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Research Article

IN-VITRO ANTI OXIDANT AND ANTI BACTERIAL STUDIES OF BETACYANIN ISOLATED FROM THE BRACTS OF *BOUGAINVILLEA GLABRA*

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ABSTRACT

Over the last few years researchers from all over the world have aimed at identifying and isolating plant derived substances for the treatment of various diseases. The synthetic products of the modern medicine surpassed the importance of medicinal plants for a while and moreover they are toxic and possess various side effects. Now people turning back to the natural drugs of plant origin with the hope of safety and efficacy. In the present work betacyanins were isolated from the bracts of *Bougainvillea glabra* by using flash chromatography and the isolated betacyanins were characterized by FT-IR, Mass Specrtoscopy. Betacyacins were screened for their *in-vitro* antioxidant activity by DPPH radical scavenging assay using ascorbic as standard and antibacterial studies were also carried out by well-diffusion method using ampicillin as standard. Results obtained showed that betacyanins posess good radical scavenging activity with 75.92 percent inhibition at 100 µg/ml of sample concentration. *In-vitro* antibacterial studies showed that betacyanins possess better antibacterial efficacy against *Bacillus subtilis* and *Pseudomonas aeroginosa.*

Keywords: *Bougainvillea Glabra*, Betacyanins, Flash chromatography, Antioxidant, DPPH, Ascorbic acid, Antibacterial, Ampicillin.

INTRODUCTION

The importance of medicinal plants is increasing dramatically day by day and a large body of evidence has been collected to show the immense potential of plants in drug discovery and development. Now a day's traditional system of plant medicine continue to be widely practiced all over the world mainly because of increase in population, inadequate supply of drugs, high cost of drugs, side effects of synthetic drugs, and development of resistance to currently used synthetic drugs by infectious bacteria have thus emphasized for the wide usage of herbs for the treatment of various ailments [1-2]. Inspite of tremendous advances in synthetic drugs, and our dependence on modern medicine but still a majority of the population is turning towards herbal medicine mainly because of its safety and less side effects [3]. In most of the developing countries the use of plant material is increasing because modern synthetic drugs are beyond the reach of three quarters of the total population inspite of spending 40-50% of their total wealth on drugs and health. So in order to reduce the financial burden on developing countries, it is quite obvious that the usage of herbal medicine will be followed in near future.

Green plants generally synthesize a wide variety of biochemicals, many of which are extractable and used as raw material for scientific investigations, as chemical feed stocks. Most of the plants secondary metabolites are having good commercial importance and useful for the development of potent pharmaceutical agents for drug discovery and development [4-5]. Plants especially used in ayurveda provide valuable information and generates lead structures useful for the modifying already existing drugs having enhanced activity with or without toxicity. So far it has been investigated that a small fraction of flowering plants have yielded 140 therapeutic agents of known structures from about 100 species of plants. Some of the plant drugs include camptothecin, vinblastine, vincristine, phodophyllotoxin, taxol, reserpine, atropine, pillocarpine, curcumin, marmelosin, artemecinin, ephedrine, codeine, morphine, lupeol, allicin, and gitoxigenin among others [6-8]. Sometimes crude extracts of plant material itself can be used as medicaments on the other hand isolation, structural elucidation, investigating active molecules along with their mechanism of action is paramount importance [9-10].

The scientific study of traditional medicines, derivation of novel herbal drugs through systemic conservation of medicinal plants and bioprospecting are thus of great importance. There are nearly 121 plant drugs currently available in literature from plant origin, but only few of them were producing through synthetic route. So for developing potential therapeutic phytomedicines it would be essential to approach holistic interdisciplinary sciences by having scientific basis of understanding plant drugs, new innovations, and its preservation for utilization in future on sustainable basis is needed for future development. In the present work betacyanins were isolated from the bracts of bougainvillea. The isolated compounds were further screened for its *in-vitro* antioxidant and antibacterial studies by using ascorbic acid and ampicillin as standards [11].

