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Original Article

Eugenol Found by GC-MS Analysis of The Methanolic Extract of The Fruit Pulp of Indigenous Mango (*Mangifera indica* L.) Variety, Champa from Murshidabad District of West Bengal, India

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Abstract

Indigenous mango (*Mangifera indica* L.) varieties have unique aroma, taste, flavour, texture and size. The interactions among volatile compounds and with sugars and acids have a role in the distinct mango flavor. Murshidabad district of West Bengal has several indigenous mango varieties that have unique attributes like taste and aroma. The indigenous Champa mango variety of Murshidabad district has a distinct aroma very similar to that of the champaka flower (*Michelia champaca* L.). The present investigation was to determine if there was any phyto-chemical which could be responsible for the distinct and unique aroma present in the mango variety Champa. Eugenol, a phytogenic bioactive component frequently found in diversified herbal plants was found by GC-MS analysis of the methanolic extract of the fruit pulp of indigenous mango variety, Champa.

Keywords: *Eugenol; Aroma; Champa; Indigenous mango variety; Murshidabad.*

Introduction

Mango genetic diversity in India is very high (Karihaloo *et al.* 2003). Ravishankar *et al.* (2000) have reported that *Mangifera indica* L. has extensive diversity due to alloploidy, outbreeding, continuous grafting and phenotypic differences arising from varied agro climatic conditions in different mango growing regions. Different varieties have unique aroma, taste, flavour, texture and size. From the research work of Malundo and co workers it is believed that from the 150 volatile compounds isolated from mango, it is probable that only a few are critical to characteristic mango flavor (Malundo *et al,* 1996). The interactions among volatile compounds and with sugars and acids have a role in the distinct mango flavor. Sugars and acids enhance human perception of specific flavor notes in mango, including aromatics (Malundo *et al*, 2001). Aroma components in fruits also consist of aldehydes, alcohols, esters, lactones, ketones, quinones and terpenes (Costa *et al,* 2013). Both physical and chemical characteristics of mango viz. colour, texture, flavor, sweetness are some of the qualities which determine consumer acceptability of the different mango varieties (Mamiro *et al,* 2007). Aroma volatiles of mango (*Mangifera indica* L.) determine consumer acceptability and influence selection of parents for mango breeding programs (Pandit *et al*, 2009).

Mango (*Mangifera indica* L.) of the family Anacardiaceae is an economically important tropical fruit globally. Mango is often referred to as the 'king of fruits.' Mango is the national fruit of India, Pakistan and Philippines. It is the national tree of Bangladesh. Mango has high production, marketing and consumption rate (Musharraf *et al* , 2016). Mango is cultivated in about 89 countries of the world (Yadav and Singh, 2017).

Indigenous mango varieties of West Bengal

Mango has rich intra-specific diversity . In India there are about one thousand indigenous or traditional varieties of *Mangifera indica* L. (Mukherjee, 1950). Malda and adjoining district Murshidabad in West Bengal form one of the largest mango yielding regions of the country producing over 5 lakh tonnes. Mango is the prime economic fruit in these two districts. But after independence this mango germplasm came under threat due to rapid industrialization (Mukherjee, 1953). More than 200 varieties of mango were known from Malda and Murshidabad districts only as mentioned but extensive survey in 2011-2014 showed that only 53 varieties are now available (Pal *et al,* 2017).

However even after massive genetic erosion there still exists a rich collection of indigenous mango varieties in Murshidabad district (Dutta *et al,* 2020). There are many indigenous varieties are rarely found outside Murshidabad districts viz. Anaras, Begum Pasand, Champa, Kohitoor, Kahinoor, Mulayam Jaam, Ranipasand, Saranga. Generally, old traditional mango cultivars are maintained either in home or village gardens, personal orchards, within the precincts of temples and on public sites such as schools. This system continues due to the mango's religious and cultural importance. Custodian farmers have been maintaining, promoting and adapting a number of indigenous mango varieties on their farms. These custodian farmers protect, nurture and propagate the rich mango legacy of India (De, 2015).

