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## Pharmacognostical and Phytochemical evaluation of three different species of citrus fruit Nimbuka, Mahalunga & Jambira (*Citrus aurantifolia* Linn, *Citrus medica* Linn, *Citrus jambiri* Linn)

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### Abstract

*Amla Varga* is collection of herb with sour taste which is used frequently for the different processes like trituration, *Shodhana*, *Jarana* while preparing medicines from different herbal and mineral raw materials. *Mahalung* (*Citrus Medica* Linn), *Jambira* (*Citrus Jambiri* Linn) and *Nimuka* (*Citrus Aurantifolia* Linn.) –different citrus fruits were commonly used herbs from the *amla varga*. *Nimbuka* was later added in *amla varga* after 8<sup>th</sup> century in the *Nighantu* and *Rasa* literature due to easy availability, large scale production with maximum yield of juice.

All citrus fruits present the same anatomical structures with variation in size and weight. Even though less is size and weight, *Citrus aurantifolia* has more percentage of juice yields than *medica* and *jambiri*. *Nimbuka* fruit juice was more acidic may be due to more percentage of citric acid content. Maximum total solid content of *Medica* may be due to presence of non acidic substances like ions which makes it less acidic. *Jambira* fruit juice has more percentage of ascorbic acid than other two citrus fruits.

**Keywords:** Citrus fruit, ascorbic acid, citric acid

### Introduction

*Rasashastra* deals with use of metal, mineral as a medicine after converting them into bioassmiable form by virtue of different procedures like *Shodhana*, *Marana*, *Bhavana*, *Swedana*. These procedures were carried out by using organic material from herbs which were classified into different groups (*Amla Varga*, *Kshara Varga*, *Ksheer Varga*) [1, 2, 3]. Juice of *Amla Varga* is used for *shodhana*, *jarana* while *mala* (Unwanted part) is removed by *kshar* group of organic drugs [4]. *Amla Varga* is combination of herbs having sour test, which is dominated by fruits. (See Table No.1) *Ashtang Hrudyam* was the pioneer followed by all *nighantus* and *Rasa shastra* related literature to mention *Amla* group. Among the herbs mainly citrus fruit were commonly used for different procedures in medicine preparations. Contemplation of ancient literature reveals that mainly *Matulung* and *Jambhira* was mainly used in *samhita* period (1500 B.C.) which is replaced by *Nimbuka* in later period (8<sup>th</sup> century). There is need to find the difference in three citrus fruits at pharmacognostical and phytochemical level. So present work is carried out why *Nimbuka* was commonly used in later period instead of *Matulung* and *Jambiri* fruit.

### Material and Methods

**Collection and Authentication of Material:** Fresh Fruits of *Citrus Medica*, *Aurangtifolia* and *Jambiri* were collected from local market of pune region. It was authenticated at the Research lab, College of Ayurveda and Research Centre, Akurdi, pune. Organoleptic characters like color, taste, touch and odor were recorded. Thin free hand sections were studied without staining. Physicochemical profiling was carried out from fresh Juice extracted from all citrus fruits [7, 8, 9].

### HPLC method for determination of ascorbic acid and citric acid

**Sample Preparation:** The fresh juice was extracted by traditional method from all fruits. The Juice was centrifuged at 5000 rpm for 7 min. Then 4.5 ml was taken from the transparent supernatant fluid and diluted with 10 times distilled water. 1 ml from above solution is further diluted with distilled water upto 20 ml mark.

**Instrumentation:** HPLC was performed on Hitachi Lachrom with column C 18(250 x 4.60 mm i.d.). 20 ul of standard solution and sample solution were injected.

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**Table 1:** List of Herbs mentioned under the *Amla* group by different *Acharyas*

Name of Plant	<i>Ashtang Hrudyam</i> <sup>5</sup>	<i>Rasa Ratna Samuchaya</i> <sup>1</sup>	<i>Rasa Targini</i> <sup>6</sup>
Amalaki ( <i>Embllica officinalis</i> Gaertn.)	+	+	+
Amalika ( <i>Tamarindus indica</i> Linn.)	+	+	+
Matulung ( <i>Citrus medica</i> )	+	+	+
<i>Nimbuka</i> ( <i>Citrus aurantifolia</i> )	+	+	+
<i>Jambir nimbu</i> ( <i>Citrus jambhiri</i> )	+	+	-
Amlavetas ( <i>Garcina indica</i> Chois.)	+	+	+
Dadima ( <i>Punica granatum</i> Linn.)	+	+	+
Rajat ( <i>Argentimum</i> )	+	-	-
Chukra ( <i>Rumex vesicarius</i> Linn.)	+	-	+
Dahi (Curd)	+	-	-
Amra ( <i>Mangifera indica</i> Linn.)	+	-	-
Kapitha ( <i>Feronia elephantum</i> Correa.)	+	-	-
Karmard ( <i>Carissa carandas</i> Linn.)	+	+	+
<i>Changeri</i> ( <i>Oxalis corniculata</i> Linn.)	+	+	+
<i>Chanakamla</i> ( <i>Cicer arietinum</i> Linn.)	+	+	+
<i>Kola</i> ( <i>Ziziphus jujuba</i> Lam.)	-	+	-
<i>Ambhastha</i> ( <i>Spondias mangifera</i> Willd)	-	+	-
<i>Narang</i> ( <i>Citrus reticulata</i> Blanco)	-	+	+

