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Diversified morphological and phytochemical screening of Wild *Begonia* of Sikkim Himalaya

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ABSTRACT

Begonia is one of the largest genera of angiosperm under *Begonia*ceae, with more than 1500 species distributed throughout tropical and subtropical regions. With diverse genera and a high degree of morphological diversity, *Begonia* is also a good sources phytochemicals. In this experiment, morphological assessment of three wild *Begonia* and preliminary phytochemicals screening by using different solvent extracts *viz.* ethyl acetate, hexane and methanol were carried out along with the determination of total flavonoids, phenolic content and anthocyanin. The results showed that the methanolic extract was found to be best among the tested solvents, which reveals that the all three *Begonia* species consist of phenols, tannin, flavonoids, alkaloids, saponins, carbohydrates, glycosides, amino acid and terpenoids. Among the tested species, hexane extract of *Begonia palmata* has maximum flavonoids (3.12 mg QE /100g) and ethyl acetate extract of *Begonia palmata* shows highest phenolic content (4.51 mg GA/g), whereas, *Begonia xanthina* has the highest anthocyanin (88.00g/mg) content. The present study reveals that wild *Begonia* species has varied morphology and a potential source of antioxidant properties and anthocyanin present in the leaves may conclude that the species may use for the production of natural dye.

Key words : *Begonia, Wild Begonia, Morphology, Phytochemical*

Introduction

Begonia L. (Linnaeus) is one of the largest genera of angiosperm under *Begonia*ceae, widely distributed throughout tropical and subtropical regions of the world except for Australia. *Begonia* is a pan-tropical genus of often shade-loving herbs and shrubs, with a number being very popular horticulturally. With diverse genera and high degree of morphological diversity, it is also very important ornamental group of plants due to their large, showy foliage, and multicolor flowers, ranging from white to pink, red, and yellow for displaying in hanging basket, garden plants, potted plants, and as greenhouse flowers (Nada *et al*., 2011).

Traditionally *Begonia*s have been used as potherbs or leafy vegetables in many parts of the world, leaves are used as a flavouring agent, soup or salad. In several countries *Begonia* species are used for medicine, Vitamin C and a source of food (Manandhar, 2002; Shrestha and Dhillion, 2006; Guan *et al.*, 2007; Girmansyah, 2009; Kar *et al.*, 2013; Rajbhandary, 2013; Isaivani *et al.,* 2014; Joshi *et al.* 2015) and some species have been reported to possess antimicrobial activities and are used to treat different ailments (Ramesh *et al*. 2002; Basurto-Pena *et al.*, 2003; Rop *et al., 2012*; Thorat *et al.* 2018). Due to its magnificent value, *Begonia* is a major component of the floriculture industry in around the world and new species continuing to be discovered in various parts of the world (Ambrish and Uddin, 2006; Shui, 2007; Phutthai and Sridith, 2010; Utley and Utley,

2011; Hughes, 2011; Thomas *et al*., 2011; Chong *et al.,* 2015; Hughes and Takeuchi, 2015; Odyuo *et al.,* 2018; Ly *et al.,* 2018).

Phytochemical constituents such as flavonoids, alkaloids, phenols, and other compounds are responsible for antioxidant property and anti-inflammatory, antimicrobial, antihemorrhagic activities, anticancer activity and anti-HIV activity (Kiritikar and Basu 2004; Zhang *et al*., 2010; Liu *et al.,* 2012; Divya *et al.,* 2014; Sofna and Banjarnahor 2014). Due to the active growth promoting activities and such important properties, they are key components in pharmacology, medicinal and cosmetics industries. *Begonia* species are also reported to possess some amounts of phytochemical constituents (Ramesh *et al.,* 2002; Karima *et al*., 2017; Shrestha *et al*. 2018). Anthocyanin is one of the important phytochemicals present in *Begonia* species and is a potential source of natural antioxidant (Awasthy *et al.,* 2016; Kwon *et al*., 2019). There are very fewer reports available regarding the morphological characterization and phytochemical studies of wild *Begonia* found in Sikkim Himalaya. Keeping this in view an attempt has been made to study morphology and phytochemical constituents present in three wild *Begonia* species viz. *Begonia xanthina*, *Begonia megaptera* and *Begonia palmata*. The main goal of this paper is to study the morphology and investigate preliminary phytochemical studies of *Begonia* plant extract in three different solvents *viz.* methanol, hexane and ethyl acetate and study the total phenolic, flavonoid and anthocyanin content in three wild *Begonia* species.