Most of the fruit crops growing in India had special characters which are distinct from the fruits growing in other countries. These special characters facilitates the registration of fruit crops under GI but the number of registered crops under GI was very low in number than the crops actually eligible for registration. So, there is an urgent need to aware about the community/farmers about the importance of GI

Volatile Organic Compounds (VOC) in mango

Jorge A. Pino and co workers at the Food Industry Research Institute, Havana have worked extensively on the Volatile Organic Compounds (VOCs) in various fruit like papaya (Pino *et al*, 2003), bullock's heart fruit (Pino *et al*, 2003), guava (Pino *et al*, 2002) and mango ((Pino *et al*, 1989). As more research work was undertaken to study the aromatic profiles of mango cultivars it was found that this group of compounds that determine the characteristic aroma of the fruit is commonly present in small quantities, approximately 50 ppm or less. They are usually comprised of mixtures of monoterpenes, sesquiterpenes, and volatile oxygenates (monoterpenes, sesquiterpenes, esters, lactones, alcohols, aldehydes, ketones, volatile fatty acids, some degradation product of phenols, and some carotenoids) (Bender *et al.,* 2000; Pino *et al.,* 2005; Lebrun *et al.,* 2008; Pandit et al., 2010; Li et al., 2017).

Several research groups have worked on the volatile components in different mango cultivars in several countries viz. Australia (Bartley, 1988), Brazil (Andrade et al, 2000), Cuba (Pino *et al,* 1989). In 2005 Pino's research group carried out studies to determine the Volatile organic compounds (VOCs) profile of different mango cultivars. The research work led to the identification of more than 300 compounds including esters, aldehydes, ketones, aliphatic alcohols, γ and δ-lactones, and mono- and sesquiterpenes (Pino *et al,* 2005). Researchers are of the opinion that the volatile components of mango vary between different mango varieties and consequently the aroma profile also differs (MacLeod *et al,* 1984).

Aroma chemistry

Aroma chemistry is complex. The fragrance/aroma/smell of any flower is never really the consequence of a single chemical compound. Flowers give off a complex mix of volatile organic chemicals, and

whilst not all of these contribute to the aroma, a significant number impact the fragrance to varying degrees. So often we can't point to single chemical compounds as being the cause of flowers' scents, we can identify those compounds that have a major impact on the aroma that our noses detect. In 2007 Jorge A. Pino's research group found aroma volatile constituents of Colombian varieties of mango (Quijano *et al,* 2007).

Unique scented indigenous Mango varieties of Murshidabad district

The indigenous mango variety Anaras has a distinct aroma of pineapple. If a person is blindfolded and asked to taste the fruit there is a high probability that the mango variety Anaras could be mistaken for a pineapple. In face the literal Bengali translation of 'Anaras' is pineapple. The famous but very rare mango variety Kohitur is said to have a unique taste and aroma. The Champa mango variety has a distinct aroma very similar to that of the champaka flower (*Michelia champaca* L.). The word Champa is the Bengali word for the scented flower champaka. *Michelia champaca* L. belongs to the family Magnoliaceae

Objective of the study

The objective of this study was to determine if there was any volatile substance which could be responsible for the distinct and unique aroma present in the mango variety Champa. Also we wanted to see if there was any bio-active compound which was similar to any compound reported in *Michelia champaca* L. using the with Gas Chromatography-Mass Spectrometry analysis method.

Materials and Methods

Collection of samples

The fruit of indigenous mango Champa variety was collected from the orchard of a 'custodian farmer' in Murshidabad.

