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# Chemical Constituents of Floral Volatiles of Plumeria rubra L. from India

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## **Abstract**

The flower volatile constituents of *Plumeria rubra* L. grown in foothills of north India were analyzed by gas chromatography (GC) and GC-mass spectrometry (GC-MS). Altogether 31 constituents, representing 94.0% of flower essential oil and 89.2% of steam volatile extract were identified. Benzyl esters (49.0%, 41.4%), aliphatic alkanes (25.8%, 7.2%), oxygenated monoterpenes (0.1%, 27.1%), oxygenated sesquiterpenes (9.5%, 8.8%), and diterpene (9.4%, 0.2%), were the major class of constituents. Benzyl salicylate (26.7%, 33.5%), benzyl benzoate (22.3%, 7.9%), geraniol (trace, 17.2%), (E,E)-geranyl linalool (9.4%, 0.2%), tricosane (8.3%, 1.1%), linalool (0.1%, 8.0%), nonadecane (7.0%, 3.8%), (E)-nerolidol (7.0%, 5.5%), and pentacosane (4.4%, 0.3%) were the major constituents identified in flower oil and hydrodistilled volatile distillate. Results were compared with reported floral compositions of P rubra that revealed considerate qualitative and quantitative variations. Alkanoic acids, neryl phenylacetate, phenylacetaldehyde, B-phenylethyl alcohol reported earlier were not present in E rubra grown in India.

**Keywords:** *Plumeria rubra*; Flower; Essential oil; Benzyl benzoate; Benzyl salicylate

# Introduction

The genus Plumeria L. (family Apocynaceae), comprises of lactiferous trees and deciduous shrubs, is a native of tropical America, and now widely distributed from southern Mexico to northern South America, tropical areas of Pacific islands, Caribbean, India [1]. Plumeria spp. are commonly grown as ornamental plants in premises, parks, gardens and graveyards because of their beautiful fragrant flowers of various color and size. The essential oil and fragrant constituents from the flowers of various Plumeria species are used in perfumery, cosmetics and aromatherapy [2,3]. Plumeria rubra L. (syn. P. acutifolia Poiret), commonly known as 'Frangipani' is one of the commonly distributed member of this genus in India [4]. Plumeria spp. are known for their diverse medicinal uses in indigenous medicine, mainly as purgative, rubefacient, errunenagogue, febrifuge or diuretic, and for treatment of dropsy dysuria, diarrhea, itch, bronchitis, cough, asthma, fever, piles, dysentery, blood disorders and tumors [5-7]. The leaves of P. rubra are used in ulcers, leprosy, inflammations, rheumatism, and as rubefacient [8,9]. The root bark is bitter, pungent, heating, carminative, laxative and traditionally used to treat asthma, leprosy, constipation, and ulcers [10]. In Indonesia, bark of *P. rubra* is being used to treat gonorrhoea, while in the Philippines, bark are used as purgative, emmenagogue and febrifuge [11]. The flowers are aromatic and bechic and widely used in pectoral syrups [12]. Flower infusions of *P. rubra* are used for the treatment of diabetes mellitus in Mexico [13]. Various biologically active phytoconstituents such as iridoids viz. plumericin, plumierides, rubrinol, allamcin and allamandin, coumarate and their glycosides, triterpenoids (rubrinol), flavones glycosides, resinic acids, sterols, lignans, terpene and non terpene esters, carboxylic acids were characterized from different parts of P. rubra [13-17]. Despite all the comprehensive scientific studies conducted on Plumeria species worldwide, review of literature revealed that very scarce information is available on the constituents of fragrant flowers of the P. rubra from India. To best of our knowledge, the fragrant volatile constitution of the flowers of P. rubra has not been investigated previously from India. Therefore, in continuation of our study to identify new perfumery materials from unexplored/underexplored aromatic plants, we describe the detailed floral composition of *P. rubra* from India.

