INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-6, Issue-3, July-Sept-2015 *Coden IJABFP-CAS-USA* *Copyrights@2015*

Received: 4th July-2015 *Revised: 19th July -2015* *Accepted: 20th July-2015*

Research article

COMPARISON OF BIOACTIVE ANTHOCYANIN COMPONENTS FROM *BEGONIA MALABARICA* LAM. AND *BEGONIA REX-CULTORUM* 'BABY RAINBOW' L.H.BAILEY BY GC-MS ANALYSIS

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ABSTRACT: Anthocyanins, derivatives of 2-phenylbenzopyryliumare are water-soluble natural pigments. Owing to extensive scientific research they have become not only the colouring food products but also therapeutic sources of bioactive compounds and continue to play role in the maintenance of human health. *Begonia malabarica* Lam. and *Begonia rex-cultorum* 'baby rainbow' L.H. Bailey are common ornamentals with varied leaf colour combinations were analyzed by Gas Chromatography-Mass Spectrometry to determine the bioactive constituents present in the methanolic extracts of the leaves. Powdered leaf samples were subjected to Soxhlet extraction method with methanol and the extract was fractioned by GC-MS. Totally, 7 different compounds each from *B. malabarica* [phenol, 2,4-bis (1,1-dimethylethyl), hexadecanoic acid, methyl ester, 9,12-octadecadienoic acid (Z,Z), methyl ester, 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z), 1,2-benzenedicarboxylic acid, diisooctyl ester, Spiro[furan-2(5H),2'(1'H)-naphtho[2,1-b]furan]-5-one, 3'a,4',5',5'a,6',7',8',9',9'a,9'b-decahydro-3,3'a,6',6',9'a-pentamethyl and androst-1-en-3-one, 4,4-dimethyl-, (5.alpha.)] and *B. rex* 'baby rainbow' [diethyl Phthalate, 1-(3,6,6-trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethanone, hexadecanoic acid, methyl ester, 7,10-octadecadienoic acid, methyl ester, 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z), 5,9 methanobenzocycloocten-1(2H)-one,3,4,5,6,7,8,9,10-octahydro-5,10-dihydroxy-3,3,7,7,9-pentamethyl and 1,2-benzenedicarboxylic acid, diisooctyl ester] were identified. All the compounds identified were medicinally proven for the treatment of various human ailments. Further studies are warranted to purify the lead molecules and to evaluate their biological properties including toxicological aspects for the development of novel drugs.

Key words: *Begonia*, Medicinal plant, phyto-chemical constituents, GC-MS analysis

INTRODUCTION

Begonia, a perennial flowering plant belongs to Begoniaceae. The genus contains about 1,600 species. Most species are grown indoors as ornamental house plants in cooler climates. The species are terrestrial (sometimes epiphytic) herbs or under shrubs, and occur in subtropical and tropical moist climates of South Central America, Africa, and southern Asia. Terrestrial species in the wild are commonly have upright rhizomatous or tuberous stem. The leaves, which are often large, variously stotched or variegated and are usually asymmetric (Forrest *et al*., 2005). Malabar Begonia are important medicinal plants due to their pool of polyphenols such as luteolin, quercetin and β-sitosterol. The leaves are commonly used for the treatment of respiratory infections, diarrhea, blood cancer and skin borne diseases. Limited reports on cultivation, breeding and phytochemical studies of *B. malabarica* are available despite its commercial importance.

Anthocyanins constitute the largest and unique group of water-soluble natural pigments (Takeoka *et al*., 2002). 635 anthocyanins types are identified in plants, and such a versatile group is responsible for the vivid blue, purple, and red colour of fruits, vegetables and flowers (Andersen and Jordheim, 2008). The colour comes from charged anthocyanin pigments related with pH of the intracellular medium. Many leaves frequently develop red coloration during development and also at senescence. Similarly, members of Caryophyllales produce unique nitrogenous pigments, the betacyanins. Anthocyanins are mostly accumulated in the vacuoles (Meyer *et al*., 1995; Witham *et al*., 1971).

Anthocyanins in plants attract animals, leading to seed dispersal and pollination. Owing to strong absorption of light, they may also be important in protecting plants from UV-induced damage. Anthocyanins belong to polyphenolics named flavonoids, which are secondary metabolites synthesized by higher plants via phenylpropanoid pathway. Their aglycones share a C-6 (A ring)-C-3 (C ring)-C-6 (B ring) carbon skeleton (Harborne 1998). Based on the characteristics of the aglycones, flavonoids are divided into different subclasses. Based upon *in vitro* cell-line studies, animal models, and human clinical trials, it has been suggested that anthocyanins possess anti-inflammatory and anticarcinogenic, cardiovascular disease prevention, obesity control, and diabetes alleviation properties, all of which are associated with their potent ROS scavenging property. The present investigation is aimed to isolate and fractionate the bioactive anthocyanin components from *Begonia* species using GC-MS.