Fig. 1. Indigenous Mango variety Champa, collected from Ajimgange, Murshidabad

Brief description of the fruit of indigenous mango variety, Champa from Murshidabad district, West Bengal:

Mature fruit is short in length (9 cm), with a narrow width, ratio of length/width is small, shape of cross section is circular, color of the skin is green and yellow, and density of lenticels is dense. The color contrast between lenticels and skin is strong, with large size of the lenticels. The corkiness is due to the presence of the lenticels is present, shallow cavity at stalk is present. Shallow neck is present. Shape of ventral shoulder is rounded outwards, shape of dorsal shoulder is rounded downwards. Groove in the ventral shoulder is absent, and bulging on the ventral shoulder is also absent. Shallow sinus is present; depth of sinus is low. Bulging at the proximal stylar scar is absent, point at the stylar scar is medium. Diameter of the stalk attachment is small. Predominant color of skin of the ripe fruit is yellow green, speckling of skin is weak, thickness of skin is thin, adherence of the flesh to the skin is medium. Main color of the flesh of the ripe fruit is medium yellow. Firmness of the flesh of the ripe fruit is soft, with medium juiciness, and medium texture of flesh (Fig. 1, 2 & 3).

Fig. 2. Fruit of the indigenous mango variety, Champa from Murshidabad district of West Bengal

Fig. 3. Fruit of the indigenous mango variety, Champa (close up view).

Fruit pulp collection

Fruit pulp from the mango variety Champa was collected from Murshidabad district. The mango fruit pulp was stored in the refrigerator for further use. Methanolic extract were prepared for the analysis.

GC-MS

The experiment was carried out in the Central Instrumentation Facility, Bose Institute, Kolkata, India. For GC-MS Thermofisher GC was used. The column (HP5) was fused silica 50 m × 0.25 mm I.D. Analysis conditions were 20 minutes at 100°C, 3 minutes at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1 µl) was evaporated in a split less injector at 300°C. Run time was 22 minutes. The components were identified by GC coupled with MS.

Identification of components

Interpretation of the mass spectrum, GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results

The identification of phytochemical compounds was based on the peak area, retention time and molecular formula. The compound was identified as eugenol (Table 1). Eugenol, a phytogenic bioactive component is frequently found in diversified herbal plants possessing well-defined functional attributes (Fig.4 & 5). Eugenol is mainly found in clove, cinnamon, nutmeg, tulsi and pepper. Eugenol is the major volatile constituent of clove bud essential oil. Eugenol is declared as GRAS (generally recognized as safe) by World Health Organization (WHO) and is considered as non-mutagenic (Khalil *et al*, 2017). Methyl eugenol is directly derived from eugenol, a product from phenylalanine (an essential amino acid) through caffeic acid and ferulic acid via the shikimate pathway (Herrmann and Weaver, 2009). It is a common phenylpropanoid found in many plant species, particularly in spices and medicinal plants (Tan and Nishida, 2012). Methyl eugenol has been reported to be present in *Michelia champaca* L (Sanimah *et al,* 2008) and is postulated to be a compound impacting the sweet fragrance of *Michelia champaca* L.