## Materials and Methods

# Plant materials and extraction of essential oils

The fresh flowers of P. rubra (white flowered) were collected from field gene bank of CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Pantnagar (Uttarakhand) in the month of April, 2013. The plant material was authenticated at Botany Department of CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre Pantnagar (India) by one of the authors (Dr Amit Chauhan). The essential oil was obtained from the fresh flowers (500 g) by hydrodistillation using a Clevenger's type apparatus for 3 h. Essential oil, light yellow in color, collected over the water surface of the extraction burette of clevenger apparatus was measured and dehydrated over anhydrous sodium sulphate and kept in a cool and dark place until further analyses. The essential oil content (%) was calculated as volume (mL) of essential oil per 100 g of fresh flower weight. The flower volatile extract was obtained by hydrodistillation of fresh flowers (800 g) in Clevenger's type apparatus for 3 h and the distillate (1.5 L) was collected. The distillate was saturated with NaCl, and the volatile constituents from this aqueous distillate were extracted using n-hexane and dichloromethane. The organic phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and then the solvent was removed under vacuum in a rotary vacuum evaporator at 30°C temperature to recover the floral extract (0.296 g). The percentage content was calculated based on fresh weight of plant materials.

# Gas chromatography (GC/FID)

GC analysis of the essential oils was carried out on a PerkinElmer AutoSystem XL gas chromatograph, equipped with DB-5 capillary

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column (60 m  $\times$  0.32 mm i.d., film thickness 0.25  $\mu m)$  and flame ionization detector (FID). The oven column temperature ranged from 70-250°C, programmed at 3°C/min, with initial and final hold time of 2.0 min, using  $H_2$  as carrier gas at 10 psi constant pressure, a split ratio of 1:35, an injection size of 0.03  $\mu L$  neat, and injector and detector temperatures were maintained at 250°C and 280°C, respectively.

# Gas chromatography-mass spectrometry (GC-MS)

GC/MS analyses of the essential was carried out on a Clarus 680 GC interfaced with a Clarus SQ 8C mass spectrometer (Perkin-Elmer, Shelton, USA) fitted with Elite-5 MS fused-silica capillary column (30 m  $\times$  0.25 mm i.d., film thickness 0.25  $\mu m$ ). The column temperature was programmed from 60 to 240°C, at 3°C /min, and to 270°C at 5°C/min. Helium was used as carrier gas at a flow rate of 1.0 mL /min. The injector, ion source and transfer line temperatures were 250°C. The injection volume was 0.03  $\mu L$  neat with split ratio 1:30, electron impact ionization mode (EI), with ionization energy 70 eV and mass scan range of 40-400 amu.

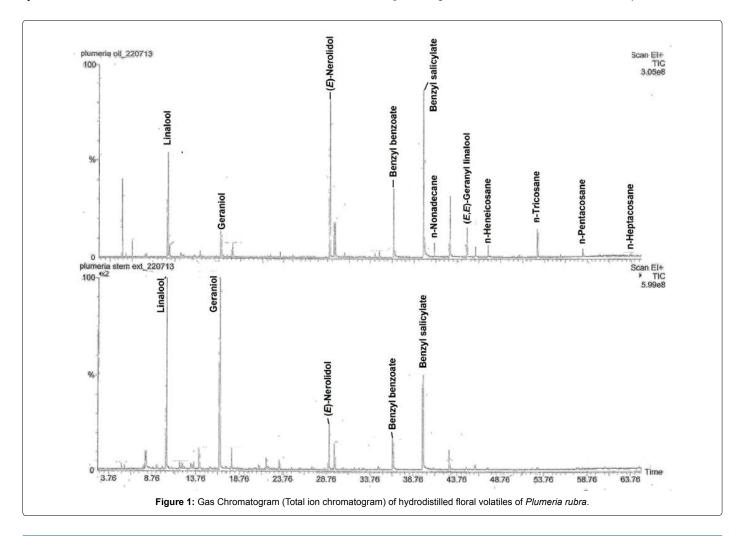
#### Identification of volatile constituents

Identification of the essential oil constituents was done on the basis of retention index (RI, determined with reference to homologous series of n-alkanes,  $C_s$ - $C_{30}$ ; Supleco Analytical, Bellefonte PA, USA) under identical experimental conditions and by comparing the mass spectral and retention data with literature [18]. The relative amounts of

individual components were calculated based on the GC/FID peak area without using a correction factor.

