## PAPER

# COMPARISON OF BIOACTIVE COMPOUNDS AND SENSORY EVALUATION ON EDIBLE FLOWERS TEA INFUSION

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#### ABSTRACT

France Rose Buds, Jasmine Flower, and Osmanthus Flower are three edible flowers commonly available in Malaysia market. Composition of these 3 edible flowers is not widely studied. Hence, the caffeine, total phenolic content (TPC), volatile compounds, and overall acceptability of tea infusion from France Rose Buds, Jasmine Flower, and Osmanthus Flower were compared. Tea infusion from the edible flowers was prepared by boiling it with distilled water. None solvent extraction was carried out to determine the bioactive compounds. Tea infusion of Osmanthus Flower contains the highest caffeine ( $4.96\pm1.94 \ \mu g/ml$ ), total phenolic content ( $4.33\pm0.03 \ mg GAE/g$ ) and overall acceptability ( $6.16\pm2.05$ ) compared to France Rose Buds and Jasmine Flower. The Jasmine Flower was found to have the highest number of volatile compounds (13) compared to France Rose Buds and Osmanthus Flower. This study indicates that the edible flowers have the potential for application as food ingredient.

*Keywords*: France Rose Buds, Jasmine Flower, Osmanthus Flower, caffeine, volatile compounds, sensory evaluation

#### 1. INTRODUCTION

Edible flowers commonly seen include cauliflower, broccoli, and artichokes (CARTER *et al.*, 2007). The edible part of cauliflower and broccoli is made of fleshy flower stalks and clusters of flower buds (CARTER *et al.*, 2007). Edible flower petals and flower buds can be eaten raw in salads. Refreshing tea infusion can also be made from the flower petals (Lim, 2014). In terms of nutritional value of edible flowers; petal of the flowers is a source of vitamins, minerals, and antioxidants thus contribute to an increase interests for consumption (MLCEK and ROP, 2011). Other than as a decoration and culinary purposes, the nutritive and chemoprotective properties of certain edible flowers are well-documented or under study. Edible flowers can also be classified as nutraceutical food (MLCEK and ROP, 2011). Besides the well-recognized wholesome effects of green and black teas prepared from young leaves of *Camellia sinensis*, hot water infusions (teas) of many other plants also may have health benefits.

Flavonoids and phenolic acids have been used as antioxidants to prevent oxidative damage and control of diseases caused by oxidative stress (NA *et al.*, 2014). Flower, seeds, and root of Osmanthus flower (*Osmanthus fragrans*) is also used as a acesodyne and as a folk medicine for the treatment of liver, stomachache and for other therapeutic purposes such as aerodontalgia, halistosis, rheumatism and physical pain (PENG and JI, 2004). The main bioactive components in the extracts of *Osmanthus fragrans* flowers are flavonoids and phenolic acids (XIONG *et al.*, 2014), carotenoids, carotenoid-derivatives and volatile constituents (LEFFINGWELL, 2002).

Jasmine Flower (*Trachelospermum jasminoides*) is used in the perfume industry, and the scent has been included in at least 55 commercially sold perfumes (Basenotes Fragrance Search, 2014). The inner bark yields a strong fiber that is utilized for making rope, sacks and paper and the stem is used for treating rheumatism and injury in traditional Chinese medicine (III CHAN NOH, 2011; SHEU *et al.*, 2009). Most publications on Jasmine Flower are rather concerned about the content of the plant (JING *et al.*, 2012) than about the flowers (JOULAIN, 1987).

France Rose Buds (*Rosa* sp. var. Rosa de Castillo) are known as edible flowers and have been used for centuries as food components, either in the fresh form or in processed products, such as confectionary and beverages (GIRARD-LAGORCE *et al.*, 2001). The combination of health benefits with recognized applicability in cuisine raises the possibility of using rose flowers in functional food products. Although the health benefits of some well-known edible flowers are commonly studied, the caffeine content, total phenolic content and volatiles compounds in tea infusion from France Rose Buds, Jasmine Flower, and Osmanthus Flower (*Osmanthus fragrans*) still remain unknown.