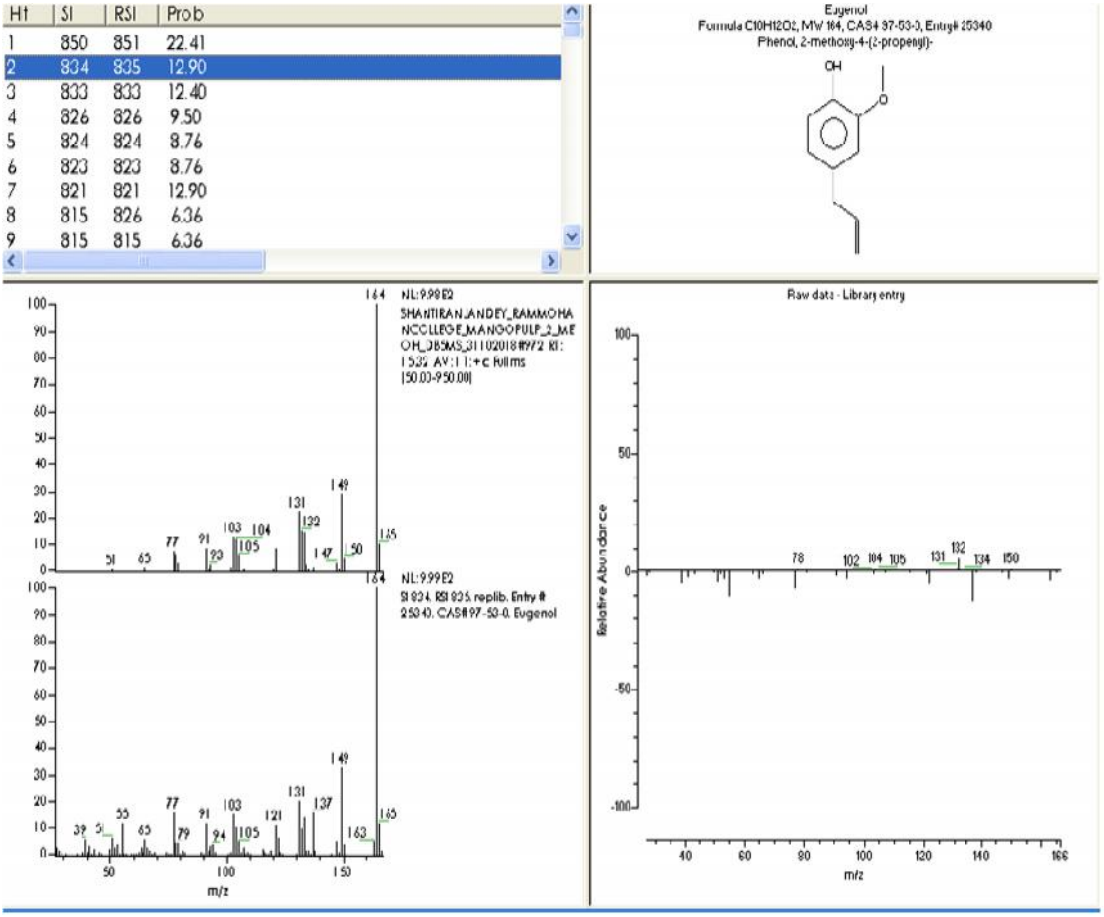

Figure 4. GC MS spectra and NIST result for identification of compound, eugenol.

Table 1. The compound identified as eugenol. Following details were observed from https://pubchem.ncbi.nlm.nih.gov/compound:

Compound	IUPAC Name	Molecular Formula	Molecular Weight	Synonyms
Eugenol	2-methoxy-4-prop-2-enylphenol	$C_{10}H_{12}O_2$	164.2 g/mol	4-Allyl-2-methoxyphenol, Allylguaiacol, 1,4-Eugenol; 1-Hydroxy-2-methoxy-4-propenylbenzene, 2-Methoxy-4-allylphenol, Eugenolum, 5-Allylguaiacol, Eugenic acid

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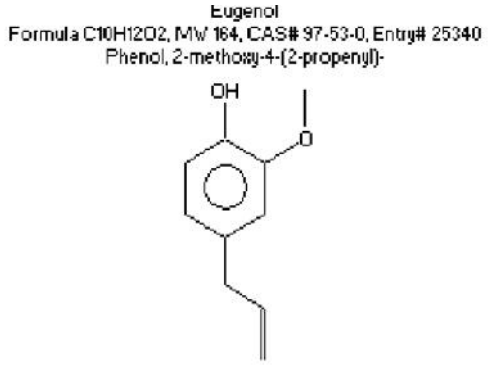

Fig. 5. Structure of Eugenol.

