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Original Article

FLORAL EXTRACTS OF *ALLAMANDA BLANCHETII* AND *ALLAMANDA CATHARTICA* ARE COMPARATIVELY HIGHER RESOURCE OF ANTI-OXIDANTS AND POLYSACCHARIDES THAN LEAF AND STEM EXTRACTS

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ABSTRACT

Objective: The present study undertakes a comparative analysis of the level of secondary metabolites present in the leaf, flower and stem of the two ornamental plants, *Allamanda blanchetii* and *Allamanda cathartica*.

Methods: The two plant species, *Allamanda blanchetii* and *Allamanda cathartica* were collected, washed, shade dried in room temperature and powered in mechanical grinder. Phytochemicals were extracted from the power with methanol and double distilled water. The estimation of flavonoids, polyphenols, polysaccharide were done by standard methods and the anti-oxidant activity was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) discoloration assay.

Results: Our study reveals that the flower of both species contain highest amount of secondary metabolites in crude methanolic and aqueous extracts. In case of leaf, the methanolic extracts contain higher amount of polyphenol, flavonoid and anti-oxidant property in comparison to aqueous extracts, where as the aqueous extract contain higher amount of polysaccharide content than its counterpart. In stem, crude organic extract has higher amount of polyphenol and flavonoid and the aqueous extract has higher amount of polysaccharide and anti-oxidant property.

Conclusion: The flower of *Allamanda cathartica* and *Allamanda blanchetii* has higher amount of flavonoids, polyphenols, polysaccharide and the floral extracts display comparatively higher anti-oxidant property.

Keywords: *Allamanda cathartica, Allamanda blanchetii,* Phytochemicals, Leaf, Stem, Flower

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INTRODUCTION

For several years nature has been the primary source of medicinal agents. Historical evidence depicts that plant products obtained from barks, leaves, flowers, roots, fruits and seeds have been used to cure many diseases since centuries back. An estimated (14-28) % of higher plant species possess medicinal value [1]. World Health Organization estimated that in developing countries such as India and China 80% of the population still relies on plant sources for traditional medicines, which are extensively used in primary healthcare. The popularity of herbal medicine stems from the popular belief that herbal medicines have fewer side effects, safe to use and are easily available [1].

A wide range of chemical compounds are synthesized by plants which are further classified as primary and secondary metabolites according to their chemical class, functional group and biosynthetic origin. While primary metabolites are directly involved in the growth and development of plants, secondary metabolites usually work as biocatalysts, often displaying medicinal properties [2]. The primary metabolites such as chlorophyll, amino acids, nucleotides, carbohydrates are widely distributed in nature and they have a key role in metabolic processes such as respiration, nutrient assimilation and photosynthesis. Secondary metabolites are synthesized due to secondary metabolism of plants and for their medicinal properties they are used extensively in pharmaceutical industries. The major classes of secondary metabolites include alkaloids, tannins, flavonoids, saponins, and cardiac glycosides. Secondary metabolites exhibit diverse biological activities with saponins showing antifungal activity, alkaloids being administered against HIV infection, tannins predominantly displaying antimicrobial activity and flavonoids having strong anticancer activity [2].

Allamanda cathartica, also known as trumpetvine, golden trumpet or yellow allamanda is an ornamental flowering plant. The beauty of the plant is the fact that the plant extracts have medicinal properties whereas, at the same time, the extracts can be toxic if they are not properly prepared [3]. A recent report suggests that *Allamanda cathartica* extracts have been found useful not only for treating malaria, jaundice, cough, wounds and constipation, but also shows activity against leukemia and human carcinomamia [3]. On the other hand *Allamanda blanchetti*, popularly known as purple allamanda is also an ornamental flowering plant with extensive medicinal properties. While ethanolic extracts of roots, stems and leaves of the plant have cytostatic and cytotoxic activity, floral extracts own anti-dyslipidemic, anti-diabetic and anti-oxidant activity [4, 5]. The present study is a comparative account of the secondary metabolites present in the aqueous and the methanolic extracts of the yellow and purple allamanda.