Materials and Methods

Plant collection and evaluation of morphological characters

The whole plant of *Begonia palmata, Begonia megaptera* and *Begonia xanthina was* collected from the different location of East District, Sikkim, India. Observation for various taxonomical parameters was taken during the vegetative and flowering stage of the plant as per the procedure of Doorenbos *et al.* (1998).

Preparation of extracts for phytochemical analysis

Fresh plant parts were allowed to shade dry and powdered and successively extraction was made with hexane, ethyl acetate and methanol. The solvents were removed completely under reduced pressure and a semi solid mass was obtained.

Preliminary phytochemical screening

The extracts have qualitatively analyzed the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, carbohydrates, glycosides and amino acid following standard methods (Kandelwal, 2001 and Kokatae, 2002; Edeoga *et al*., 2005; Gopinath *et al*., 2012 and Yadav *et al*., 2014)

Estimation of Total phenol content.

Total phenolic content of *Begonia* plant extract was determined by using Folin–Ciocalteu assay (Meda *et al.*, 2005). Solutions of each extract (100 µL; 1 mg/mL) were taken individually in test tubes. To this solution, 2.5 mL of 10-fold diluted Folin–Ciocalteu reagent was added, and the test tubes were thoroughly shaken. After 3 min, 2.0 mL of 7.5 % Na_2CO_3 solution was added and the mixtures were incubated for 30 min. The absorbance of the reaction mixtures was measured at 760 nm by using a spectrophotometer. Gallic acid was used as a standard and TPC of *Begonia* extracts was expressed in milligram gallic acid equivalents (mg GAE/g extract).

Estimation of Total Flavonoid content

Total flavonoid content was determined by the aluminium chloride colourimetric method (Lamaison and Carnet 1990), with some modifications. Briefly, the test samples were individually dissolved in a different solvent. Then, the sample solution (2 mL) was mixed with 2 mL of 2% $AlCl_3$. The absorbance of the solution was measured at 435 nm by using a spectrophotometer, after 10 min of incubation at ambient temperature. The flavonoid content was expressed as milligram quercetin equivalent (mg QE/g extract).

Anthocyanin content

Estimation of anthocyanin content of *Begonia* plant extract was carried out by weighing 1g of leaf sample and homogenized in 3 mL methanol with 1% HCl and the anthocyanin content was quantified by the standard protocol of Sutharut and Sudarat (2012). The absorbance of each dilution was read at 510 and 700 nm against blank distilled water.

DPPH radical scavenging activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) test is widely used to determine antioxidant activity in

plant extracts. The test was performed according to the methodology described by (Shen *et al.* 2010). 0.1mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of all the extracts in methanol at different concentration (50, 100, 200 and 400 µg/mL). The mixture was vortex and kept in dark for 30 minutes. The change in colour from dark blue to yellow was determined by measuring the absorbance at 517 nm using UV-vis Spectrophotometer. Ascorbic acid was used as standard and control was prepared using DPPH and methanol but without sample extract, whereas baseline correction was done using methanol. A lower absorbance value indicates a high radical scavenging activity. The scavenging activity of the plant extracts was calculated using the formula:

Scavenging activity %= [(A-B)/A] ×100

where, A is the absorbance of the control, and B is the absorbance of the extract samples and reference. The entire test was performed in triplicates and the results were averaged. The IC_{50} (the microgram of extract to scavenge 50% of the radicals) value was calculated using linear regression analysis. Lower IC_{50} value indicates greater antioxidant activity.

