



## Original Research Article

### Volatile composition of three Floral variety of *Plumeria rubra*

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## A B S T R A C T

The identification of different varieties of *P. rubra* is based, even today, the color of the corolla. Several studies were made by botanists in an attempt to discover the most characteristic morphological differences between varieties. However, the results were equivocal. The aim of this study was to investigate whether the floral volatile composition of three varieties of *Plumeria rubra* L more found in Brazil differ. The essential oils of flowers were obtained by extraction process "headspace" static cold finger, from material collected at the Federal University of Rio de Janeiro campus. Of the three varieties, oils from flowers of typica form was presented the highest number of hydrocarbons and sesquiterpenes (humuleno curcumene,  $\alpha$ - copaene, and  $\beta$ - selinene  $\alpha$ - cadineno). ( $\alpha$ -Copaene) benzenoídicos compounds (2-methoxy-4-propyl-phenyl ethyl benzoate, 2 - phenylethyl and hexenyl salicylate) and low concentrations in oil form tricolor (R & D) Woodson one sesquiterpene was identified and a monoterpene hydrocarbons. Finally, in oil form acutifolia (TIA) Woodson was identified in high concentration monoterpene linalool, and benzoic compounds (phenylacetaldehyde, phenylethanol and phenyl acetonitrile) and hydrocarbons. In the oil of flowers of this variety is no sesquiterpene found. The results showed profound differences between these three varieties aromas. Probably, these differences observed in the chromatograms and even sensory (during harvesting of flowers), are related to the type of mechanism used by these plants to attract insect pollinators (visual, olfactory and visual stimuli and olfactory stimuli).

## Keywords

Static headspace, essential oil, *Plumeria rubra*.

## Introduction

*Plumeria rubra* L. species is found throughout the tropical and subtropical

world, second in popularity only to Nerium oleander (Oleander popularly known as),

another representative of Apocynaceae (1-4) family. The identification of different varieties of *P. rubra* [*P. rubra typica* form, *Q. rubra L. lutea* form (R & P) Woodson, *P. rubra L. acutifolia* shape and *P. rubra* form tricolor (R & P) Woodson] is based, even today, the color of the corolla. Several studies were made by botanists in an attempt to discover the most characteristic morphological differences between varieties. However, the results were equivocal. The last attempt to separate them was based on the shape, size and appearance of the leaves. The difficulty of finding reliable and repeatable characteristics was due mainly deciduous foliage that made the extremely fragile herbarium specimens.

Moreover, as the corolla of the flowers used in the comparison and identification bleached during the drying processes, the chance of finding significant changes between the plants already described have become extremely difficult (3, 4). Soon, the impasse remains as to the identification of varieties of *P. rubra L.* According RE Woodson JR (3) the genus *Plumeria* has a single representative in Brazil: *Plumeria rubra L.*, since *Pl. loranthifolia* Muell. - Arg., *Plumeria acutifolia* Poir and other variants are considered first as the result of cultivation and natural hybridisation. Four varieties of *Plumeria* are accepted [*P. rubra L.*, typical form, *Q. rubra L.*, fashion lutea (R & P) Woodson, *P. rubra L.*, acutifolia shape and *P. rubra L.* tricolor form (R & P) Woodson] (3, 4). Among these three varieties are distinguished by the beauty and aroma, they are: *Q. rubra L.*, form tricolor (R & P) Woodson (predominantly white Corolla with margins of pink lobes and the center in yellow); *Q. rubra L.*, acutifolia form (Ait) Woodson (white flowers with the center of the corolla dyed yellow) and *Q. rubra L.*, typical form (corolla with various shades of pink).

Studies on the floral biology of *Plumeria rubra* with representatives *L.* revealed that this species has no nectary and similarly some orchids, they are pollinated by insects by floral mimicry. The representatives of this species are able to mimic the flavor, size and floral array of other species. The specialization of this plant has reached such a level that the period of their flowering does not coincide with the mimicked plants. This evolutionary *Plumeria rubra L.* character besides being very interesting has not been well explained by biology pollination (2-5).