MATERIALS AND METHODS

Plant materials

The two plant species, *Allamanda blanchetii* and *Allamanda cathartica* were collected from Patuli, Kolkata in November, 2017. The leaf, stem and flower of two species were washed, shade dried in room temperature and powered in mixer grinder.

Preparation of extracts

3.16 gm of leaf and stem sample of each species was used to prepare crude aqueous or methanolic extracts in 55 ml of double distilled water or 55 ml of methanol respectively. Similarly, floral extracts were prepared by using 0.28 gm of flower in 50 ml of methanol or 50 ml of double distilled water.

Chemicals and reagents

Sodium carbonate, Follin-ciocalteu reagent, Anhydrous Sodium Nitrite, Sodium Hydroxide, Anhydrous Aluminium Chloride, methanol and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were bought from Merck. Dextrose, Quercetin, Ascorbic acid and Gallic Acid were purchased from Sigma. All chemicals were analytical grade.

Instrumentation

Spectroscopic measurements were performed using Systronics UV-Vis Spectrophotometer.

Estimation of total polyphenolics in the allamanda extracts (Follin-ciocalteau assay)

The concentration of polyphenols in the flower, leaf and stem extracts was determined by initially reacting 10 ml of double distilled water in 2 ml of extract followed by addition of 1 ml of Folin Ciocalteu (1:10) solution. Then 12 ml of 7.5% sodium carbonate was added to the solution and the solution was incubated for 30 min. Absorbance of the solution was measured at 760 nm. A gallic acid standard curve was used to calculate the concentration of polyphenols in the extracts [6].

Determination the total level of flavonoid (Aluminum chloride assay)

The concentration of flavonoids in the allamanda extracts was estimated by adding 300 µl of allamanda plant extracts to 90 µl of 5% sodium nitrite and 90 µl of 10% aluminum chloride. The reaction was carried out at room temperature for 6 min and then 600 µl of 1M sodium hydroxide was added. Further, water was added to bring the final volume upto 3 ml. The reaction tubes were incubated at room temperature for 20 min in the dark. The absorbance of the samples was measured at 510 nm. By using a quercetin standard the concentration of flavanoids in the extracts was determined [7].

Estimation of polysaccharide contents

In 30 µl of extracts 470 µl of methanol or water was added followed by 500 µl of 5 % phenol and 2.5 ml of sulphuric acid. The solution was then incubated for 30 min, after which absorbance was measured at 488 nm. The calibration curve was prepared by using dextrose as a standard [1].

Estimation of the anti-oxidant potential of the extracts by DPPH (1, 1–Diphenyl-2-picrylhydrazyl) radical scavenging assay

Initially, 2.4 mg of DPPH was dissolved in 40 ml of methanol for 30 min in the dark which yielded DPPH free radicals. The absorbance of the resultant solution was adjusted to 1 by adding appropriate amount of methanol. Then, 100 µl of plant extracts were added to 2.9 ml DPPH free radical solution and the solution was incubated for 30 min in the dark. Then the absorbance of the solution was measured at 517 nm. Anti-oxidant activity was interpreted in terms of ascorbic acid equivalents [8].

RESULTS AND DISCUSSION

Crude aqueous and organic flower extracts of these two plant species possess higher amount of polyphenol as indicated in fig. 1. Polyphenol content in the aqueous flower extracts of *Allamanda blanchetii* was found to be 4.5054 mg/gm tissue, while in *Allamanda cathartica* the level was observed at 4.04794 mg/gm tissue. The organic extracts *A. blanchetii* and *A. cathartica* both contained 3.1074 mg/gm tissue of polyphenol. In case of leaf and stem methanolic extracts showed higher amount of polyphenol content than aqueous extracts. Aqueous leaf extracts of *Allamanda blanchetii* extract contained 1.4018 mg/gm tissue of polyphenol where as *Allamanda cathartica* contained 1.2532 mg/gm tissue of polyphenol. In methanolic extracts the polyphenol amount was 2.7438 mg/gm tissue in purple allamanda and 2.7438 mg/gm tissue in the yellow one. The organic stem extracts contain 1.2744 mg/gm tissue and 0.9027 mg/gm tissue of polyphenol in purple and yellow allamanda respectively.