Research on sensory evaluation had been conducted by CHEN *et al.* (2010) on the attributes of taste, flavor, and overall acceptance on tea infusion from Pu-erh teas. ZHU *et al.* (2016) also conducted evaluation of volatile compounds in infusion of oolong tea (fully fermented *Camellia sinensis L.*). Instead of using hedonic scale to rate upon answering the questionnaire, 5 aroma terms were used to define the aroma by well-trained panel of ten members: 2-methylpyrazine for "roast" note, maltol for "sweet" note, hexanal for "green and grassy" note, dipropyl disulfide for "sulphur" note, phenylethyl alcohol for "floral" note. For the sensory analysis by BENVENUTI *et al.* (2016), 5 different organoleptic characteristics of which spiciness, sweetness, softness, scent, and bitterness were included in the evaluation scheme and were expressed in a scale of 1 to 100. In addition, sensory profile of tea infusion of France Rose buds, Jasmine Flower, and Osmanthus Flower corresponding to the volatile compositions is still insufficient. Therefore, this study was presented to compare the caffeine, total phenolic, volatile compounds, and overall

acceptability of the 3 types of edible flowers by untrained panelists. The flowers may be further exploit as a source of natural antioxidant for food and nutraceutical applications.

# 2. MATERIAL AND METHODS

#### 2.1. Sample preparation

France Rose Buds, Jasmine Flower, and Osmanthus Flower were purchased from Yin Onn Shd. Bhd. (Mid Valley City) at Lower Ground Floor 035 & 036, Mid Valley City Megamall, Mid Valley City, Lingkaran Syed Putra,Kuala Lumpur, Malaysia. Extraction was prepared using boiled distilled water by 1: 8.82 ratio (ratio of dry mass sample to boil distilled water). The ratio used was modified from BISPO *et al.* (2002).

## 2.2. Chemicals

Methanols and acetic acid from Fisher Scientific, UK and caffeine standard from Sigma-Aldrich, China were used for High Performance Liquid Chromatography (HPLC) analysis. Folin-Ciocalteu and gallic acid from Merck, Germany and sodium carbonate from Fisher Scientific, UK were also used for Total Phenolic Content analysis.

#### 2.3. Determination of caffeine content using HPLC

HPLC (Shimadzu Corporation, Japan) was carried out to determine and compare the caffeine content of France Rose Buds, Jasmine Flower, and Osmanthus Flower. The experimental procedure was adopted from BISPO *et al.* (2002) with some modifications. The modifications were on the amount of France Rose Buds, Jasmine Flower, and Osmanthus Flower applied in this study. About 17 g of dried edible flowers were used, instead of 4 g as referred to the method BISPO *et al.* (2002). The extraction time was also changed from 3 min to 10 min. Aqueous extract was obtained using 150 ml boiled distilled water with 17 g of dried edible flower for 10 min with continuous stirring. The extracts were filtered using syringe filter (Chemolab Supplies, Malaysia) of 0.45  $\mu$ m twice (double filter) prior injected into HPLC.

#### 2.4. Total Phenolic Content (TPC) analysis

TPC of France Rose Buds, Jasmine Flower, and Osmanthus Flower were determined and compared using Folin-Ciocalteu method. The assay was conducted as described by BHEBHE *et al.* (2015). Total phenolic content was expressed as gallic acid equivalents (GAE). The results were obtained from representative samples prepared from each concentration. Calibration and linear curves were determined.

# 2.5. Analysis of volatile compounds using automated Gas Chromatography-Mass Spectrometry (GC-MS)

The volatile flavor constituents of each sample were analyzed using Trace GC Ultra Gas Chromatography system coupled with TSQ Quantum XLS Mass Spectrometer System (Serial No: TQU03227) from Thermo Fisher Scientific (USA) and capillary column of 30 m × 0.25 mm, 0.25  $\mu$ m film thickness Model TG-5MS (Thermo Scientific, USA). The detail of experimental procedure was adopted from LI *et al.* (2013).