# **Results and Discussion**

The flower volatile constituents of Plumeria rubra L. grown in foothills of north India (29°N, 79.38°E) was analyzed by gas chromatography (GC) and GC-mass spectrometry (GC-MS) (Figure 1). The fresh flowers of *P. rubra* yielded 0.016% and 0.037% of essential oil and hydrodistilled floral extract, respectively on hydrodistillation. Although the flowers of various *Plumeria* species are fragrant in nature, but in general percentage yields of the volatile constituents are very low. The essential oil yield reported 0.03% in *P. rubra* (yellow flowered), 0.06% in *P. rubra* (pink flowered) and 0.39% in *P. rubra* (orange flowered) grown at Peninsular Malaysia [5]. In another report, the flower essential oil yield was reported 0.03% to 0.12% in P. rubra from Malaysian origin [17]. However, floral volatile yields 0.0167%, 0.0045% and 0.0342% were reported in P. obtusa from Thailand by water, steam and watersteam distillations, respectively [19]. Moreover, 0.08% essential oil yield was reported for P. alba; and 0.04%-0.07% for P. rubra grown in India [20,21]. The variations in the yield of floral volatile constituents of *Plumeria* species may be possible due to their diverse geographic origin, soil and climatic conditions of regions where they are grown, time of flower collections, as well as various method of their extraction. GC and GC-MS analysis resulted identification of 31 constituents, representing 889.2%-94.0% of essential oil and hydrodistilled floral



extract, respectively (Table 1). Results showed that Benzyl esters (49.0%, 41.4%), aliphatic alkanes (25.8%, 7.2%), oxygenated monoterpenes (0.1%, 27.1%), oxygenated sesquiterpenes (9.5%, 8.8%), and diterpene (9.4%, 0.2%) were the main constituents distributed in *P. rubra*. The flower essential oil was characterized by, relatively, a higher content of benzyl ester (49.0%) comprised of benzyl salicylate (26.7%) and benzyl benzoate (22.3%), followed by aliphatic alkanes (25.8%) represented by tricosane (8.3%), nonadecane (7.0%), pentacosane (4.4%), heneicosane

Campa	RI <sub>Exp</sub>	RI	Content (%)	
Compound*			FO	FE
β-Pinene	970	974	0.1	1.1
p-Cymene	1015	1020	t	0.2
Limonene	1020	1024	t	0.3
1,8-Cineole	1024	1026	t	0.2
Benzyl alcohol	1028	1026	t	0.3
Terpinolene	1085	1086	t	0.3
Linalool	1101	1095	0.1	8.0
n-Nonanol	1106	1100	0.1	0.3
iso-3-Thujanol	1128	1134	t	0.2
Camphor	1139	1141	t	0.2
α-Terpineol	1180	1186	t	0.9
Geraniol	1251	1249	t	17.2
neoiso-Thujanyl acetate	1283	1281	t	0.2
α-Copaene	1378	1374	t	1.0
(E)-Methyl cinnamate	1380	1376	t	0.2
β-Caryophyllene	1420	1417	t	1.0
(E)-Nerolidol	1560	1561	7.0	5.5
Dendrolasin	1573	1570	2.4	2.6
β-Copaen-4α-ol	1588	1590	t	0.1
n-Heptadecane	1701	1700	0.3	t
(E)-Nerolidyl acetate	1714	1716	0.1	0.6
Benzyl benzoate	1762	1759	22.3	7.9
Benzyl salicylate	1863	1864	26.7	33.5
n-Nonadecane	1901	1900	7.0	3.8
(E,E)-Geranyl linalool	2021	2026	9.4	0.2
n-Heneicosane	2100	2100	2.1	0.3
n-Tricosane	2301	2300	8.3	1.1
n-Tetracosane	2401	2400	1.3	0.5
n-Pentacosane	2500	2500	4.4	0.3
n-Hexacoasane	2600	2600	0.4	0.5
n-Heptacosane	2700	2700	2.0	0.7
Class compositions				
Benzyl esters			49.0	41.4
Aliphatic alkanes			25.8	7.2
Monoterpene hydrocarbons			0.1	1.9
Oxygenated monoterpenes			0.1	27.1
Sesquiterpene hydrocarbons			t	2.0
Oxygenated sesquiterpenes			9.5	8.8
Diterpenes			9.4	0.2
Benzyl alcohols			t	0.3
Aliphatic alcohols			0.1	0.3
Total			94.0	89.2

Identification methods: RI, MS; t=trace ( $\leq$  0.05%), RI<sub>Exp</sub>: Experimental retention index determined on DB-5 column (30 m × 0.25 mm) using a homologous series of *n*-alkanes; RI<sub>Exp</sub>: Retention index from literature [18]; FO: Flower oil (By hydrodistillation for 3 h and collected pure oil using a Clevenger's apparatus); FE: Floral extract (By hydrodistillation and the distillate collected and the volatile constituents extracted using organic solvents)

 Table 1: Composition of the hydrodistilled floral volatiles of Plumeria rubra.