Bougainvillea is a tropical and subtropical woody, evergreen shrubby vine. It is usually multi-trunked having an average height of up to 20 feet [12]. It climbs by sending out delicate, slender arching canes with curved thorns. As the age progress the stem turns from mid-green to dull green-brown. Generally *Bougainvillea* is deciduous when it grows in areas with a long dry season. The bracts are usually 1/2-2-inch long structures to which the flowers are attached at the terminal region of mid-rib. Leaves are simple and alternate, with undulate margin, and the leaf blade is 2-4 inches long, with lot variation in shape like globular, elliptical, obivate, ovate, or cordate. Colour of the leaves is mid to deep green, and the vibrant colour of *Bougainvillea* is not from the inconspicuous white or yellow flower but it is from the bracts that surround each flower. Fruit is generally elongated with less than ½ inch long [13].

Bougainvilleas grow best in full sun light and moreover high light intensity is required for good flowering. Shady areas and low light areas are not suitable, as a result plants can drop their bracts. Elevation about 10 to 2500 feet provides better conditions for *Bougainvillea* to survive. *Bougainvillea* grows well in rich, acidic, well drained soil of pH nearly 5.5-6.0. It is wind resistant, drought tolerant, salt tolerant and very susceptible to girdling during a storm. The flowers of *Bougainvillea glabra* are white to cream coloured while thorns are small and curved at its tips which was first identified by a swiss botanist Jacques Denys Choisy in 1850. The deep red colour of *Bougainvillea* is mainly due to the presence of betalins [14]. Betalins are a class of indole-derived pigments synthesized from tyrosine which is commonly found normally in plants of caryaphyllates as well as some higher order fungi. Unlike anthocyanin pigments which was normally found in most plants, betalins may occur in any part of the plant including the petals, fruits, leaves, stems and roots. Betalins are of two types, betacyanins including reddish to violet betalin pigments and betaxanthins are those betalin pigments which appear yellow to orange. Betacyanins [**Fig. 1**] possess various biological activities like antioxidant, anticancer, natural colorant, antibacterial and anti-inflammatory.

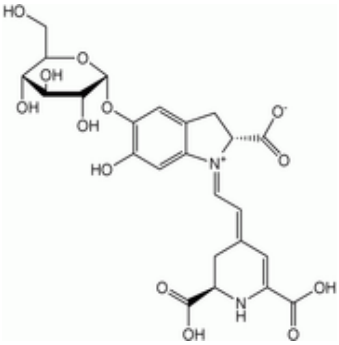

Fig. 1: Structure of Betacyanin

MATERIALS AND METHODS

Collection of plant material

Bougainvillea glabra choicy were collected from GVK Biosciences PVT Ltd, Nacharam branch, Hyderabad, Andhra Pradesh, India.

Extraction

Freshly harvested bracts (18 g) were frozen in liquid N_2, and homogenized in a motar. The resulting powder was suspended in methanol and further allowed to stand at 4℃ for 2 hours with continuous stirring. The resulting mixture was filtered, centrifuged and the residue re-extracted twice with 50 % aqueous methanol. The combined supernatants were taken to dryness at 30℃ under reduced pressure (**Fig. 2**). The residue was re-dissolved in 60 ml H_2O [15].

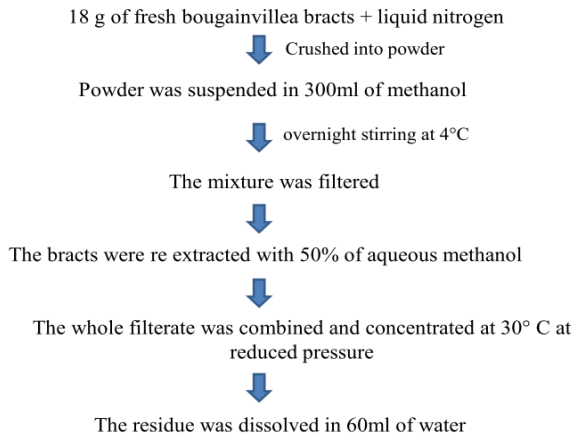

Fig. 2: Extraction of betacyanins from bracts of bougainvillea.