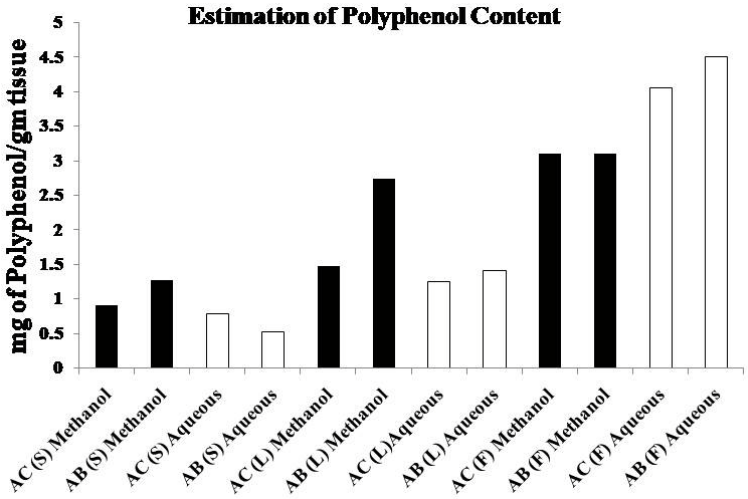

Fig. 1: Comparison of polyphenol content in aqueous and methanolic extracts, here AC and AB indicates *Allamanda blanchetti* and *Allamanda catharica respectively,* the notations S, L and F indicate atem, leaf and flower respectively

Estimation of flavonoid showed that crude aqueous and organic flower extracts were found to contain higher amount of flavonoid (fig. 2). Aqueous extract of purple allamanda had 14.9961 mg/gm tissue of flavonoid while the flavonoid content in yellow allamanda was estimated at 5.6695 mg/gm tissue. In methanolic flower extracts, flavonoid amount was 19.6349 mg/gm tissue for *Allamanda blanchetii* and 7.9275 mg/gm tissue for *Allamanda cathartica*. Methanolic extract of leaf had 2.1530 mg/gm tissue of flavonoids in *Allamanda blanchetii* and 7.0969 mg/gm tissue in *Allamanda cathartica.* Aqueous extract of leaves had 4.5452 mg/gm tissue of flavonoid in *Allamanda cathartica*, while 1.0366 mg/gm tissue of flavonoid was present in *Allamanda blanchetii*. In methanolic stem extracts, purple allamanda contained 1.3990 mg/gm tissue of flavonoids and yellow allamanda had 3.1171 mg/gm tissue of flavonoid. In aqueous extract of stem the flavonoid amount was found to be at 0.6814 mg/gm tissue for *Allamanda blanchetii,* and 1.6310 mg/gm tissue for *Allamanda cathartica*.

Like polyphenol and flavonoid, aqueous and organic flower extracts contained higher amount of polysaccharide (fig. 3). In aqueous extract the amount of polysaccharide content was 932.4809 mg/gm tissue in *Allamanda blanchetii* and 924.5562 mg/gm tissue in *Allamanda cathartica*. On the other hand, the methanolic extracts of *Allamanda blanchetii* had 725.1162 mg/gm tissue of polysaccharide, while the polysaccharide content in *Allamanda cathartica* was estimated at 524.355 mg/gm tissue. In aqueous extracts of leaf the amount of polysaccharide was found to be 360.8890 mg/gm tissue (*Allamanda blanchetii*) and 287.8997 mg/gm tissue (*Allamanda cathartica*). Methanolic leaf extracts had lower amount of polysaccharide at 231.7242 mg/gm tissue for *Allamanda blanchetii* and 208.31772 mg/gm

tissue for *Allamanda cathartica.* For stem, *Allamanda cathartica* aqueous extract had higher amount of polysaccharide (243.8175 mg/gm tissue). However, in *Allamanda blanchetii* methanolic extract had higher amount of polysaccharide content (86.21389 mg/gm tissue).

