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RESEARCH ARTICLE :

Analysis of bioactive constituents from selected genotypes of annatto seed extract through Gc-Ms

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SUMMARY : The term "annatto" in industrialized countries is commonly referred to *Bixaorellana* seed extract containing carotenoid-type pigments. In India, it is well distributed in Kerala, Karnataka, Tamil Nadu, Andhra Pradesh, Odisha, West Bengal, Gujarat, Maharashtra, Madhya Pradesh and Chhattisgarh and also reported to be cultivated commercially. The GC-MS results of bixa genotype TNBi -13 and KLBi -3 confirms the presence of 18 compounds and 22 compounds each, respectively.Apart from these compounds some new compounds like Himachol (1.17%) and Synaptogenin (1.13%) were found in bixa genotype TNBi -13 whereas compounds like α-Terpinene (1.06%) and α-bulnesene(0.76%) were found to be present in bixa genotype KLBi -3.These results suggest that there are chances of variation among the secondary metabolites not only at a quantitative level but also at a qualitative level. Hence profiling of each and every screened genotype is required in order to attain the best selection.

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KEY WORDS :
GC-MS, Carotenoids, Sequiterpenes, Geranylgeraniol, Spathulenol, Anticancerous

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BACKGROUND AND OBJECTIVES

Dye yielding plants are matter of study in the recent past (Garg *et al.*, 2010; Choudhary and Upadhyay, 2011; Saravanan *et al*., 2012 and Choudhary *et al*., 2012). *Bixa orellana* L. (Bixaceae), is one such natural dye crop, native to Central and South America, is also known as annatto, uruc'u, or achiote (Coelho-Ferreira, 2009 and Carvalho, 1989). In India, it is well distributed in Kerala, Karnataka, Tamil Nadu, Andhra Pradesh, Odisha, West Bengal, Gujarat, Maharashtra, Madhya Pradesh and Chhattisgarh and also reported to be cultivated commercially. Annatto has adapted itself to a wide range of edaphic and ecological conditions; it implies that considerable amount of existing genetic variability to be exploited for potential realization.

Keeping this in view, this study was designed to explore the variation in bioactive chemical constituents among the screened genotypes for their economic characters for future breeding and improvement programmes.

RESOURCES AND METHODS

Collection of seed :

The Bixageno types, TN Bi – 13 selected from Coimbatore, Tamil Nadu and KL Bi – 3

selected from Kuttichathanpadi, Keralaare utilized for this study which were collected from an established Bixa gene park at Horticulture College and Research Institute, Periyakulam, Tamil Nadu.The study was carried out during 2015 - 2016.

Preparation of sample extract :

A sample of 5g seed of bixagenotype (TN Bi – 13 and KL Bi -3) was extracted in 25 mL of hexane (HPLC grade) in automated soxhlet apparatus (SOXTEC 2043 FOSS). It was kept overnight to remove the traces of hexane and the obtained extract was stored in vials which were kept in an airtight container at 4^0C for further use.

GC - MS analysis :

The chemical composition of the seed extract was analysed using Thermo GC - Trace Ultra Ver: 5.0 and Thermo MS DSQ II fitted with a DB 35 - MS capillary standard non - polar column (30 m, ID: 0.25 mm and film thickness of 0.25 µm). 0.5 µl of methanol extract was injected for analysis and Helium was used as a carrier gas at 1 mlit./ min. The instrument was set as follows, Injector port temperature set to 250^0C, source kept at 220^0C. The oven temperature was programmed from 70^0C to 260^0C at the 6^0C/ min rate. The MS was set to scan from 50 - 650 Da. The MS also had inbuilt pre - filter which reduced the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum, NIST4 and WILEY9 containing more than five million references.

Identification of compounds :

Interpretation of mass spectrum of GC - MS was done using the database of National Institute Standard and Technology (NIST4) and WILEY9 (Dool and Kratz, 1963).

OBSERVATIONS AND ANALYSIS

The GC-MS results of bixa genotype TNBi -13 confirmed the presence of 18 compounds liketrans-geranylgeraniol, spathulenol, phthalic acid, pulegone, longifolene (V4), α - guaiene, aromadendrene, α-cadinene, methylhydrogen-(9'Z)-6,6',-dioateand odecane (Table 1 and Fig. 1).