Results

Morphological characters

The present study shows varied morphology within the species of wild *Begonia*. The details of botanical description are as follows:

1. *Begonia megaptera* A. DC.

B.megaptera belongs to rhizomatous rootstocks and its stem up to a height of 45 cm. Leaves base is unequally cordate and margin undulates to angular and scarcely dentate, leaf diameter is 20-22 × 10-15 cm, acuminate, leaf colour of both adaxial and abaxial is green and absent of leaf pubescent. Total plant spread was recorded 46.4 cm, rosette form, the number of leaves /plant is 7-10, root length is 5.2 cm. Length of petioles is 21.3 cm, stipules lanceolate, 1 cm, glabrous, persistent. Peduncles elongate up to 10 cm; bracts 2-2.5 cm, glabrous, deciduous. Flowers pink; outer perianth segments oblong, 1.5 ×1 cm. Stamens numerous, united at base. Styles 2, 7mm, Capsules becoming inverted, 12-15 × 5-7 mm, glabrous; longest wing attached below apex, up to 2 cm long, smaller wings 3mm, longest wing attached below apex, up to 2 cm long, smaller wings 3 mm. The number of flowers/plant is about 15-20. Distributed on cliff edges and moist rocks in shady places, Altitude; 600-2000 m.

2. *Begonia xanthina* Hook. F.

As per the data presented in Table 1 and 2 *B. xanthina* is rhizomatous plants in a rosette form, up to a height of 40 cm and a plant spread around 30 cm, Leaf margin is entire, ovate to narrowly triangular and sinuate denticulate. Leaf base of *B. xanthina*is cordate and apex acuminate to cuspidate. Leaf colour of the adaxial surface is dark green with pale green and grayish spots between the veins and abaxial surface is with red spots in the main veins. The number of leaves per plant is 3-4, leaves diameter is around 20-25 x 15-20 cm. Petioles length is 20-

Table 1. Comparison of morphological studies of three *Begonia* species.

	B. xanthina	*B. palmate*	*B. megaptera*
Leaf Margin	Entire, ovate to narrowly triangular Sinuately denticulate	Ovate or suborbicular, Acutely toothed or lobed	Undulate-angular, scarsely dentate
Leaf base	Obliquely cordate	Obliquely cordate	Unequally cordate
Leaf apex	Acuminate to cuspidate	Acuminate to long acuminate	Acuminate
Leaf colour (Adaxial)/upper	Dark Green with pale green and grayish spots between main veins	Green with creamish white colour lining.	Green
Leaf colour (abaxial)/ lower	Dark red in the main veins	Reddish and green	Green
Leaf pubescent	Absent	Densely	Absent
Petioles colour	Reddish brown	Greenish brown	Green
Petioles hair	Absent	Present	Absent
Flower colour	Yellow	Whitish pink	Pink
Rootstock	Rhizomatous	Rhizomatous	Rhizomatous

Table 2. Showing morphological data of three Begonia species.

	B. xanthina	*B. plamata*	*B. megaptera*
Plant height (cm)	40-50	60-70	35-45
Plant spread (cm)	25-35	45-55	40-50
No. of leaves/plant	3-4	5-10	4-8
Shoot length (cm)	Rosette form	20-30	Rosette form
No of shoots/plant	-	3-6	-
Nodes and internodes	-	2.8	-
Root length (cm)	5-10	6-12	5-10
Stem diameter (cm)	-	2-5	-
Length of petioles (cm)	20-25	15-20	15-20
Length of peduncles (cm)	15-20	10-15	10-15
No of flowers/plant	10-15	10-20	15-20

25 cm, colour reddish - brown, and petioles hair is sparsely present. Peduncles length is about 15-20 cm, the flower is in yellow, the number of flowers/ plant is 10-15, fruit becoming inverted, the largest wing 2.3 × 0.7 cm, rounded, striate. Distributed in nearby streams, shady and in a subtropical forest, altitude 1200-1800 m, flowering season: July- September. Details morphology of this species was presented in Figure 2.