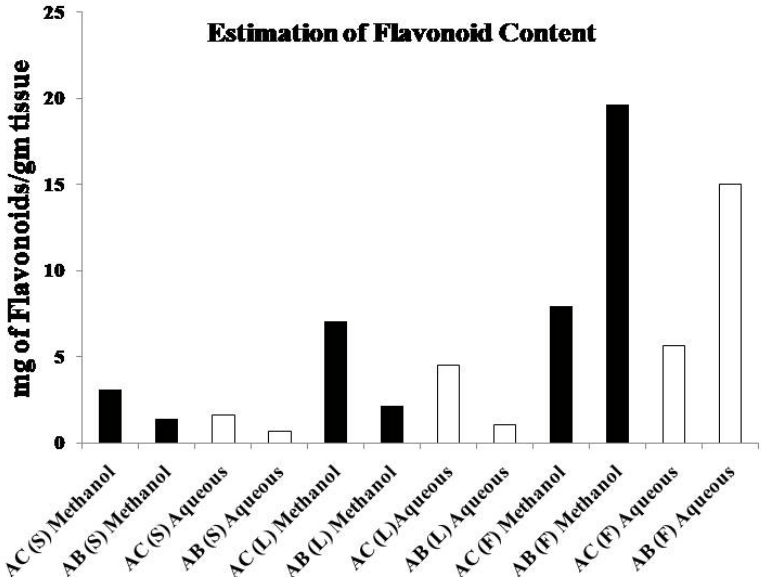

Fig. 2: Comparison of flavonoid content in aqueous and methanolic extracts, here AC and AB indicates *Allamanda blanchetti* and *Allamanda catharica respectively,* the notations S, L and F indicate atem, leaf and flower respectively

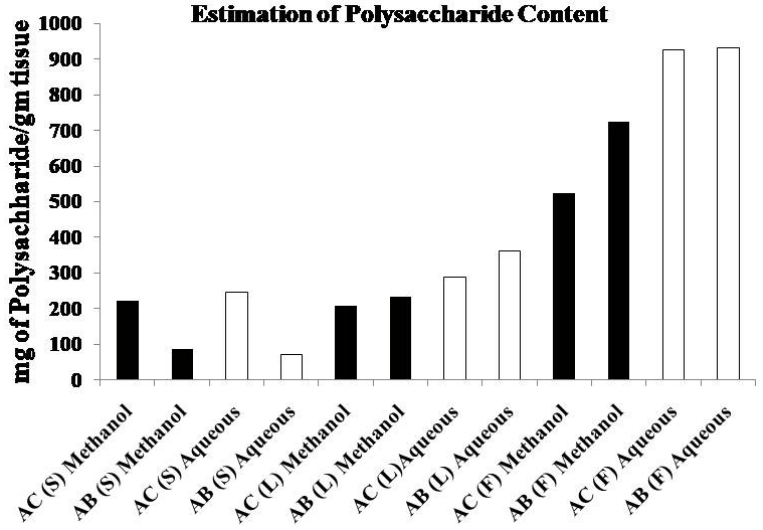

Fig. 3: Comparison of polysaccharide content in aqueous and methanolic extracts, here AC and AB indicates *Allamanda blanchetti* and *Allamanda catharica respectively,* the notations S, L and F indicate atem, leaf and flower respectively

As shown in fig. 4, methanolic and aqueous extracts of flower possessed the maximum anti-oxidant property. Anti-oxidant property was measured in milligram of ascorbic acid equivalent/gm tissue. In case of methanolic extracts of *Allamanda cathartica* and *Allamanda blanchetii* the amount of polyphenol was found to be 6.0486 mg/gm tissue and 9.9877 mg/gm tissue, respectively and for aqueous extracts the amount was estimated at 6.9590 mg/gm tissue and 9.3574 mg/gm tissue, respectively. As for the leaves, the organic extracts exhibited higher anti-oxidant property at 0.8694 mg/gm tissue (*Allamanda cathartica)* and 0.7981 mg/gm tissue (*Allamanda blanchetii*). In case of stem, aqueous extract showed more anti-oxidant property than methanolic extract at 0.4801 mg/gm tissue for yellow allamanda and 0.5390 mg/gm tissue for purple allamanda. Interestingly, methanolic extracts both the stem contained equal amount of anti-oxidant property at 0.3172 mg/gm tissue.

