WHO guidelines for the assessment of herbal medicines, WHO expert committee on specification for pharmaceutical preparation. Technical Report Series no. 863, Geneva.

Yousufmalla, M. (2013)."Phytochemical screening and spectroscopic determination of total phenolic and flavonoid contents of Eclipta alba L."Journal of Natural Product and Plant Resources 3(2): 86-91.

Yu, C. Huang, T. Ding, Z. Goa, X.and Zhang, Z. (1993). "Eneyclopedia of Chinese medicines." Chinese Medicinal Science and Technology, Beijing.