(2.1%), heptacosane (2.0%), tetracosane (1.3%), hexacosane (0.4%), and heptadecane (0.3%). Besides, these other major constituents identified were (E,E)-geranyl linalool (9.4%), (E)-nerolidol (7.0%), and dendrolasin (2.4%). Moreover, the flower volatile extract of P. rubra was also dominated by benzyl esters (41.4%) with benzyl salicylate (33.5%) and benzyl benzoate (7.9%) as major constituents. Contrary to aliphatic alkane's dominance in flower essential oil the volatile extract of P. rubra comprised of 27.1% of oxygenated monoterpenes with geraniol (17.2%) and linalool (8.0%) as major constituents. Aliphatic alkanes constitute 7.2% of the composition, with nonadecane (3.8%) and tricosane (1.1%) as main constituents. Other constituents identified in considerable content were (E)-nerolidol (5.5%), dendrolasin (2.6%), β-pinene (1.1%),  $\beta$ -caryophyllene (1.0%), and  $\beta$ -copaene (1.0%). Although the volatile constituents identified in essential oil and steam volatile extract of P. rubra were qualitatively same, but they differed considerably in their quantitative compositions (Table 1).

The fragrant flower volatile constitutions of P. rubra have been studied in the past from diverse geographic origin. The flower essential oil extracted by simultaneous distillation and extraction of P. rubra L. cv. 'Irma Bryan' grown Hawaii reported to have β-phenylethyl alcohol, phenylacetaldehyde and methyl cinnamate as major constituents, while its other cv. 'Common Yellow' contained linalool, phenylacetaldehyde, β-phenylethyl alcohol, geraniol, α-terpineol, and citral as major constituents [22,23]. The volatile oil from P. rubra from Chinese origin is characterized by linalool (20.7%), geraniol (16.2%) and (E)-nerolidol (14.1%) [24]. However, the flower oil compositions of three Malaysian P. rubra cultivars showed intricate compositions. The oil of P. rubra (white flowered) was characterized by benzyl salicylate (39.0%), benzyl benzoate (17.2%), (E)-nerolidol (10.6%), and neryl phenylacetate (10.5%). Phenyl ethyl benzoate (12.3%), lauric acid (11.8%), palmitic acid (9.3%), linalool (5.3%), benzyl benzoate (4.0%) and benzyl salicylate (4.1%) were the major constituents reported in P. rubra (reddish-orange flowered), while palmitic acid (27.2%) and linoleic acid (20.7%), myrstic acid (18.9%), lauric acid (10.6%), with no benzyl salicylate and benzoate characterized the flower essential oil of P. rubra (red flowered) [17]. In an another report on flower oil of P. rubra (pink flowered), alkanoic acids (58.0%) viz. lauric acid (30.8%), myristic acid (17.4%) and palmitic acid (9.8%) were reported as major constituents, while P. rubra (orange flowered) was reported to be dominated by alkanoic acid with palmitic acid (36.0%), linoleic acid (16.8%), lauric acid (10.4%), myristic acid (10.3%) as major constituents. However, P. rubra (orange flowered) was reported to be dominated by benzyl salicylate (20.9%), (E)-nerolidol (14.4%) and benzyl benzoate (8.6%) [2]. Further, microwave distilled flower essential oil composition of two cultivars of P. rubra grown in China was also reported. The essential oil of one cultivars (PRL) of P. rubra was reported to be characterized by high hydrocarbon content (38.6%) with 9-hexacosene (14.6%) as major constituent. On contrary, the second cultivar (PRLA) was characterized by high content of carboxylic acids (59.7%) with palmitic acid (35.8%) and myrstic acid (11.2%) as major constituents [25]. However, the flower oil composition P. rubra of Nigerian origin was reported to contain heneicosane (19.15%), nonadecane (15.63%), citronellol (14.63%), geraniol (9.17%), tricosane (6.06%), and nonadecene (5.86%) as major constituents [26]. Variability in volatile constituents of the flowers of Plumeria rubra from different geographic regions was compared in Table 2. The variation in the flower volatile constituents of P. rubra from various geographic reasons might be due to different cultivars, climatic conditions as well as due to different extraction and analytical processes during analysis. Comparison of results of present study on flower volatile constituents of P. rubra grown in India revealed