MATERIALS AND METHODS

Plant materials

For the whole attempted work, the fresh healthy *Begonia* plants belonging to two species such as *Begonia malabarica* Lam. and *Begonia rex-cultorum* 'baby rainbow' L.H.Bailey, (Bailey 1951; Bailey 1976) were collected from the department garden. Leaf sample at specific growth stage was selected for the entire analysis.

Estimation of anthocyanin content

1g leaf sample was homogenized in 3 ml methanol with 1% HCl and vortexed for 30 sec and kept in water bath at 60^{o} C for 20 min. Subsequently, the samples were centrifuged at 10000 rpm for 10 min. The supernatant was transferred to 10 ml volumetric flask. The residue was again mixed with 3 ml of methanol. The supernatant was again centrifuged and combined with the previous supernatant and made up to 10 ml. The final extract was kept at 0^{o}C for further analysis.

1ml of extract was taken and transferred to 10 ml volumetric flask for preparing two dilutions of the sample, one adjusted with KCl buffer, pH 1.0 and the other with sodium acetate buffer pH 4.5. These dilutions were equilibrated for 15 min. The absorbance of each dilutions was read at 510 and 700 nm against distilled water as blank (Sutharut and Sudarat, 2012).

GC-MS (Gas chromatography – Mass spectrum) analysis

The leaf sample was extracted with methanol using Soxhlet hot continuous extraction method. GC-MS is an analytical technique of gas chromatography and mass spectrometer. The gas chromatographic technique basically fractionates diverse phytochemicals present in a mixture. Further, the separated molecules can be analyzed with respect to its mass in a mass spectrometer which helps to identify the compounds. GC MS analysis of the sample was carried out by using Agilant FC MS system and identification of compounds is based on NIST Mass Spectral Library.

High resolution JEOL GCMATE II GC-MS with Data system with maximum resolution of about 6000 and calibrated to mass 1500 Daltons were used for the GC-MS measurement of the samples. The positive ion source is used for the ionization process. The obtained spectral data were compared with that of the United States National Institute of Standards and Technology (NIST) data base.

RESULTS AND DISCUSSION

Begonia species show wide variations in their morphological characters especially in the leaves. A wide range of *Begonia* morphoforms were reported from all over the world. Cultivar types can be easily identified with the help of variations in the morphology of their leaves. Leaves are varied in many features like colour, shape and size of the leaf. Meanwhile, flowers of *Begonia* cultivars are almost similar. The content of anthocyanin varies among the species and cultivars. Natural dyes have been extracted from plants and possess applications in food, textiles and pharmaceutical industries. Initially, an attempt was made to analyze the anthocyanin content and subsequently, its characterization using GC-MS analysis.

Anthocyanin content

Anthocyanin content was quantified according to the method of Sutharut and Sudarat, (2012) *B. rex-cultorum* 'baby rainbow' (69.64 mg/g) showed the highest anthocyanin content compared to *B. malabarica* (22.88 mg/g).

GC MS analysis

GC MS analysis revealed a pool of compounds in the methanolic extract with 1% HCl of *Begonia malabarica* and *B. rex- cultorum* 'baby rainbow'. The major shared compound in *B. malabarica* and *B. rex- cultorum* 'baby rainbow' was 9, 12, 15-octadecatrienoic acid, methyl ester, (Z, Z, Z) - with 46.5% and 22.21% respectively. The identification of the phytochemicals was confirmed based on the peak area, retention time and molecular formula. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area in percentage are presented in Table- 1 & 2 and Fig. 1 & 2.

The phytocomponents observed in *B. malabarica* were (1) phenol, 2,4-bis (1,1-dimethylethyl)-(6.13%), (2) hexadecanoic acid, methyl ester (11.64%), (3) 9,12-octadecadienoic acid (Z,Z)-, methyl ester (10.47), (4) 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- or (alpha-linolenic acid)- PUFA (46.95%), (5) 1,2-benzenedicarboxylic acid, diisooctyl ester or (Phthalic acid) (11.23%),(6) Spiro[furan-2(5H),2'(1'H)-naphtho[2,1-b]furan]-5-one, 3'a,4',5',5'a,6',7',8',9',9'a,9'b-decahydro-3,3'a,6',6',9'a-pentamethyl (4.07%) and (7) androst-1-en-3-one, 4,4-dimethyl-, (5.alpha.)-(9.51%) (Table-1 and fig. 1).