3. *Begonia palmata* D. Don; *B. laciniata*Roxb.

Data obtained from Table 1 and 2 of *B. palmata* is also having rhizomatous rootstock, stem up to a height of 60-70 cm tall, and a plant spread around 50 cm. Leaves margin is ovate and acutely toothed or lobed, leaf base is obliquely cordate and the apex is acuminate. Leaf diameter is around 15-20 x 10-15 cm, adaxial leaf colour is green with creamish white colour lining, the abaxial surface having pale to reddish and light green colour lining. Petioles colour is brownish - green in colour and length up to 15-20 cm. stipules triangular, 10-15 mm, acuminate, peduncles are about 10 cm, the number of flowers/ plant is about 10-15, flower colour is white to pink,

Fig. 1. Leaf Morphology of three *Begonia* species (A. *Begonia xanthina*, B. *Begonia megaptera*and C. *Begonia palmata*.).

capsule becoming inverted, 20 × 7 mm, with one long wing 2.7 × 15 cm and two smaller wings 6 mm. *Begonia plamata* is distributed in moist and shady areas in the subtropical and broad- leaved forest, altitude 1500-2100 m, flowering season: May-June.

Fig. 2. *Begonia palamata* D Don. *Begonia palmate* D. Don. A- Habit, B- Roots and stipules arising from stem, C- Staminate flower, D- fruit, E and G- Carpellate flower, F- Leaf apex and H- Leaf base.

Preliminary phytochemical screening

The results of the phytochemical analysis of three *Begonia* plants showed the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, carbohydrates, glycosides and amino acid. All the extracts were reported to show the positive result in colour reaction only for phenols, flavonoids and carbohydrates. The phytochemical constituents of the plants investigated are summarized in Table 3.

Total Phenol, flavonoid, anthocyanin content:

The results in figure 5 showed that the total phenolic concentration varies appreciably from one species to another in respect of different solvent. The highest

Fig. 3. *Begonia xanthina* Hook. F. A- Whole plant and habitat, B- Stipules and petioles arising from rhizome, C- Leaf base, D- Leaf apex, E -G- Staminate flower, G- Carpellate flower and capsule.

total phenolic content was found in methanolic extract of *B. palmata* at 24.00 GAE/g and hexane extract of *B. xanthina* was recorded lowest at 1.67 GAE/g. The level of flavonoids, expressed in Quercetin equivalent (QE) in mg/g of plant extract, varied among the species. According to the Fig. 6, highest flavonoid content was recorded in methanolic extract of *B. palmata* at 10.76 mg QE/g and lowest was observed in the hexane extract of *B. megaptera* (3.09 mg QE/g).

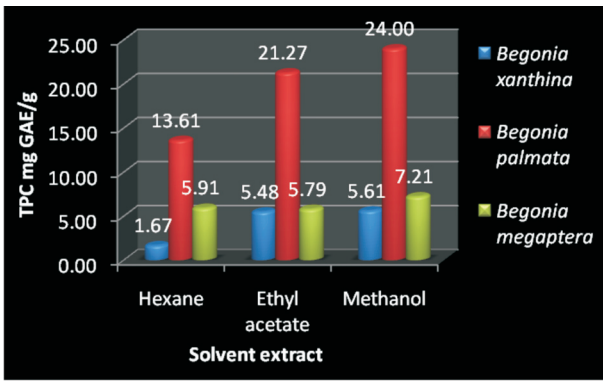

Fig. 5. Total Phenol content GAE/g.

Whereas, total Anthocyanin content varies from *B.xanthina* (88.00 mg/g), 68.26 mg/g in *B.palmate* and 20.08mg/g in *B. megaptera* (Fig. 7).