Origin/habitat	Cultivar/Flower color	Major reported constituents of essential oils	Ref.			
Malaysia	Pink	Lauric acid (30.8%), myristic acid (17.4%) and Palmitic acid (9.8%)				
	Orange	Palmitic acid (36.0%), linoleic acid (16.8%), lauric acid (10.4%), and myristic acid (10.3%)				
	Orange-pink	Benzyl salicylate (20.9%), (E)-nerolidol (14.4%) and benzyl benzoate (8.6%)				
Malaysia	White	Benzyl salicylate (39.0%), benzyl benzoate (17.2%), (E)-nerolidol (10.6%), and neryl phenylacetate (10.5%).				
	Reddish-orange	Phenyl ethyl benzoate (12.3%), lauric acid (11.8%), palmitic acid (9.3%), and linalool (5.3%),				
	Red flowered	Palmitic acid (27.2%) and linoleic acid (20.7%), myrstic acid (18.9%), and lauric acid (10.6%),				
Hawaii	cv. 'Irma Bryan'	β-Phenylethyl alcohol (31.6%), phenylacetaldehyde (12.1%), and 2-methylbutan-1-o1 (10.5%)				
	cv. 'Common Yellow'	Linalool (16.1%), phenylacetaldehyde (14.4%), ( $E$ , $E$ )-farnesol (10.4%), $\beta$ -phenylethyl alcohol (8.8%), and geraniol (5.4%)	[22,23]			
China	White	Linalool (20.7%), geraniol (16.2%) and ( <i>E</i> )-nerolidol (14.1%)				
China	cv. PRL	Hydrocarbons (38.6%) with 9-hexacosene (14.6%)	[05]			
	cv. PRLA	Carboxylic acids (59.7%) with palmitic acid (35.8%), and myrstic acid (11.2%)	[25]			
Nigeria	Reddish-orange	Heneicosane (19.15%), nonadecane (15.63%), citronellol (14.63%), geraniol (9.17%), tricosane (6.06%), and nonadecene (5.86%)				
Nigeria	Reddish-orange	( <i>E</i> )-non-2-en-1-ol (15.7%), limonene (10.8%), phenyl acetaldehyde (9.0%), <i>n</i> -tetradecanol (8.8%), and γ-elemene (6.5%)				
China	White	enzenedicarboxylic acid (66.11%)				
Cuba	na	Butyl oleate (13.8%), butyl palmitate (11.5%), methyl palmitate (9.7%), methyl oleate (9.3%), and linalool (8.2%)				
Egypt	na	α-Pinene, 2-carene, β-pinene, β-phellandrene, p-cymene, linalool, phenylalcohol, and citral				
Egypt	na	Farnesol, geraniol, phenylethyl benzoate, methyl pentadecane, and terpinolene	[31]			
India	White	Benzyl salicylate (26.7%-33.5%), benzyl benzoate (7.9%-22.3%), geraniol ( $\leq$ 0.05%-17.2%), ( <i>E</i> , <i>E</i> )-geranyl linalool (0.2%-9.4%), tricosane (1.1%-8.3%), linalool (0.1%-8.0%), nonadecane (3.8%-7.0%), ( <i>E</i> )-nerolidol (5.5%-7.0%)	Presen report			

na: not available/mentioned

Table 2: Variability in constituents of flowers of Plumeria rubra from different geographic regions/origin.

remarkable qualitative and quantitative differences in the compositions with earlier reportes on chemical compositions of P. rubra from other countries. The composition of P. rubra grown in India was exclusively dominated by benzyl salicylate (26.75%-33.50%), benzyl benzoate (7.87%-22.30%), (EE)-geranyl linalool (0.17%-9.36%), linalool (0.15%-7.99%), *n*-tricosane (1.08%-8.31%), and *n*-nonadecane (3.79%-7.01%). Composition reported in present study was very close to the P. rubra (cv. white flowered and orange flowered) grown in Malaysia, although neryl phenylacetate, phenylacetaldehyde, \beta-phenylethyl alcohol and alkanoic acids were not present in P. rubra of present analysis. These variations are likely to be due to both biotic and abiotic factors affecting plant growth and biosynthesis as well as due to occurrence of differently flower colored cultivars of P. rubra worldwide. To best of our knowledge, this is for the first time the detailed flower steam volatile composition of P. rubra grown in India was analysed and reported. Further, due to the presence of benzyl esters, mainly benzyl salicylate having mild sweet floral-balsamic or floral-woody odour, as major constituent of flower distillates of P. rubra, floral extract could be used as fragrance materials for incense preparations.

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