The bioactive phytoconstituents in *B. rex- cultorum* 'baby rainbow' were (1) diethyl Phthalate (20.60%), (2) 1-(3,6,6-trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethanone (17.57%), (3) hexadecanoic acid, methyl ester (10.03%), (4) 7,10-octadecadienoic acid, methyl ester (6.71%) , (5) 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- or (alpha-linolenic acid)- PUFA (22.21%), (6) 5,9 methanobenzocycloocten-1(2H)-one, 3,4,5,6,7,8,9,10-octahydro-5,10-dihydroxy-3,3,7,7,9-pentamethyl-(13.49%) and (7) 1,2-benzenedicarboxylic acid, diisooctyl ester or (Phthalic acid) (9.40%) (Table- 2 and fig. 2).

The compound identified with less retention time (21.602 min) was diethyl phthalate, whereas 1,2-Benzenedicarboxylic acid, diisooctyl ester becomes the final compound with longest retention time (53.444 min) in *Begonia rex- cultorum* 'baby rainbow'. Meanwhile, in *Begonia malabarica* within 21.927 min phenol, 2,4-bis(1,1-dimethylethyl)- was eluted and androst-1-en-3-one, 4,4-dimethyl-, (5.alpha.)- took 56.691 min to be fractionated. The phytochemicals identified through GC-MS analysis were proven in many plants for their biological activities (Table-1 & 2).

Hexadecanoic acid, methyl ester; 9, 12, 15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- and 1,2-benzenedicarboxylic acid, diisooctyl ester were found to be common among the species of *Begonia*.

The identified compounds displayed many biological properties. For instance, 9,12,15-octadecatrienoic acid, (Z,Z,Z)-linolenic acid a fatty acid ester was evaluated for anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties. The compound is seen in Purslane seed fixed oil (Osman and Mohammed, 2015) with proven antioxidant and anti-diabetic activities.

n-Hexadecanoic acid-palmitic acid a fatty acid ester was reported to show antioxidant property, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha reductase inhibitions. 1,2-benzenedicarboxylic acid and di-isooctyl ester were present in the extract of *Memecylon umbellatum* (Murugesan *et al*., 2011) having anti fouling and antimicrobial activities.

Table 1: GC-MS profile of the identified compounds from methanolic extract of *B.malabarica* with the retention time and percentage of area

S.No	Compound	Retention time (min)	Area (%)
1	Phenol, 2,4-bis(1,1-dimethylethyl)-	21.927	6.13
2	Hexadecanoic acid, methyl ester	36.292	11.64
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	41.290	10.47
4	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- or (alpha-Linolenic acid)- PUFA	41.468	46.95
5	1,2-Benzenedicarboxylic acid, diisooctyl ester or (Phthalic acid)	53.498	11.23
6	Spiro[furan-2(5H),2'(1'H)-naphtho[2,1-b]furan]-5-one, 3'a,4',5',5'a,6',7',8',9',9'a,9'b-decahydro-3,3'a,6',6',9'a-pentamethyl	56.535	4.07
7	Androst-1-en-3-one, 4,4-dimethyl-, (5.alpha.)-	56.691	9.51

Table 2: GC-MS profile of identified compounds from methanolic extract of *B.rex-cultorum* 'baby rainbow' with the retention time and percentage of area

S.No	Compound	Retention time (min)	Area (%)
1	Diethyl Phthalate	21.602	20.60
2	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone	21.856	17.57
3	Hexadecanoic acid, methyl ester	36.237	10.03
4	7,10-Octadecadienoic acid, methyl ester	41.261	6.71
5	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- or (alpha-Linolenic acid)- PUFA	41.443	22.21
6	5,9-Methanobenzocycloocten-1(2H)-one,3,4,5,6,7,8,9,10-octahydro-5,10-dihydroxy-3,3,7,7,9-pentamethyl-	49.490	13.49
7	1,2-Benzenedicarboxylic acid, diisooctyl ester or (Phthalic acid)	53.444	9.40