#### 2.6. Sensory evaluation

Sensory analysis of France Rose Buds, Jasmine Flower, and Osmanthus Flower were conducted at room temperature  $(25\pm2^{\circ}C)$  by 50 untrained panelists who need to complete the questionnaire containing 9-point hedonic scales. The evaluation covered the attributes of taste, color, aroma, and overall acceptability where scale 9 represented like extremely and 1 dislike extremely (HAJMOHAMMADI *et al.*, 2016; LIM, 2011; ZHENG *et al.*, 2014).

#### 2.7. Statistical analysis

The experimental design was completely randomized. Statistical analysis and comparisons among means were carried out using the statistical package Minitab 17. The data collected was analyzed by one-way analysis of variance. The Tukey's post hoc test was applied for comparison of means, and differences are considered significant at the level of p<0.05. Linear regression for correlation analysis was performed using Microsoft Office Excel (2007).

#### 3. RESULTS AND DISCUSSION

#### 3.1. Caffeine content

Caffeine content of France Rose Buds, Jasmine Flower, and Osmanthus Flower were determined as shown in Table 1 with 17 g of each samples were brewed using boiled distilled water. From the data collected, it shows that Osmanthus Flower contain significantly (p<0.05) the highest amount of caffeine ( $4.96\pm1.94 \ \mu g/ml$ ) than France Rose Buds and Jasmine Flower, at  $0.17\pm0.00 \ \mu g/ml$  and  $0.67\pm0.03 \ \mu g/ml$  respectively.

EL-SHAHAWI *et al.* (2012) reported that caffeine in tea infusion from tea (*Camellia sinensis* L.) is well-known as natural powerful antioxidant and help in the prevention of cardiovascular diseases and cancers. EL-SHAHAWI *et al.* (2012) also revealed that every consumption of 100 ml of brewed tea infusion (*Camellia sinensis* L.), about 1.04 to 212 mg of total catechins and 0.194 to 5.04 mg of caffeine were found in the tea infusion from 29 commercial green tea samples (*Camellia* sinensis L.) in Saudi Arabia. Coffee, tea, and fruits were the most important food sources of total polyphenols. A total of 437 different individual polyphenols were consumed, including 94 of them consumed at a level 1 mg/day (ZAMORA-ROS *et al.*, 2015). To date, no specific dietary intake of caffeine in any food product including edible flowers tea infusion related to antioxidant activity has been reported.

EL-SHAHAWI *et al.* (2012) reported that twenty-nine green tea samples (*Camellia sinensis L.*) of different origins (China, Japan, Indonesia, Sri Lanka and Taiwan) from Saudi Arabian local market have caffeine content ranged from 0.09 to 2.23 mg/g. D'ARCHIVIO *et al.* (2016) also reported that average of  $2.5\pm0.2 \text{ mg/g}$  of caffeine was detected in tea infusion of oolong tea (semi-fermented *Camellia sinensis L.*). Caffeine content in tea infusion of France Rose Buds, Jasmine Flower, and Osmanthus Flower were relatively much lower than both of the green tea and oolong tea infusion as discussed above.

Type of flower	Caffeine (µg/ml)		
France Rose Buds	0.17±0.00 <sup>b</sup>		
Jasmine Flower	0.67±0.03 <sup>ab</sup>		
Osmanthus Flower	4.96±1.94 <sup>a</sup>		

**Table 1.** Caffeine Content of France Rose Buds, Jasmine Flower, and Osmanthus Flower using HPLC.

Values expressed as mean $\pm$ standard deviation (n=2). Means with different letters within the same column are significantly different at the level of p<0.05.