The literature cites some work in identifying volatile organic compounds of different forms of *Plumeria rubra L.* Basically, the researchers used the techniques of simultaneous distillation and extraction (SDE) and solvent extraction for the isolation of volatile organic compounds from *Plumeria rubra L.* [according to the description of the author it is the *Plumeria rubra L.*, form tricolor (R & P) Woodson (tricolor flowers; DES)] *Plumeria rubra L.* and (7), acutifolia form (Ait.) Woodson [white flowers (extraction with solvents) and SDE] (6, 8). Techniques used above resulted in a very different chemical profile, which is very plausible, since the isolation method chosen has a predominant influence on the composition of volatile organic compounds (8-10). Although the method of "headspace" is considered by most researchers as the best procedure for getting closer to the original composition, the majority of studies with the genus *Plumeria* flower essences stopped in the methods of distillation and extraction (6-8).

The objective of this study floral volatile composition of three varieties of *Plumeria rubra L.* that differ among themselves by the color of the corolla [was to investigate *P.*

*rubra* L., *acutifolia* form (Ait) Woodson (white flowers), *Q. rubra* L., form *tricolor* (R & P) Woodson (intermediate flowers) and *Q. rubra* L., *typica* form (pink flowers)]. Was chosen as the extraction method, the "headspace" cold finger, as this is the method that leads to the nearest chemical composition of the original and floral essence to be the method that leads to production of fewer artifacts.

## Materials and Methods

### Plant Material

Respectively 702.05 g of white flowers, 514.09 g and 416.91 g flowers rosy red flowers of *Plumeria rubra* L. in the campus of the Federal University of Rio de Janeiro (UFRJ) in October 1996 were collected. Flowers were collected by morning (8:00) obeying a time randomly set only to standardize the results. Although white flowers are as fragrant as the other, it was necessary to collect a greater number of flowers, as the detection limit (threshold) of the substances was very low. A specimen was deposited at the Institute of Biology of the Federal University of Rio de Janeiro, under number 24346.

### Varieties of essential oils

The flowers were subjected separately to two static headspace cold finger with a capacity of 5000mL and 1000mL. Subsequently, samples of the flowers were transferred into the flask, and sealed with the cold finger filled with dry ice. As the layer of water crystals were deposited on the wall of the cold finger, volatile substances in the sample migrate, due to the difference in pressure existing inside the flask, and this condensed on cold surface. When the layer of water crystals was around 1mm thick were scraped, the cold finger was returned to

the flask taking care to replace, where necessary, the dry ice.

At the end of each day the cold fingers were washed with dichloromethane PA, joining the collected material samples previously obtained. The amount of dichloromethane the sample was greater than the volume of water, so that the partition to be as efficient as possible. The collected samples were kept in the freezer throughout the isolation procedure. Subsequently, the samples were dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated in a distillation apparatus. The material was placed in a round bottom flask of 250 mL, together with a magnet of approximately 1 cm. The flask was placed in a tub with water and heated over a stir plate with the temperature of the water bath around 40 °C. A Vigreux column approximately 15 cm in length was connected to the flask and the upper portion of the column was placed a distillation head (Claisen) and a round bottom flask (250 mL) which was collected distillate solvent. Took care to use a cooling device, in order to keep the water temperature constant (approximately -5 to °C).

The entire distillation apparatus after being duly attached by claws and rings the two metal brackets, has been involved with foil, keeping the temperature constant throughout the system. The samples were reduced to approximately 1.5mL and transferred to "vials" to 4mL. Subsequently, these samples were subjected to analysis on capillary GC and GC-MS.

### Instrumentation

Essential oils from flowers of *Plumeria rubra* L. were analyzed by gas chromatograph Hewlett-Packard 5790A gas chromatograph and Hewlett-Packard 5980 Mass Spectrometer coupled to

Computerized (HP-5980A) of quadrupole analyzer (Impact Ionisation electrons, 70eV used a DB-5 60 m, ID = 0.25 mm, SD = 0.25  $\mu$ m column analysis conditions were the same for both instruments the gas [splitless; programming temperature = 30°C (8min) - 2°C/min - 290°C (10min.) gun = 270°C; detector = 310°C carrier gas H<sub>2</sub> =] the substances were identified by comparison of their molecular ions and ions resulting from fragmentation characteristic routes of the main classes of compounds volatile Kovats indices (C<sub>5</sub>-C<sub>30</sub>) with the mass spectral data reported in the literature data (13) and Wiley espectroteca 275 T. the solvents used in the process of extraction of essential oils were purified by treating, drying and distillation (12).