DPPH

The free radical scavenging activity of methanol extract of three *Begonia* against DPPH radicals was

Fig. 4. *Begonia megaptera* D. Don, A and B- Habit and whole plant, C- Stipule and petioles arising from the rhizome, D: Carpellate flower, E- Leaf apex, F- Stipules, G and H- Staminate flower, and I- Rhizome and roots.

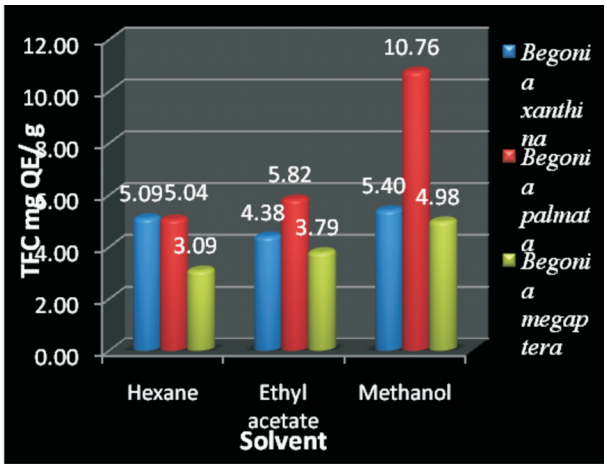

Fig. 6. Total flavonoid content

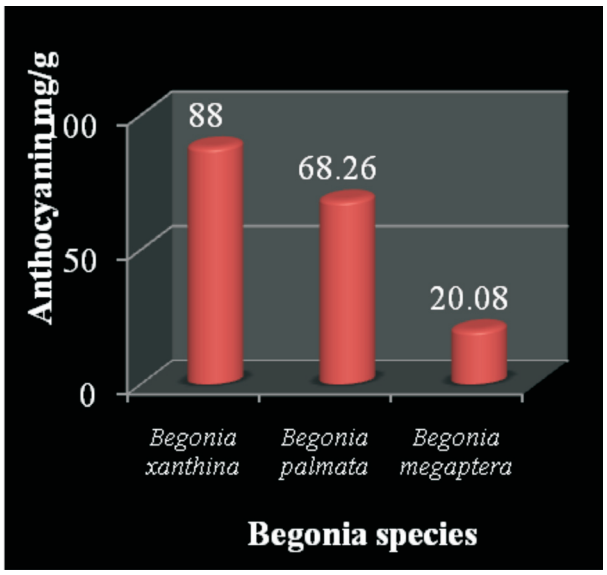

Fig. 7. Anthocyanin content mg/g

shown in Figure 8. Methanolic extract of *B. palmata, B.xanthina, B.megaptera* and Ascorbic acid showed antioxidant activity in dose dependent manner in the rage of 50-400 µg/mL and produced maximum scavenging activity at a dose of 400 ug/mL. The mean IC_{50} values of three *Begonia* species and ascorbic acid were in the range of 177.007 to 17.89 µg/mL. Among all the tested samples of *Begonia* species, methanolic extract of *B. palmata* (90.89 µg/mL) was lower which showed that the radical scaveng-

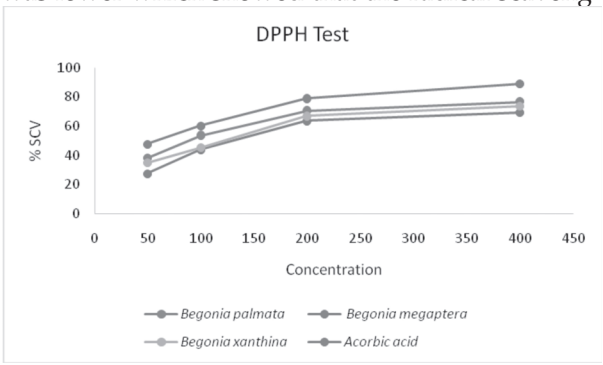

Fig. 8. DPPH radical scavenging activity of methanolic extract of three wild *Begonia* species.