#### 3.2. Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined by Folin-Ciocalteu assay and the result was shown in Table 2.The result was expressed as gallic acid equivalent (GAE). Table 2 shows that Osmanthus Flower had significantly the highest TPC value ( $4.33\pm0.03$  mg GAE/g) among the three edible flowers in this study (p<0.05). In addition, the highest caffeine content was also detected in Osmanthus Flower at  $4.96\pm1.94 \ \mu g/ml$  (Table 1) and this could also be related to the highest total phenolic content of Osmanthus Flower. Similarly, NA *et al.* (2014) reported that the total phenolic contents of *Osmanthus fragrans* was higher  $16.00\pm0.57$ mg GAE/g than the other 50 edible types in a range of  $0.63\pm0.03$  to  $35.84\pm1.67$  mg GAE/g wet weight.

The TPC value of tea infusion of Osmanthus Flower in this study was lower than Na *et al.* (2014). The different results obtained may be due to the different parts of plant used, where leaves had higher content of TPC than fruit, stem, or brunches. Species type, age, maturity and or environmental stress may also contribute to differences in phenolic content of a plant (Watson, 2014). Generally, amount of polyphenols in plants is affected by genetics and environment. External environment also triggers the presence and content of polyphenols for the protection of the plant (WATSON, 2014). The most efficient solvent for polyphenols extraction was 50% DMF for black tea (fully fermented *Camellia sinensis L.*) and 50% acetone for mate tea. Higher phenolic content often linked with higher antioxidant activity where it is health benefiting in terms of medicinal properties such as antibiotic, anti-inflammation, anti-cancer and anti-allergic (BHEBHE *et al.*, 2015).

Type of flower	Total Phenolic Content (mg GAE/g)
France Rose Buds	3.75±0.03 <sup>b</sup>
Jasmine Flower	4.27±0.02 <sup>a</sup>
Osmanthus Flower	4 33+0 03 <sup>a</sup>

Table 2. Total phenolic content of France Rose Buds, Jasmine Flower, and Osmanthus Flower.

Results expressed in Gallic Acid Equivalent. Values are expressed as mean $\pm$ standard deviation (n=5). Means with different letters within the same column are significantly different at the level of p<0.05

#### 3.3. Volatile compounds

Volatile compounds of France Rose Buds, Jasmine Flower, and Osmanthus Flower were determined by automated headspace GCMS with the total retention time of 18.30 min. The

chromatography of volatile compounds detected in tea infusion of edible flowers and their relative content were summarized in Table 3. About 5 volatile compounds were detected in France Rose Buds by automated headspace GCMS. Phenylethyl alcohol was the major volatile compound identified from tea infusion of France Rose Buds at 57.12%, and this agrees with DUDAREVA and PICHERSKY (2006) that phenylethyl alcohol can be detected in France Rose Buds. Phenylethyl alcohol, also known as benzyl carbinol, 2-phenylethanol, and  $\beta$ -phenylethyl alcohol, is a colorless and viscous liquid, rose-like odor, initially a slightly bitter taste then sweet and reminiscent of peach (FENAROLI *et al.*, 2000). Phenylethyl alcohol has been used as an antimicrobial, antiseptic, disinfectant, fragrance in perfumes and preservatives (BURDOCK, 1997).

Compound 1-Iodo-2-methylundecane (15.75%), tridecane (3.22%), cis-2-Methyl-7octadecene (3.18%), and 1-Iodo-2-methylnonane (2.67%), classified as hydrocarbon group were detected in tea infusion of France Rose Buds. Antonelli et al. (1997) showed similar findings where the main component in 24 different rose varieties was phenylethanol, but some roses showed unusually high levels of benzyl alcohol. Antonelli et al. (1997) also 4-ethenylphenol, detected 4-Vinylphenol, also known as p-Vinylphenol, p-Hydroxystyrene, and 4-VP, in two samples of Rosa gallica out of 24 different rose varieties, but those volatile compounds was not found as analyzed in this study. Tridecane detected in this study was similar to Lin et al. (2013) where tridecane was extracted by HS-SPME in a total of 75 oolong tea (fully fermented *Camellia sinensis L*.) at the ranged of 0.30 to 2.50%.