## Results and Discussion

The results of the analysis by capillary GC and GC-MS of flower essences obtained by static headspace cold finger of the three varieties of *Plumeria rubra L.* are presented in Table-1 and 2. For the detection of minor compounds in Total ion chromatogram was used fragmentography the ions with specific masses of different classes of components. Ion chromatograms of m / z 204 (molecular ion weight between common sesquiterpenes), m / z 220 (common ion molecular weight alcohols and sesquiterpenes oxides) and m / z 57 (referring to butyl fragment ion quite abundant in hydrocarbons) were obtained for all chromatograms (Table 1).

The results observed in the chromatograms show different profiles for the oils of three varieties of *P. rubra L.* The oil derived from the flowers of *P. rubra L.*, typical form presented more volatile constituents. Three varieties of oils from flowers of *P. rubra L.* typical way that showed a higher amount of hydrocarbons and sesquiterpenes. The oil from the flowers *P. rubra L.* tricolor form (R & P) Woodson a single sesquiterpene the

$\alpha$ -copaene was identified. This oil has a fairly different chemical profile of the typical form.

The oil tricolor form showed the highest amount of benzenoídicos compounds and low concentrations of monoterpenes and hydrocarbons. In oil acutifolia form (Ait) Woodson was not detected the presence of sesquiterpenes. This oil has a fairly similar way to the tricolor chemical profile. The oil acutifolia form showed a lot of benzoic compounds and low concentrations of monoterpenes (except the monoterpene linalool). Table-1 and 2 illustrates the main differences between the oils from flowers of three varieties of *Plumeria rubra L.*

The results obtained from the essential oil of typical form are novel in the literature. The composition of the essential oils of two varieties of *Plumeria rubra L.* (acutifolia shape and form tricolor) obtained with the technique of static headspace, was quite different than quoted in the literature 6-8. This is not at all surprise there since Winterhalter and Shreier in their work, admit that the mild extraction techniques such as "Headspace", are the proper techniques for the determination of genuine volatile organic compounds (1, 9-10).

The results showed profound differences between these three varieties aromas. Probably, these differences observed in the chromatograms and even sensory (during harvesting of flowers), are related to the type of mechanism used by these plants to attract insect pollinators, that is, the stimuli used for the attraction (visual, olfactory and visual and olfactory) (5, 11). Given that the species of *Plumeria rubra L.* mimic the aroma of different species of flowers pollinated by moths (5, 11). Probably the pollinators of these varieties of *Plumeria rubra L.* are of different species, which would explain the difference between them fragrant.

**Table.1** Compounds identified the essential oils of flowers *Plumeria rubra* L., *forma typica* (1), *Plumeria rubra* L., *forma tricolor* (R & P) Woodson (2) and *Plumeria rubra* L., *forma acutifolia* (Ait) Woodson (3)

compostos identificados	t <sub>r</sub>	RI (DB-5)	1	2	3
<b>DERIVADOS DO ÁCIDO GRAXO</b>					
<b>Alkanes</b>					
Nonane	17.29	899 <sup>a, c</sup>	x		
Decane	24.28	999 <sup>a, c</sup>	x		
2- Methyl pentane	25.65	1013 <sup>c</sup>	x		
2- Methyl decane	26.66	1031 <sup>c</sup>	x		
2,6,6-Trimethyl decane	27.81	1051 <sup>c</sup>	x		
2, 9,9-Trimethyl decane	28.93	1071 <sup>c</sup>	x		
Undecane	29. 69	1099 <sup>c</sup>	x		
2, 5-Dimethyl undecane	30.87	1105 <sup>c</sup>	x		
4-Methy dodecane	31.11	1110 <sup>c</sup>	x		
4-Methy-nonane	31.76	1121 <sup>c</sup>	x		
2, 6,11- Trimethyl dodecane	44.67	1349 <sup>c</sup>	x		
3, 5-Dimethyl l dodecane	45.16	1357 <sup>c</sup>	x		
Pentadecane	53.03	1500 <sup>a, c</sup>			x
Hexadecane	58.87	1600 <sup>a, c</sup>	x	x	x
Heptadecane	64.59	1700 <sup>a, c</sup>	x	x	
Octadecane	70.46	1800 <sup>a, c</sup>	x	x	
Nonadecane	80.63	1900 <sup>a, c</sup>	x	x	x
Eicosane	85.39	2000 <sup>a, c</sup>			x
Cyclohexadecane	89.96	2200 <sup>c</sup>	x	x	
Tricosane	98.41	2300 <sup>a, c</sup>		x	x
Tetracosane	102.64 <sup>b</sup>	N.d	x	x	
Heptacosane	110.09 <sup>b</sup>	N.d	x	x	x
Octacosane	117.12 <sup>b</sup>	N.d	x	x	x
Nonacosane	123.69 <sup>b</sup>	N.d	x	x	x
17, Pentatriacontane	144.52 <sup>b</sup>	N.d	x		
<b>Alkenes</b>					