The GC-MS results of bixa genotype KLBi -3 confirms the presence of 22 compounds among which trans-geranylgeraniol, aromadendrene, longifolene (V4), α-selinene, germacrene D, α-cubebene, dodecane, methylhydrogen-(9'Z)-6,6',-dioate, spathulenol and pulegone were the major constituents (Table 2 and Fig. 2). Some new compounds likehimachol and synaptogenin were also found in bixa genotype TNBi -13 whereas compounds like α-terpinene and α-bulnesene were found

Table 1 : Chemical compounds in seed extract of Bixagenotype (TN Bi – 13)

Sr. No.	Retention time	Compound name	Molecular formula	Molecular weight	Area %
1.	6.74	Dodecane	$C_{13}H_{28}$	184	1.15
2.	7.44	Methylhydrogen-(9'Z)-6,6',-dioate	$C_{25}H_{30}O_4$	394	1.15
3.	10.63	α-elemene	$C_{15}H_{24}$	204	0.36
4.	12.61	Longifolene-(V4)	$C_{15}H_{24}$	204	2.41
5.	12.61	α-Guaiene	$C_{15}H_{24}$	204	2.41
6.	12.61	Aromadendrene	$C_{15}H_{24}$	204	2.41
7.	12.94	α-Cadinene	$C_{15}H_{24}$	204	1.44
8.	12.94	α-Selinene	$C_{15}H_{24}$	204	1.44
9.	15.29	Pulegone	$C_{10}H_{16}O$	152	2.60
10.	17.13	(+) spathulenol	$C_{15}H_{24}O$	220	11.46
11.	17.69	5-cedranone	$C_{15}H_{24}O$	220	1.39
12.	18.52	Longiborneol	$C_{15}H_{26}O$	222	1.72
13.	18.52	α-Cedrol	$C_{15}H_{26}O$	222	1.72
14.	19.08	Himachalol	$C_{15}H_{26}O$	222	1.17
15.	24.97	Phthalic acid	$C_{19}H_{28}O_4$	320	3.35
16.	27.80	trans-Geranylgeraniol	$C_{20}H_{34}O$	290	58.88
17.	28.53	trans-Farnesol	$C_{15}H_{26}O$	222	0.29
18.	30.01	Synaptogenin b	$C_{30}H_{46}O_4$	470	1.13

to be present in bixagenotype KLBi -3.Ten compounds were found to be in common for both the genotypes.

The main compounds found in *Bixaorellana* are carotenoids and apocarotenoids. Most of the carotenoids have been isolated from seed and seed coats. Bixin [methylhydrogen-(90 Z)-6,60 -diapocarotene-6,60 -dioate] is the major carotenoid compound present in *B. orellana* seed coat and accounts for 80% in addition to the presence of other carotenoids in trace amounts (Lauro, 1991 and Preston and Richard, 1980). Trans-geranylgeraniol have been reported for anticancer activity on B16F-10 melanoma cell line(Ohizumi *et al.*,1995).The

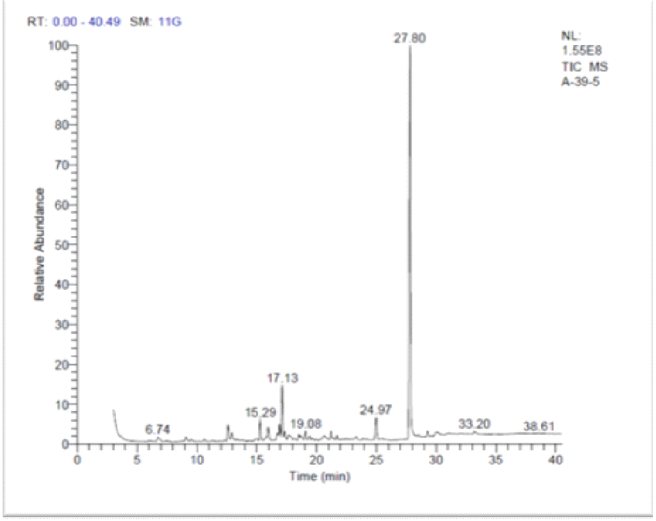

Fig. 1 : GC-MS chromatograph of seed extract of Bixagenotype (TN Bi – 13)

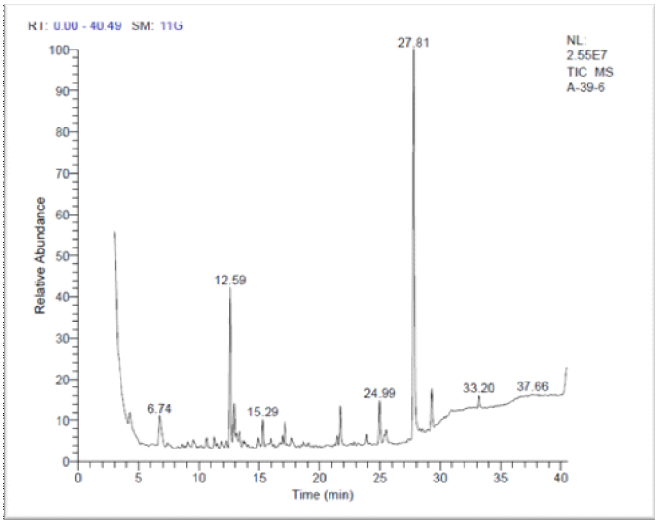

Fig. 2 : GC-MS chromatograph of seed extract of Bixagenotype (KL Bi – 3)