Benzyl benzoate (6.81%) and 1,6-Octadien-3-ol,3,7-dimethyl- (3.81%), also known as linalool, were detected in both tea infusion of Jasmine Flower and Osmanthus Flower, similarly as Lim (2014). The (R)- (-)-linalool was found to be the key odorants of Jasmine tea flavor. Linalool was identified in a high proportion using different solid-phase micro extraction fibres. A total of 13 constituents were identified in the headspace of Jasmine Flower, with compound linalool (25.01%) was the highest in proportion, followed by benzyl acetate (23.71%) and 3-hexenyl acetate (13.80%) (LIM, 2014). Pregna-5, 14-diene-3, 20-diol-18-carboxylic acid, 3-acetate-, lactone were the major volatile compound detected in tea infusion of Jasmine Flower at 31.98%. However, this volatile compound has never been reported in the similar research as reported by LIM (2014). This might be due to the different part of the plants used in the experiments as different part consists of different volatile compounds. Volatile compositions vary according to genetics, soil, climate, and agricultural practices (TOCI and FARAH, 2008). Jasmine Flower can be further extracted and incorporated as new food ingredient due to its high volatile compounds.

#### 3.4. Sensory evaluation

Sample preparation for sensory evaluation was conducted under the same preparation method for all the above analysis where the tea infusion was prepared by brewing it with boiling water without addition of sugar or honey. The prepared sample was kept at room temperature  $25\pm2^{\circ}$ C. About 50 untrained panelists were required to complete the questionnaire to score the attribute of taste, aroma, color, and overall acceptability. The result was recorded in Table 4. Although Jasmine Flower and Osmanthus Flower are originated from the same order and family, France Rose Buds and Osmanthus Flowers show significant different (p> 0.05) in sensory attributes, in terms of taste, aroma, color, and overall acceptability.

No	Retention time (min)	Compound name	Molecular weight (g/mol)	France Rose Buds (%)	Jasmine Flower (%)	Osmanthus Flower (%)
•	4.48	Cyclohexene,methyl-5- (1-methylethenyl)-, (R)-		-	5.90	2.79
•	4.93	Ethyl2-(5-methyl-5- vinyltetrahydrofuran-2- vl)propan-2-vlcarbonate		-	-	13.38
	5.21	1,6-Octadien-3-ol,3,7- dimethyl- (Linalool)	154.25	-	3.81	13.04
•	5.49	Phenylethyl Alcohol	122.16	57.12	-	-
•	6.07	2H-Pyran-3-01,6- ethenyltetrahydro-2,2,6- trimethyl-		-	-	5.42
•	6.18	Heptanediamide,N,N'-di- benzoyloxy-		-	2.07	-
•	6.79	1,6-Octadien-3-ol,3,7- dimethyl-,2- aminobenzoate		-	1.68	8.55
	7.88	Megastigma-4,6(E),8(Z)- triene 1H-3a,7-		-	-	8.05
	8.44	Methanoazulene,2,3,4,7, 8,8a-hexahydro-3,6,8,8- tetramethyl-,		-	1.97	-
•	8.95	3-Buten-2-one,4-(2,6,6- trimethyl-1-cyclohexen- 1-yl)- 1 3 6 10-		-	-	3.53
•	9.04	Dodecatetraene,3,7,11- trimethyl-, (Z,E)-		-	2.21	-
•	9.68	3-Hexen-1-ol benzoate			1.92	-
•	10.62	Tridecane 1-I2-O-benzovl-3.5-O-	184.37	3.22	-	-
	11.19	dibenzyl-alpha-D- ribosyl]-5,6- dimethylbenzimide		-	1.07	-
•	11.44	Benzyl Benzoate	212.25	-	6.81	4.74
	11 77	Phenylmalonic acid		-	9 60	6.21
	12.06	monobenzyl ester cis-2-Methyl-7-		3 18	-	-
	10.02	octadecene 1-lodo-2-		15 75		
•	12.23	methylundecane		15.75	-	-
•	12.31	Ethanone,2,2- dimethoxy-1,2-diphenyl-		-	6.52	3.55
•	13.51	1-lodo-2-methylnonane		2.67	-	-
	13.56	z-l4-methylcyclohex-1- enyl)hexa-1,3,5- trienyl]cyclohex-1-en-1- carboxaldehyde		-	3.96	-
•	13.85	Cholest-1-eno[2,1- a]naphthalene, 3',4'- dihydro-		-	-	2.38
•	14.23	Pregna-5,14-diene-3,20- diol-18-carboxylic acid,3- acetate-, lactone		-	31.98	

**Table 3.** Volatile compounds and their relative contents detected in France Rose Buds, Jasmine Flower, and Osmanthus Flower using HS GCMS.