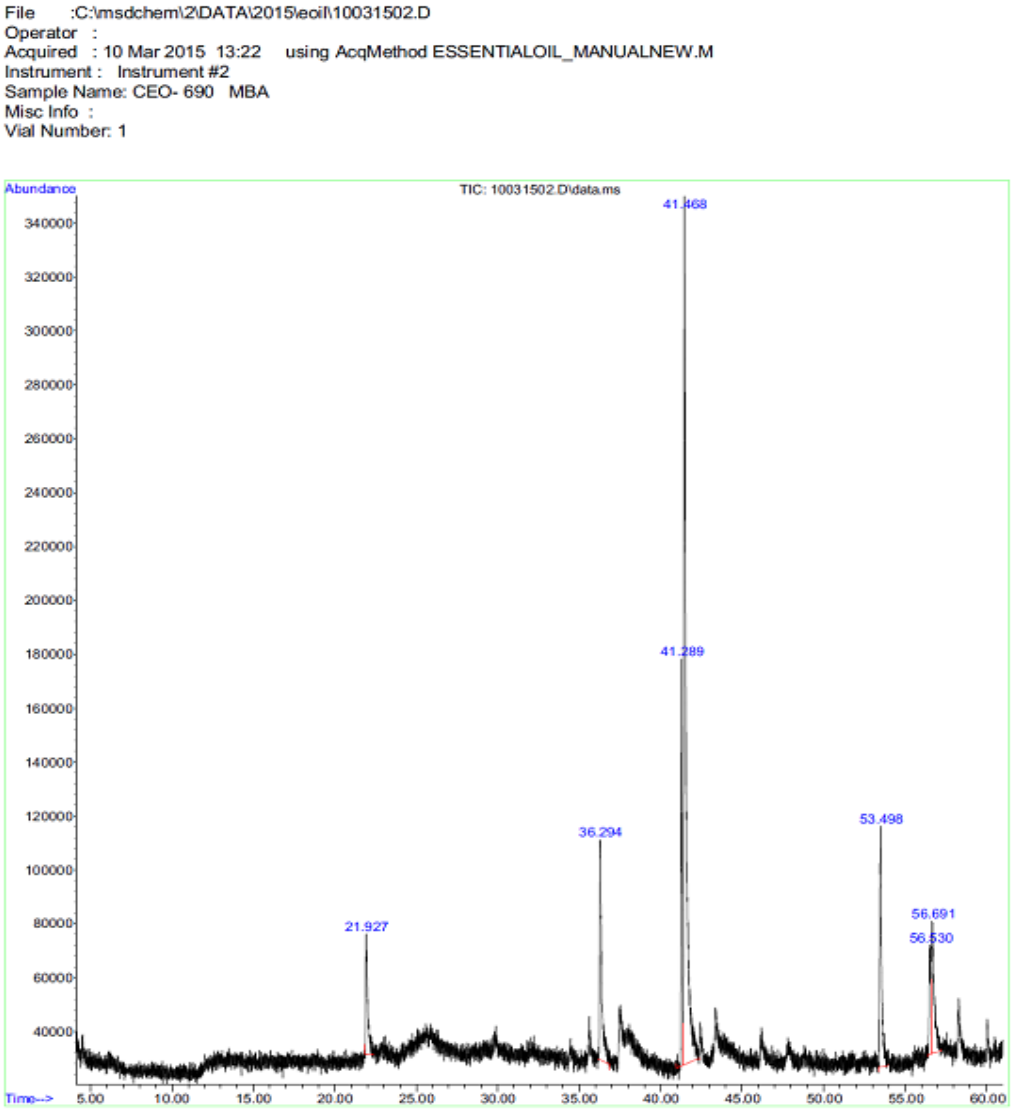

Figure 1. GC- MS profile of the seven bioactive constituents of *Begoina malabarica* in methanolic extract