Isolation

The isolation of betacyanins was done by combi flash chromatography using alumina column. Water was used as eluent, and the resultant betacyanins were obtained by the concentration of the solvent by lyophilization. As it is a well known fact that betacyanins are heat sensitive pigments hence very low temperature has to be maintained throughout the extraction as well as isolation process.

Characterization

The isolated betacyanins were characterized by IR spectroscopy and LC-MS spectroscopy.

Biological studies of Betacyanins

Antioxidant studies

Betacyanins are known for its excellent antioxidant activity. The antioxidant property of betacyanins was carried out by using DPPH method.

Antioxidants block the process of oxidation by neutralizing free radicals, in doing so, the antioxidants themselves become oxidized. That is why there is a constant need to replenish our antioxidant resources. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions [16-17].

DPPH Radical Scavenging Assay

Radical scavenging activity of compounds against stable DPPH (2, 2-diphenyl-2-picrylhydrayl hydrate) was determined spetrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep-violet to light yellow) were measured at 517 nm on a UV-Vis light spectrophotometer. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, which give rise to the reduced form with the loss of violet colour [18-19]. The reaction is:

$DPPH\cdot + RH = DPPH + R\cdot$

Where RH is the reduced form and R· is free radical

Method

Compound solutions were prepared by dissolving various concentrations of each (10, 50, 100 mg/ml) compound in ethanol. The solution of DPPH in ethanol was prepared just before UV measurements. 3 ml of sample and 1 ml of DPPH solutions were mixed and kept in the dark for 30 min at room temperature and then the decrease in absorption was measured. For the control absorption blank sample containing the same amount of ethanol and DPPH solution was measured. Ascorbic acid was used as standard and the experiment was carried out in triplicate. Radical scavenging activity was calculated by following formula:

$$I\% \text{ (percentage inhibition)} = [AB\text{-}AA/AB] \times 100$$

Where A_B = absorption of blank sample

A_A = absorption of sample

Antibacterial studies

Screening of antimicrobial activity was performed by well diffusion method. The tested compounds were dissolved in DMSO (10mg/ml). Mueller-Hinton agar medium was used for the preparation of inoculum.

Preparation of Muller-Hinton Agar Medium

Muller-Hinton agar is considered to be best for routine susceptibility testing of drug resistant bacteria. It is prepared from a commercially available dehydrated base according to the manufacture's instruction.

Well Diffusion Method

The organisms were cultured in nutrient broth and the tests carried out on Muller-Hinton agar plates. The inoculate of the microbial strains were prepared form 24 h broth cultures and suspensions. Muller-Hinton agar was poured in to petridishes and after solidification 0.5 ml of test strains were inoculated in the media separately by using sterile cotton swab. Appropriate care was taken to ensure proper homogenization [20-23].

The experiment was performed under strict aseptic conditions. After the medium solidified, a well was made in the plates with sterile borer (5mm). The test sample with different concentrations namely (40µl, 60µl,) was introduced in to the well. Ampicillin was taken as standard. The inoculated plates were incubated at 37 ºC for 24 hours. After incubation zone diameters were measured to the nearest millimetre (mm).

RESULTS AND DISCUSSION

The FT-IR spectra of the compound betacyanin shows absorption peak at 2928.35 cm^{-1} for OH stretch and 1649.32 cm^{-1} for the existence of C=N, C=O stretch. The existence of C-O stretch is observed at 1255.82 cm^{-1} and 1103.92 cm^{-1} respectively. The existence of C-H deformation (benzene ring) is observed at 898.02 cm^{-1} (Supplement **Fig. 1**). LC-MS spectra of betacyanin confirm the mass as 561.24 which match with the molecular weight of the compound in literature (560) (Supplement **Fig. 2**).