3,4-dimethyl decene	27.33	1043 <sup>c</sup>			x
<b>Acids</b>					
Nonanoic acid	40.54	1280 <sup>a, c</sup>	x		
Dodecanoic acid	63.03	1568 <sup>a, c</sup>	x		
<b>Álcohol</b>					
Octanol	30.31	1070 <sup>a, c</sup>			x
2- Decenol	69.71	1790 <sup>c</sup>			
<b>Cetona</b>					
2,6-Dimethyl-7-octen-4-one	27.61	1048 <sup>c</sup>			x
<b>Éster</b>					
Methyl octadecanoate	86.60	2128 <sup>a, c</sup>		x	x
Ethyl octadecanoate	89.27	2194 <sup>a, c</sup>		x	
Hexadecyl hexadecanoate	134.79 <sup>b</sup>	N.d.	x		
Octadecyl hexadecanoate	140.29 <sup>b</sup>	N.d	x		
<b>BENZENOIDES</b>					
<b>Benzenes</b>					
1,3-Dimethyl benzene	15.65	870 <sup>c</sup>	x		
Estirene	17.03	889 <sup>c</sup>	x		
1,2-Dimethyl benzene	17.19	892 <sup>c</sup>	x		
Cumene	23.38	926 <sup>a, c</sup>	x		
1,2,4-Trimethyl benzene	26.52	1023 <sup>a, c</sup>	x		
<i>p</i> -Cymene	30.68	1026 <sup>a, c</sup>	x		
Benzil alcohol	26.73	1032 <sup>a, c</sup>	x		
alpha, methyl benzenemethanol	27.48	1060 <sup>a, c</sup>		x	x
Phenyl ethyl alcohol	33.31	1110 <sup>a, c</sup>	x		x
Phenyl-terct-butanol	33.75	1156 <sup>a, c</sup>	x		
1,2,3,5 Tetramethyl benzene	33.78	1157 <sup>c</sup>	x		
<i>p</i> -Cymen-9-ol	36.07	1197 <sup>a, c</sup>			x
<i>p</i> -Methyl-1-en-3-ol, trans	36.50	1205 <sup>a, c</sup>	x		
(Z)- Anethole	39.14	1251 <sup>a, c</sup>			x
Phenyl propanol	42.30	1307 <sup>c</sup>			x
<i>cis</i> -Calamenene	54.47	1521 <sup>a, c</sup>		x	