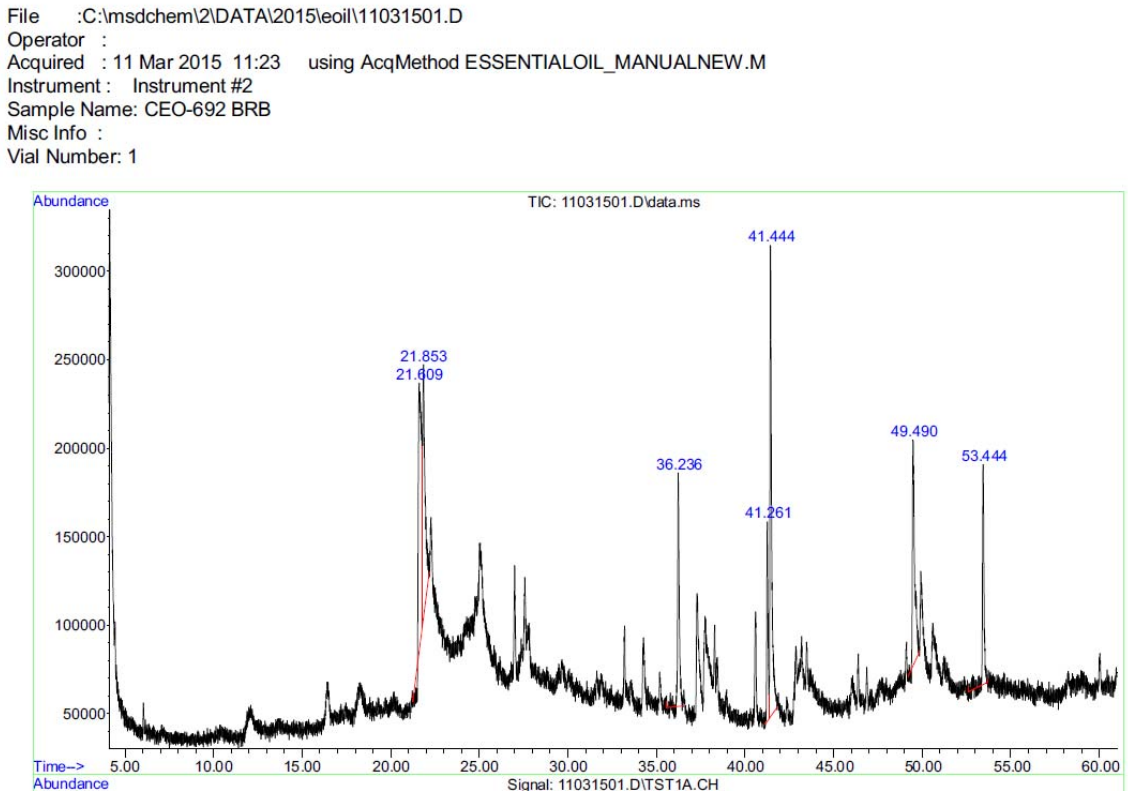

Figure 2. GC- MS profile of the seven bioactive constituents of *Begoina rex-cultorum* 'baby rainbow' in methanolic extract

Diethyl phthalate is one among the fourteen compounds of the present study was reported in *Scolopendra subspinipes* (Govindappa *et al*., 2014) and possess antioxidant potentiality. 1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta [c]pyran-1-yl) ethanone reported in *Casimiroa edulis* is insecticidal and antifeedant (Barakat 2011). Similarly, phenol, 2,4-bis(1,1-dimethylethyl) possessed antimalarial and antibacterial activities in *Balanites aegyptiaca* and *Monochaetia kansensis* extracts (Govindappa *et al*., 2014).

9,12-Octadecadienoic acid (Z,Z)-, methyl ester (Linoleic acid) possess similar activities as that of 9,12,15-octadecatrienoic acid. Hexadenoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata* (Grace and Davis, 2002) and *Melissa officinalis* (Sharafzadeh *et al*., 2011). Parasuraman *et al*., (1985) identified 17 compounds with n-hexadecanoic acid and octadecanoic acid as the major phytochemicals in the leaves of *Cleistanthus collinus*. GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-hexadecanoic acid (Ibrahim *et al*., 2009). n-Hexadecanoic acid, hexadecanoic acid, phytol, 9, 12 - octadecadienoic acid, 9, 12, 15-octadecatrienoic acid and squalene were identified in the ethanol leaf extract of *Aloe vera* (Arunkumar and Muthuselvam, 2009) and *Vitex negundo* (Praveen *et al*., 2010). Squalene is used in cosmetics as a natural moisturizer. Devi *et al.,* (2009) reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and 9, 12-Octadecadienoic acid with therapeutic values.

CONCLUSION

Structural characterization realized by GC-MS indicated the presence of compounds which are characteristics of the molecular structure of anthocyanins. Compounds identified were bioactive and possessed several proven functions in many herbals. Thus, present study revealed the chemical characteristic profiles and the identification of probable phytochemicals associated to the colour of anthocyanin in *Begonia* species. Many of them were active ingredients of several medicinal preparations hence further studies are planned to isolate and purify the principle compound of the extract and to analyze its therapeutic values.

ACKNOWLEDGMENT

The authors acknowledge the Kerala State Council for Science, Technology and Environment (KSCSTE), Govt. of Kerala for providing funding in connection with the major project.

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ISSN : 0976-4550

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