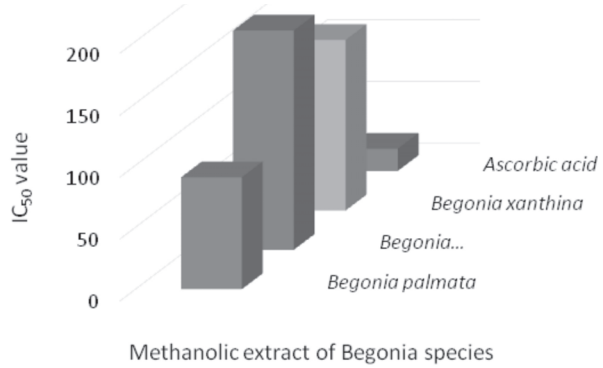

Fig. 9. IC_{50} values of methanolic extract of three wild *Begonia* species.

Table 3. Phytochemical screening of three *Begonia* species.

	Begonia xanthina			Begonia palmate			Begonia megaptera		
	Methanol	Ethyl acetate	Hexane	Methanol	Ethyl acetate	Hexane	Methanol	Ethyl acetate	Hexane
Flavonoid	+	+	+	+	+	+	+	+	+
Alkaloids	-	-	-	+	-	-	-	-	-
Tannins	+	-	+	-	-	-	+	+	-
Phenol	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+
Glycosides	+	-	-	+	-	-	+	-	-
Saponins	+	-	-	+	-	-	-	-	-
Amino acids	+	+	-	+	-	-	+	+	+
Terpenoids	+	-	-	-	-	-	+	-	-

ing activity was shown to be effective. The percentage inhibition of activity was calculated and the results are given in Fig. 9.

Discussion

Morphology

The results obtained from the present study shows diverse morphological traits among the species or within the species. Comparing the morphological traits of three wild *Begonia*s it was found that they contribute diverse in phenotypic traits, results obtained in Table 1 found that *B. xanthina* and *B. palmata*are having reddish colour in abaxial leaf surface, whereas *B. megaptera* having green in colour. Leaf margin of tested species are also found distinct, *B. palmata* having palmately/acutely lobed, whereas *B. xanthina* having entire, ovate to sinuate denticulate and *B. megaptera* is having undulated to angular and scarcely dentate. Flower colour also differs in the respective species, *B. xanthina* is in yellow, *B. palmata* having whitish pink and *B. megaptera* blooms pinkish flower. Details morphological trait which was taken during the flowering time/ season at maximum attainable height were evaluated and presented in Table 1 & 2, which shows distinct phenotypic characters for each species.

Phytochemical screening

Phytochemical constituents are biologically active compounds and are responsible for various activities such as antioxidants, antimicrobial, antifungal and anti-cancer. Phytochemicals present in the plant samples are the source of various treatment of the health hazards and different compounds have different therapeutic value. It is interesting to note that the methanolic extract of *Begonia* species showed the presence of flavonoids and phenols in abundant quantity.

Total phenol

Phenolic compounds are considered to be the most important class of antioxidants. Phenolic compounds can donate hydrogen atoms to free radicals and possess ideal structural properties for free radical scavenging properties. Phenolic compounds commonly found in edible and medicinal plants have various biological effects including antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities. A synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) butyl hydroxy quinone, propyl gallate and gallic acid esters are commercially available but their usages have been limited due to their adverse effects to human health due to their toxicity (Kahl, 1984; Kahl and Kappus, 1993). Hence, consumer concern regarding their safety has motivated the food industry to look for natural source of antioxidants (Bravo, 1998). Consumption of wild edible plants may provide natural source of antioxidant to be best against synthetic antioxidant and are harmless too (Anubudhasan *et al.* 2014; Barlow, 1990). In the present study, the highest total phenolic content was found in methanolic extract of *B. palmata* at 24.00 GAE/g, which shows plant may possess a significant amount of antioxidants. Similar results were also obtained by various author countering phenolic content in the *Begonia* species (Han *et al*. 2013; Geetha *et al.,* 2016; Shrestha *et al.,* 2016; Isaivani *et al.,* 2014; Deinghdoh, 2017; Jose *et al*., 2016). It has also confirmed that the pharmacological effect of phenol is correlating with their antioxidant activities.