2-Ethyl-chromone	54.47	1607 <sup>a, c</sup>	x		
<b>Ácidos</b>					
Benzoic acid	37.44	1221 <sup>c</sup>		x	
<b>Aldeídos</b>					
Benzaldehyde	21.86	961 <sup>a, c</sup>	x	x	x
Benzene acetaldehyde	28.06	1056 <sup>c</sup>	x	x	x
Benzaldehyde, 4-metoxy	39.17	1252 <sup>a, c</sup>	x		
Benzaldehyde, 3-ethoxy-4-hydroxy	52.83	1452 <sup>a, c</sup>	x		x
Trans-2-hexenyl benzoate	58.20	1587 <sup>a, c</sup>	x		
Amyl cinnamaldehyde (Z)	61.16	1645 <sup>a, c</sup>		x	
<b>Álcoois</b>					
Phenol	23.91	986 <sup>c</sup>			x
2-Cresol	27.48	1053 <sup>a, c</sup>		x	x
Phenol 2,6-dimethyl	30.87	1102 <sup>a, c</sup>	x		
Curcuphenol	65.45	1715 <sup>a, c</sup>	x		
Coniferyl alcohol	66.30	1729 <sup>a, c</sup>			
Octyl phenol <sup>b</sup>	70.89	1812 <sup>b</sup>	x	x	
4-Nonyl phenol <sup>b</sup>	71.47	1823 <sup>b</sup>	x		
Nonyl phenol <sup>b</sup>	71.99	1833 <sup>b</sup>	x	x	
<b>Estheres</b>					
2-hydroxy methyl benzoate	31.99	1125 <sup>c</sup>	x		x
propanol phenyl acetate	39.14	1251 <sup>a, a</sup>			x
Pentyl Benzoate	55.32	1536 <sup>a, c</sup>		x	
Dihydroeugenol acetate	55.32	1536 <sup>a, c</sup>	x		x
trans-Isoeugenol acetate	59.98	1611 <sup>a, c</sup>	x	x	x
Hexyl phenyl acetate	60.67	1630 <sup>a, c</sup>	x		x
Hexyl salicylate	63.30	1675 <sup>a, c</sup>		x	
Benzyl salicylate	73.97	1863 <sup>a, c</sup>		x	
Benzeneacetic acid, 2-phenylethyl ester	78.55	1908 <sup>a, c</sup>		x	
<b>ISOPRENOIDS</b>					
<b>Monoterpenes</b>					
Limonene	27.00	1031 <sup>a, c</sup>			x

<i>trans</i> , Ocimene	27.76	1050 <sup>a, c</sup>			X
$\gamma$ terpinene	28.52	1063 <sup>a, c</sup>			X
Myrcenol	31.46	1118 <sup>a, c</sup>	X		X
<i>trans</i> - Pinan-2-ol	31.49	1118 <sup>a, c</sup>	X		
Dihydro linalool	32.51	1134 <sup>a, c</sup>	X	X	X
<i>cis</i> - Carveol	37.74	1229 <sup>a, c</sup>			X
<b>Sesquiterpenes</b>					
Humulene	49.88	1440 <sup>a, c</sup>	X		
$\alpha$ -Copaene	51.64	1376 <sup>a, c</sup>	X		X
Geranyl n-propanoate	51.88	1475 <sup>a, c</sup>	X		X
Curcumene	52.54	1483 <sup>a, c</sup>	X		
$\beta$ -Selinene	52.59	1485 <sup>a, c</sup>	X		
oxido Calamenene	52.60	1489 <sup>a, c</sup>	X		
(E,E)-alpha- Farnesene	53.59	1508 <sup>a, c</sup>	X		
<i>cis</i> -Calamenene	54.43	1521 <sup>a, c</sup>	X		
$\alpha$ -Cadinene	55.32	1537 <sup>a, c</sup>	X		X
<i>cis</i> -Sesquisabinene hydrate (MM= 222)	55.79	1545 <sup>a, c</sup>	X		X
Germacrene B	56.55	1558 <sup>a, c</sup>	X		
Nerolidol	57.00	1564 <sup>a, c</sup>	X		
cariophyllene oxide	57.90	1581 <sup>a, c</sup>	X		
Cubenol (MM=222)	59.65	1614 <sup>a, c</sup>	X		X
$\gamma$ -Eudesmol (MM= 222)	60.67	1630 <sup>a, c</sup>	X		X
$\delta$ Cadinene	60.71	1628 <sup>a, c</sup>	X		
$\alpha$ - Cadinol (MM= 222)	61.87	1653 <sup>a, c</sup>	X		
Bisabolol (MM= 222)	62.90	1671 <sup>a, c</sup>	X		X
Germacrone	64.27	1693 <sup>a, c</sup>	X		
Curcumenol (PM= 234)	66.19	1728 <sup>a, c</sup>			X
<b>AMINE ANOTHER COMPOUNDS WITH N</b>					
Benzonitrile	23.65	982 <sup>c</sup>	X		
4-hydroxy-Benzonitrile	30.50	1099 <sup>c</sup>			X
oxy-Phenyl -acetonitrile	31.41	1115 <sup>c</sup>		X	
Phenyl acetonitrile	35.03	1179 <sup>c</sup>	X	X	X