Discussion

Aroma is a major factor that influences the quality and consumer acceptance of mango products (Liu *et al,* 2020). Detailed study of the aromatic components could improve the understanding and also facilitate the control of critical quality parameters that could influence mango processing. Eugenol is a natural compound that has several pharmacological activities, action on the redox status, and applications in the food and pharmaceutical industry. Eugenol (4-allyl-2-methoxyphenol) is a phenolic compound from the class of phenylpropanoids and the main component of clove. Often eugenol has been used in the food industry as a preservative, mainly due to its antioxidant property (Zhang, *et al.* 2009). Methyleugenol is used as a flavouring agent in jellies, baked goods, non-alcoholic beverages. It has been used as a fragrance ingredient in perfumes, toiletries and detergent. It is possible the Eugenol or its derivative in the indigenous mango variety Champa is responsible for the unique flavor.

Genetic diversity analysis of mango germplasm is not uniform throughout all the states of India viz. Bajpai *et al,* 2016; Dash and Hota, 1997. However in India, the mango gene pool has not been explored fully. Each of the traditional varieties of mango has a unique taste and flavor (Mehta, 2017). In India many mango varieties grow under specific climate. This specific type of climate is responsible for acquiring some unique characteristics for that particular indigenous mango variety. So many indigenous mango varieties which have unique taste, texture or aroma and they are found restricted to certain areas. Amongst these varieties most are of seedling origin and are found to be growing as heirloom varieties from generation to generation (Dinesh *et al,* 2015).

Some of these unique mango cultivars are popular and also registered under Geographical Indication of Goods (Registration and protection) Act 1999. Names of the indigenous mango varieties registered under geographical indication (GI) include Appemidi Mango of Western Ghats, Karnataka, Laxman Bhog Mango of Malda district, West Bengal, Khirsapati (Himsagar) Mango of Malda district, West Bengal and Fazli Mango grown Malda in West Bengal, Mango Malihabadi Dusseheri of Lucknow district, Uttar Pradesh, and Gir Kesar Mango of Gujarat state (Rani & Kishore, 2013).

Apart from the famous indigenous mango varieties which have already got a GI tag there are several unique traditional mango varieties in several pockets. Their uniqueness could be attributed to certain phyto-chemicals. But in recent years farmers are abandoning the indigenous mango varieties in favour of more lucrative commercial mango cultivars. Farmers are keen to maintain commercial mango cultivars for fresh consumption and income generation. Also the original habitats of local mango have been rapidly changing in response to biotic, economic and other pressures in recent years (Subedi *et al.* 2004).

So on one hand there is an urgent need to identify unique mango varieties and novel phyto-chemicals which account for the unique aroma and other characteristics. Detailed and well documented information about the available genetic material together with a broad, well maintained varietal diversity are essential for future mango breeding efforts. This should also include local varieties (Subedi *et al.,* 2005), which may have a low market, but high breeding value. Identifying indigenous mango varieties with unique features and marketing them could lead to higher profit among the mango orchard owners. This could be a deciding factor for conserving a indigenous mango variety and its legacy for posterity. Brand development of mango is needed in such a way that it would lead to integrated growth of that crop (Banerjee, 2011). These indigenous varieties will survive

alongside modern varieties if they are characterised by distinctive traits that make them relevant in the farming system or demanded in the market (Berg, 2009).

Conclusion

In West Bengal, the mango gene pool has not been explored fully. The scientific information relating to aromatic constituents as well as sensory characteristics of indigenous mango cultivars of West Bengal is limited. There are several unique varieties of mango, which need to be identified and if possible given a geographical identity. Further detailed investigations are needed to understand the cause of their uniqueness. Efforts are also needed to locate the places linked with a particular variety of mango and thereafter attempt for registration of geographical identity of such local varieties. The genetic diversity embedded within the indigenous mango varieties are a unique genetic resource. Plant genetic resources are among the most vulnerable of all non-renewable natural resources. Once lost, they are lost forever. It needs to be cherished and preserved otherwise there is a great risk of losing the genetic resource.

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Conflicts of Interest:

The authors declare that there are no conflicts of interest regarding the publication of this work.

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