The present study is a comparison of the secondary metabolites and the anti-oxidant property of the two allamanda species. The free radical scavenging activity of the two allamanda species was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) reducing power assay. Our research showed that the aqueous and methanolic extracts of flower of both the species have higher amount of flavonoid, polyphenol and polysaccharide content. So for making any drug which contain highest amount of flavoniod, polyphenol or polysaccharide content flower of these species should be taken for further research. Flowers bloom for a relatively short period of time and are actively involved in the reproductive machinery of a plant. It's a widely accepted belief that secondary metabolites offer protective characteristics to plants against predation. Being a key player of the reproductive machinery of a plant, flowers need to be protected due to their lesser number and short bloom period. Hence, higher secondary metabolites concentration in the flowers may actually contribute

towards offering protection against loss and damage and thereby help in increasing the chances of reproductive success.

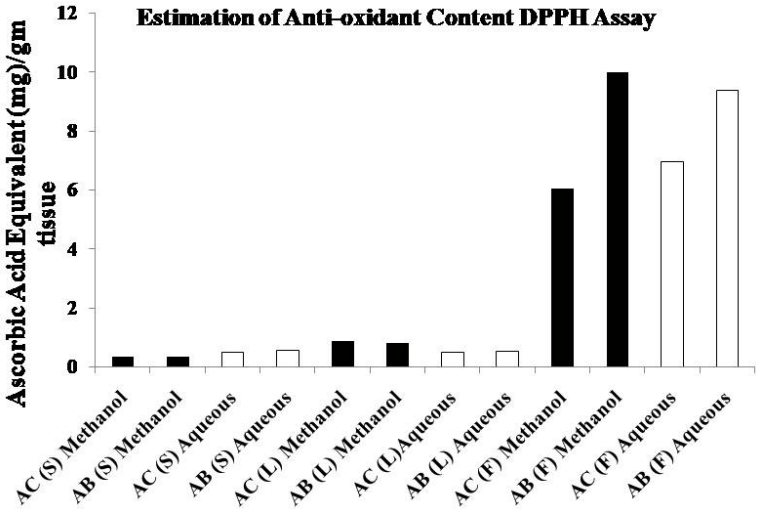

Fig. 4: Comparison of Anti-oxident content in aqueous and methanolic extracts, here AC and AB indicates *Allamanda blanchetti* and *Allamanda catharica respectively,* the notations S, L and F indicate atem, leaf and flower respectively

CONCLUSION

Medicinal plants are being used as the richest bio-resource for herbal medicine, modern medicine, folk medicine and also for traditional medicine. Stresses associated with present lifestyle calls balanced intake of carbohydrates, anti-oxidants, polyphenols and flavonoids for maintaining optimum health. In the present study we compare the secondary metabolites of two species of Allamanda that is *Allamanda blanchetii* and *Allamanda cathartica*. Our study reveals that the flower of both species contain highest amount of secondary metabolites in crude methanolic and aqueous extracts. In case of leaf, the methanolic extracts contain higher amount of polyphenol, flavonoid and anti-oxidant property in comparison to aqueous extracts, where as the aqueous extract contain higher amount of polysaccharide content than its counterpart. In stem, crude organic extract has higher amount of polyphenol and flavonoid and the aqueous extract has higher amount of polysaccharide and anti-oxidant property. Our study indicates that the usage of allamanda flowers to formulate new pharmaceutical drugs may serve as better alternative than their leaves, which is in contrast to the popular belief that the leaves are major sources of anti-oxidants in plants. A recent study demonstrating the anti-inflammatory properties of pluremide extracted from *Allamanda cathartica* flowers strengthens over prediction [9].

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

The authors declare no conflict of interest for the current study

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