In present study, isolated betacyanins was evaluated for their free radical scavenging activity using the DPPH radical assay. Reduction of DPPH radicals can be observed by decrease in absorbance at 517nm. Activity of betacyanin was compared with commercial antioxidant Ascorbic acid. The percentage inhibitions were calculated for various concentrations of betacyanin as well as ascorbic acid as standard. The scavenging activity increased with increasing concentration of the test samples. The Percentage inhibitions for 10, 50, 100 µg/ml were found to be 18.01, 49.47, and 75.92 respectively which were in comparable to that of standard Ascorbic acid. Result in Table 1 and Fig. 3.

Results obtained from antibacterial studies revealed that the isolated betacyanin posses good anti-bacterial activity against *B.subtilis, P.aeroginosa* and *E.Coli*. When tested by well diffusion method betacyanin showed significant activity against *B.subtilis*, *P.aeroginosa* and moderate activity against *E.Coli* when compared to standard ampicillin (supplement **Fig. 3**). Result in Table 2.

Table 1: Percentage inhibition of ascorbic acid and betacyanin at various concentrations

S. No.	Concentration µg/ml	Absorbance (standard)	I% (percentage inhibition for standard)	Absorbance (sample)	I% (percentage inhibition for sample)
1.	10 µg	0.1143	26.20	0.1297	18.01
2.	50 µg	0.0682	63.92	0.0792	49.47
3.	100 µg	0.0293	89.30	0.0357	75.92

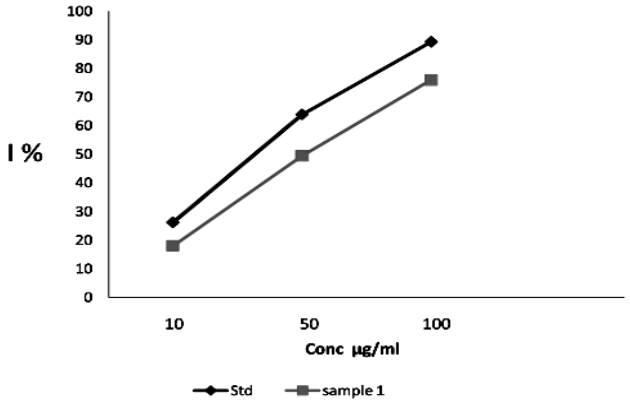

Fig. 3: Antioxidant activity of standard and sample

Table 2: Antibacterial activity of betacyanin and standard ampicillin

Compound	Strains	Zone of Inhibition		
		Standard	Sample	
		Ampicillin (60µl)	40µl	60µl
Betacyanin	*Bacillus subtilis*	8.7mm	6.7mm	7 .4mm
	Pseudomonasaeruginosa	6mm	4.8mm	5.3mm
	Escherichia coli	5.6mm	3.4mm	3.7mm

CONCLUSION

In conclusion betacyanins were successfully isolated from the bracts of *Bougainvillea glabra* and confirmed with LC-MS and IR spectroscopy. Betacyanins were also screened for its *in-vitro* antioxidant and antibacterial activity. They exhibit potent antioxidant activity which was comparable to that of standard ascorbic acid and good antibacterial activity especially against *B.subtilis* and *P.aeroginosa.*