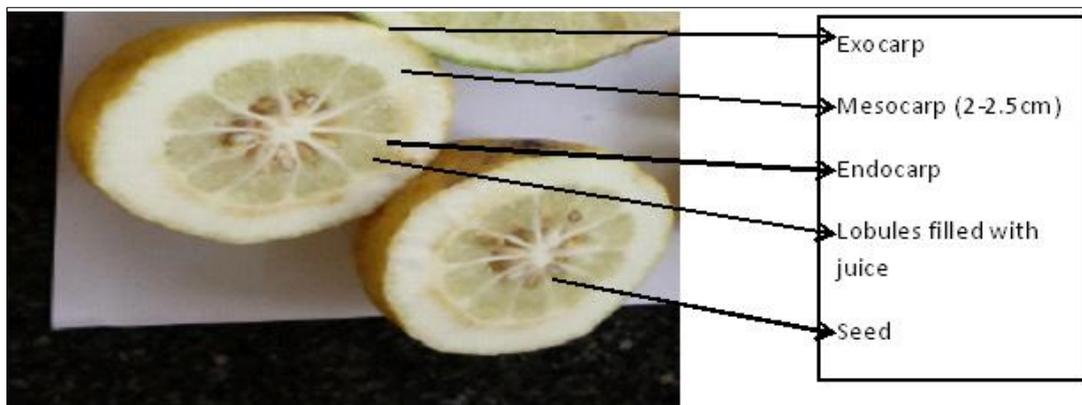
### Results and Discussions

Different herbal drugs were classified in different drugs depending upon the similarities and their use in many *Rasashastra* procedures like *Shodhana*, *Marana*, *Bhavana*. Citrus fruits variants were commonly used from *Amla Varga*. *Citrus medica* and *jambiri* were commonly used in the period of *Samhita* as *Medica* was originated from Sub Himalayan region of Northeast India<sup>[10]</sup> and *jambiri*<sup>[11]</sup> was cultivated in Dehradun. *Citrus aurantifolia* was originated from South east asia sp. from Indonesia and Malaysia, which later cultivated in European countries as hybrid variant<sup>[12]</sup>.

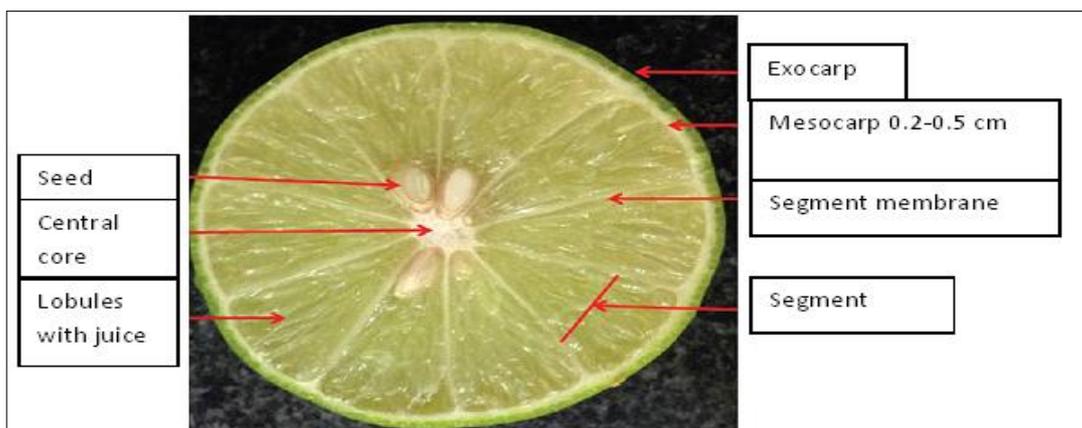
Later period was dominated by (from 8<sup>th</sup> century) *Aurantifolia* as it has more per annum yield (600-1500 kg/yr) than *Medica*

(40-100 kg/yr) and *Jambiri*. Barrett and Rhodes (1976) suggested that citron (*Citrus medica* L.), mandarin (*C. reticulata* Blanco), and pummelo (*Citrus maxima* (Burm) Merrill) species are only three 'basic' true species of *Citrus* within the subgenus *Citrus*, other species within this subgenus are hybrids derived from these true species, species of subgenus *Papeda* or closely related genera<sup>[13]</sup>. *Aurantifolia* has dominance of *tikshna* and *bhedan* property among the citrus fruit. *Citrus Medica* has *pittahar* property among the citrus fruit as having sweet and sour taste.