Indole	41.23	1288 <sup>a, c</sup>	x		x
2-Phenyl nitroethane	46.30	1377 <sup>c</sup>	x		
Benzoate methyl 2-Amine	49.14	1428 <sup>c</sup>			x
furane 2-Ethyl-	7.36	753 <sup>c</sup>	x		
furfural 5-Methyl-	23.88	962 <sup>a, c</sup>		x	
oxirane 2,2-ethyl-hexyl-1-methyl-	30.87	1105 <sup>c</sup>	x		
tiazole 2,4-dimethyl-5-acetyl	38.23	1235 <sup>c</sup>	x	x	x
Azulene	38.26	1299 <sup>a, c</sup>		x	
Naphtalene ethyl	54.03	1514 <sup>c</sup>			x

<sup>a</sup>Retention Time in a DB-5 column (according to reference 50) and Kovats Index (KI)

<sup>b</sup> likely contaminants

<sup>c</sup> Substances identified by automated comparison of mass spectra with standard spectra stored in the library masses

N.d. = not determined; MM = Molecular weight

**Table.2** Main differences between oils from flowers of three varieties of *Plumeria rubra* L.

<i>P. rubra</i> L.	Monoterpenes	Sesquiterpenes	Benzenoids	Hidrocarbonetos
<i>forma typica</i> (1)	-	+++	++	+++
<i>forma tricolor</i> (2)	+++	--	+++	++
<i>forma acutifolia</i> (3)	-	-	+++	++

(+++) **Major**; (++) **gifts**; (-)**Minority** and (--)**Absent**

(1) **pink flowers**; (2) **tricolor flowers** and (3) **white flowers**

; (-) **Minority** and (-)

(1) **pink flowers**; (2) **tricolor flowers** and (3) **whit**

Therefore, it may be that there is a major difference between them. But for these hypotheses are proven further studies are needed to identify: the main pollinators of different varieties of *Plumeria rubra L.*, the types of mechanisms (visual, olfactory and visual stimuli and olfactory stimuli) that are being used by these plants for attraction of insects (for pollination) and (s) substance (s) responsible for the scents of these flowers.

## References

1. Knudsen, J. T.; Tollsten, L.; Gunnar-Bergström, L.; *Phytochemistry* 1993, 33, 253.
2. Coliviaux, P.; *Ecology* 2, John Wiley & Sons, Inc.,1993, p. 688.
3. Woodson, R. E.; *Ann. Mo. Bot. Gard.* 1938, 25, 189.
4. Azambuja, D.;. *Arquivo do Serviço Florestal* 1947, 3, 13.
5. Haber, W. A.; *Biotropica* 1984, 16, 269.
6. Omata, A.; Yomogida, K.; Nakamura, S.; Hashimoto, S.; Arai T.; Furukawa, *Flav. Frag. J.* 1991, 6, 277.
7. Omata, A.; Nakamura, S.; Hashimoto, S.; Furukawa, K.; *Flav. Frag. J.* 1992, 7, 33.
8. Pino, J. A.; Ferrer, A.; Alvarez D.; Rosado, A.; *Flav. Frag. J.* 1994, 9, 343.
9. Bicchi, C.; Joulain, D.; *Flav. Frag. J.* 1990, 5, 131.
10. Schreier, P.; *Chromatographic Studies of Biogenesis of Plants Volatiles.* New York: Hüthing, 1984, p. 170.
11. Williamson, G. D.; *Biol. J. Linn. Soc.* 1982, 18, 49.
12. Perrin, D. D.; *Purifications of Laboratory Chemicals*, Pergamon Press: Oxford, 2<sup>a</sup> ed., 1980, p. 568.
13. Adams, R. P.; *Identification of Essential Oil Components by Gas Chromatography Mass Spectroscopy*, Allured Publishing Corporation Carol Stream, Illinois USA, 1995, p. 468.