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REFERENCES

1. Perumalsamy .R, Ignacimuthu. S, Sem A. Screening of 34 Indian medicinal plants for antibacterial properties. J. Ethnopharmacol. 2008:62: 173-182.
2. Agarwal .V.S. Rural economics of medicinal plant: Vegetation in the forest. Drug plants of India. Kalyani publishers India. 1997:1: 1-160.
3. Krithikar .K. R, Basu .B.D . Indian Medicinal Plants, International Book Distributors, Dehra Dun. 1975: 212-213.
4. C.S.I.R. "The wealth of India" National Institute of Science communication and Information Resources". 1985: I (A), 86.
5. Gupta A.K, Tondon N. "Review on Indian medicinal plants". Indian council of medicinal research, New Delhi. 2004: 312.
6. Chatopadhyay .I, Biswas . K, Bandhopadhyay.U , Banerjee R.K. "Turmeric and Curcumin: Biological actions and medicine of applications". Curr Sci.2004:87: 44.
7. Khare. C. P. Indian Medicinal Plants, Springer International Edition. 2007:329-330.
8. Chandra .P.K, Pitamber. P.D, Bikram. S.S. Developing the medicinal plants sector in northern India: Challenges and oppurtunities. J. Ethnobiol. Ethnomed. 2008:62: 173-182.
9. Edwin. E, Joshi S.B, Jain.D.C. A brief review on few Indian medicinal plants. Int J Green Pharm. 2007: 1(3-4):2-12.
10. Ashutosh. K. Pharacognosy and Pharmaco biotechnology, 2nd edition, New Age International Publishers, New Delhi. 2007: 5-7.
11. Barrett. M. The Hand Book of Clinically Tested Herbal Remedies, 1st edition, CBS Publishers and Distributers, New Delhi. 2007:3-6
12. Piattelli.M, Imperato.F. "Betacyanins from *Bougainvillea*". Phytochemistry. 1964:9:455-458.
13. Sussane. H, Sabine.R, Jorg W.M, Victor.W, Manfred.N, Bieter.S . "Betacyanins from the bracts of *Bougainvillea glabra*". Phytochemistry. 1999: 37(3):761-767.

14. Piattelli.M, Imperato.F. "Pigments of *Bougainvillea glabra"* Phytochemistry.1970:9:572-578.
15. Elena.E.S, Miguel.A.P, Marianny.Y.C. "Volatile secondaray metabolites from spilanthes americana obtained simultaneous steam distillation-solvent extraction and supercritical fluid extraction" Journal of Chromatography A. 1996:752:223-232.
16. Dharmendra.K.K, Parikshit.J, Archana. R.J. Phytochemical investigation and *in vitro* antioxidant activities of *Indigofera Cardifolia* seed extracts. Int J Pharm Pharm Sci. 2013:5(2):71-75.
17. Sivakrishnan.S, Muthu.A.K. *In-vitro* free radical scavenging activity of aerial parts of ethanolic extract of *Albizia Procera* (family:Mimosoideae). Int J Pharm Pharm Sci. 2013:5(2):352-354.
18. Rajesh.P.M, Natwar.P.J. *In vitro* antioxidant activity of coumarins compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. Journal of Advanced pharmacy Education and Research.2011:1:52-68.
19. Pourmorad.F, Hosseinimehr.S.J, Shahabimajd.N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr.J. Biotechnol. 2011: 5(11):1142-1145.
20. Gislene.G.F, Nascimento, Juliana.L, Paulo.C.F,Giuliana.L.S. "Antibacterial activity of plant extracts and phytochemicals on Antibiotic Resistant Bacteria". Brazilian Journal of Microbiology. 2000:31:247-256.
21. Rameeja.B.A, Poonkothal.M. *In- vitro* antimicrobial activity and phytochemical analysis of *Brassica Oleracea*. Int J Pharm Pharm Sci. 2013:5(2):405-408.
22. Umamaheshwari.A, Shreevidya.R, Aparna.N. "In vitro Antibacterial activity of *Bougainvillea Spectabilis* leaves extracts" Advances in Biological Research. 2008:2:01-05.
23. Gupta.V, George. M, Joseph. L, Singhal.M, Singh. H. "Evaluation of antibacterial activity of *Bougainvillea glabra* Snow white and *Bougainvillea glabra* Choicy". Journal of Chemical and Pharmaceutical Research. 2009:1(1):233-237.