Flavonoids

The result showed that these wild *Begonia* species are excellent sources of phenolic and flavonoid antioxidants. Flavonoids are associated with health promoting effects and are key components in pharmaceutical, medicinal and cosmetics industries; due to their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties (Evans *et al.* 1995; Cook and Samman, 1996; Steinmetz and Potter, 1996; Panche *et al*. 2016; Kaurinovic and Vastag, 2019). The present study carried out phytochemicals, flavonoids and phenols on the wild *Begonia* species plant extract revealed the presence of medicinally active constituents. The previous report suggests the presence of phenol and flavonoid in the *Begonia* (Ramesh *et al*. 2002; Solomon and Mari-muth, 2012; Jose and Kumar, 2016). The findings of the current study shows the plant extracts of *Begonia* species revealed similar results to previous findings. Phytochemical constituents such as alkaloids, flavonoids, phenols, tannins, saponins and several other organic compounds are secondary metabolites of plants that serve as a defense mechanism against microorganism and insects.

Anthocyanin

B. xanthina and *B. palmate* shows maximum antho-

cyanin content 88.00 mg/g and 68.26 mg/g, respectively, it was due to the fact that *B. xanthina* and *B. palmata* have red colour leaves, whereas, *B. megaptera* green leaves. Previous reports on anthocyanins suggest that it can significantly affect plant response to environmental stress, protect organs and substances involve in photosynthesis processes, relieve photo-oxidation damage to leaves (Lee and Collins, 2001; Lee, 2002; Wang *et al*. 2016). Anthocyanins may act as an effective antioxidant and can greatly improve the viability and resistant to plants (Lee, 2002; Wang *et al.* 2016). This preventive effectiveness of anthocyanin may be related to the existence of a relationship between the content of anthocyanin to the antioxidant activity resulting in cellular defenses. The extracts of *Begonia* may have excellent potential as functional ingredients representing a potential source of natural antioxidant. These results are highly correlated with the previous reports on the *Begonia* plants (Diengdoh 2017; Awasthy *et al.*, 2016; Awasthy and Murungan, 2015; Ambhujaksi *et al.,* 2018).

DPPH

DPPH assay reveals that the *Begonia* plant extract showed a relevant antioxidant activity. Previously many researches (Jose *et al*., 2016; Ganapaty *et al.* 2013; Joshi *et al.,* 2015) have demonstrated that *Begonia* species showed DPPH radical scavenging ability. The antioxidant potential of *Begonia* species between the various publications may vary both due to the methods used, as well as the method of bioactive substances extraction. In the present experiment, the methanolic extract of *Begonia* species had significant scavenging effects on the DPPH which was increasing with the decreasing in the concentration of the sample from 50-400 µg/mL. Methanolic extract of *B. palmate* showed best scavenging activity as compare to *B. xanthina* and *B. megaptera*. This might be due to the presence of flavonoid, phenol and anthocyanin content, the most required bio-compounds for scavenging activity in the extract.

Conclusion

This study shows great diversity in the morphology of *Begonia* genera and quantitative phytochemical analysis indicated that the plant contains significant amounts of phenolics compounds such as total phenolic and flavonoids. *B. xanthina, B. palmata* and *B. megaptera* were recognized as shade loving plant and exhibit horticulturally importance species, owing to have ornamental properties and some potent medicinally importance and can use for edible purposes too. Due to the high content of anthocyanin from their red colour leaves may use for the production of bio colour.

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and grayish spots between main veins on adaxial surfac

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