-, not found. Four constituents selected for analysis are indicated in bold type.

**Table 4.** Sensory evaluation on attributes including taste, aroma, color, overall acceptability of France Rose Buds, Jasmine Flower, and Osmanthus Flower.

Type of Flower	Taste	Aroma	Color	Overall acceptability
France Rose Buds	5.48±2.06 <sup>ª</sup>	6.24±1.84 <sup>a</sup>	6.56±1.47 <sup>a</sup>	5.90±1.62 <sup>a</sup>
Jasmine Flower	4.34±2.05 <sup>b</sup>	5.24±1.80 <sup>b</sup>	5.60±1.63 <sup>b</sup>	4.96±1.65 <sup>b</sup>
Osmanthus Flower	5.76±2.36 <sup>a</sup>	6.42±2.07 <sup>a</sup>	6.42±1.66 <sup>a</sup>	6.16±2.05 <sup>ª</sup>

Mean $\pm$ standard deviation (n = 50). Values that are followed by different letters within each column are significantly different (p< 0.05).

1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely.

Research on sensory evaluation had been conducted by CHEN *et al.* (2010) on the attributes of taste, flavor, and overall acceptance on tea infusion from Pu-erh teas. ZHU *et al.* (2016) also conducted evaluation of volatile compounds in infusion of oolong tea (fully fermented *Camellia sinensis* L.). Instead of using hedonic scale to rate upon answering the questionnaire, 5 aroma terms were used to define the aroma by well-trained panel of ten members: 2-methylpyrazine for "roast" note, maltol for "sweet" note, hexanal for "green and grassy" note, dipropyl disulfide for "sulphur" note, phenylethyl alcohol for "floral" note. For the sensory analysis by BENVENUTI *et al.* (2016), 5 different organoleptic characteristics of which spiciness, sweetness, softness, scent, and bitterness were included in the evaluation scheme and were expressed in a scale of 1 to 100.

Table 4 displays a trend where France Rose Buds and Osmanthus Flower were scored significantly (p<0.05) higher than Jasmine Flower in terms of taste, aroma, color, and overall acceptability. Osmanthus Flower had the most acceptable sensory attributes with the highest score in terms of taste, aroma, and overall acceptability. Despite Jasmine Flower being scored as the lowest in all sensory attributes, it contains the highest number of volatile compounds among the 3 edible flowers. It is believed that the edible flowers serve more than just as decoration and ornamental plants.

#### 4. CONCLUSIONS

This study compared the bioactive components contained in tea infusion of France Rose Buds, Jasmine Flower, and Osmanthus Flower. It was found that tea infusion of Osmanthus Flower has the highest caffeine ( $4.96\pm1.94 \ \mu g/ml$ ), total phenolic content ( $4.33\pm0.03 \ mg \ GAE/g$ ) and overall acceptability ( $6.16\pm2.05$ ) compared to tea infusion of France Rose Buds and Jasmine Flower. Meanwhile, Jasmine Flower contains the highest number of volatile compounds among the 3 edible flowers. In addition, edible flowers contain the natural bioactive components and may be used in food preparation to reduce or replace the use of synthetic bioactive components. Thus, Osmanthus Flower can be further explored and developed into functional foods, nutraceutical, and pharmaceuticals to prevent and treat diseases caused by oxidative stress.

#### ACKNOWLEDGEMENTS

The authors would like to express their gratitude to Universiti Putra Malaysia for their technical support.

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Paper Received November 3, 2017 Accepted October 30, 2018