Table 2 : Chemical compounds in seed extract of Bixagenotype (KL Bi – 3)

Sr. No.	Retention time	Compound name	Molecular formula	Molecular weight	Area %
1.	6.74	Dodecane	$C_{13}H_{28}$	184	1.15
2.	7.44	Methylhydrogen-(9'Z)-6,6',-dioate	$C_{25}H_{30}O_4$	394	3.52
3.	9.51	α-Terpinene	$C_{10}H_{16}$	136	1.06
4.	9.51	α -Humulene	$C_{15}H_{24}$	204	1.06
5.	10.62	α -Elemene	$C_{15}H_{24}$	204	0.95
6.	11.30	α -ylangene	$C_{15}H_{24}$	204	1.61
7.	11.85	α -Guaiene	$C_{15}H_{24}$	204	0.76
8.	11.85	α -Bulnesene	$C_{15}H_{24}$	204	0.76
9.	12.28	α –Copaene	$C_{15}H_{24}$	204	0.62
10.	12.59	Aromadendrene	$C_{15}H_{24}$	204	13.62
11.	12.59	Longifolene-(V4)	$C_{15}H_{24}$	204	13.62
12.	12.59	α -selinene	$C_{15}H_{24}$	204	13.62
13.	12.91	Germacrene D	$C_{15}H_{24}$	204	5.56
14.	12.91	α -Cubebene	$C_{15}H_{24}$	204	5.56
15.	13.38	α -Panasinsene	$C_{15}H_{24}$	204	1.55
16.	13.38	trans-Caryophyllene	$C_{15}H_{24}$	204	1.55
17.	13.71	α -cadinene	$C_{15}H_{24}$	204	0.85
18.	15.29	Pulegone	$C_{10}H_{16}O$	152	2.15
19.	17.14	(+) spathulenol	$C_{15}H_{24}O$	220	3.04
20.	17.69	Hexadecane, 1-(ethenyloxy)-	$C_{18}H_{36}O$	268	1.16
21.	21.44	trans- α -Farnesene	$C_{15}H_{24}$	204	0.93
22.	27.81	trans-Geranylgeraniol	$C_{20}H_{34}O$	290	37.49

spathulenol is a colorless, viscous oil coupled with immunosuppressive effect (Martins *et al.*, 2010). Several other sesquiterpenes found usually in water-soluble as well as in oil-soluble extracts include α-copaene (Galindo-Cuspinera *et al.*, 2002). α -copaene has been described as having a fresh, earthy, mango-like smell. During sesquiterpene biosynthesis several isomers are formed that can readily undergo isomerization depending on environmental conditions. Minor sesquiterpenes found in *B. orellana*include cubebene, β- and δ-cadinene. α-Cubebene has been described as having a fruity, sweet, citrus-like smell (Gertsch *et al.*, 2008). D- germacrene, mainly found in dried seeds (1.93%), is known for its antimicrobial activity (Wang *et al.*, 2006). α-humulene hasbeen reported to be a major aromatic component of hops anda major contributor to the flavor of beer (Rybacek, 1991) which is also present in bixa. It is usually accompanied byits isomer caryophyllene a compound which was also foundin annatto extracts but in smaller amounts (Arctander, 1969). Murugesan *et al.* (2016) also reported the presence of humulene in the *L. camara* essential oil which was found to be responsible for the non-preference mechanism of defoliators in the nursery and young plantations of teak and ailanthus as well as through non-preference for oviposition. α-Ylangene and α-copaene are examples of isomers which are present in bixa genotype KLBi-3 (Moss and Weedon,1976 and Clayton, 1971). Trans-caryophyllene compound is known to be an important component of many essential oils which imparts antibacterial activities (Vania *et al.*, 2016). Murugesan *et al*. (2016) reported the presence β-Selinene which is known to possess anti bacterial properties in Lantana essential oil. β-Selinene which possess antibacterial properties is also present in Bixa dye. Presence of ylangene could be due to either rearrangement of copaene during the extraction procedure or as a by product of the biosynthesis of the latter.

Conclusion :

The result of the present study is a source of secondary metabolites which suggest that there are chances of variation among the secondary metabolites not only at a quantitative level but also at a qualitative level. Hence profiling of each and every screened genotype is required in order to attain the best selection.

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