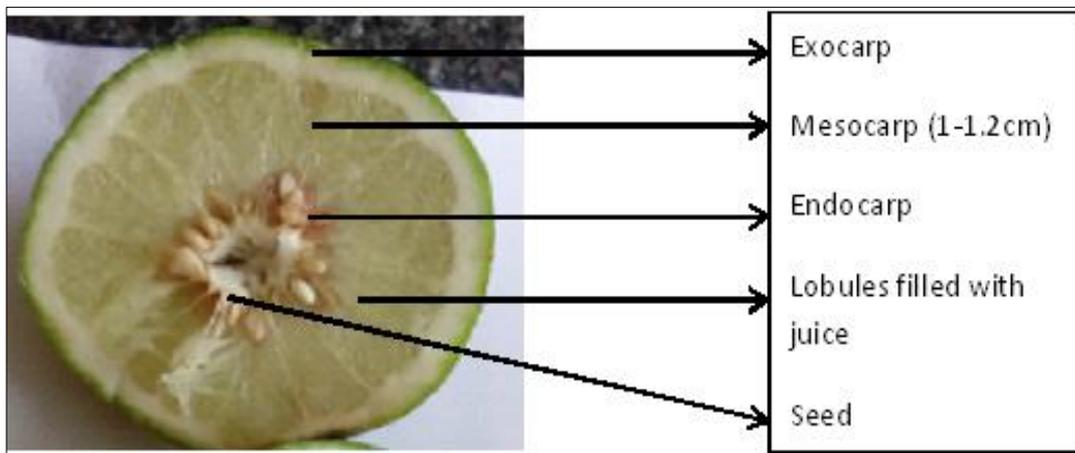
### Macroscopic variation in three citrus fruits



**Fig 1:** Macroscopic Structure of T.S. of citrus medica fruits



**Fig 2:** Macroscopic Structure of T.S. of citrus Nimbuka Fruits



**Fig 3:** Macroscopic Structure of T.S of citrus Nimbuka Fruits

All citrus fruits present the same anatomical structures [Figure 1, 2 and 3]: (1) Exocarp is the external part of the fruit and has a lot of flavonoids as its name. The outer cell wall is composed of wax and cutin for prevention of water loss from the fruit; (2) albedo is the white spongy portion, below the flavedo layer; (3) carpal membranes or septum presenting around 8–11 glandular segments, usually aligned and situated

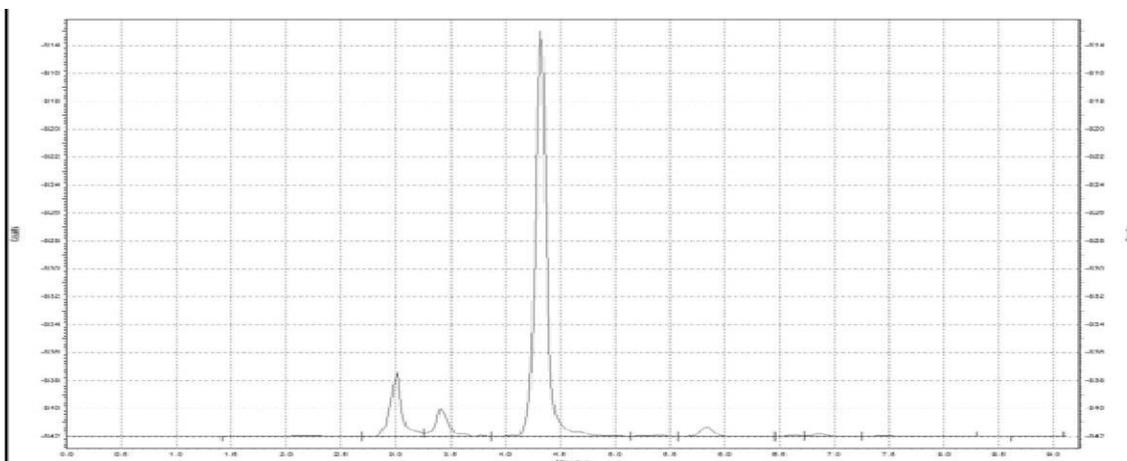
around (4) the soft central core; (5) juice sacs are yellow-green pulp vesicles; and (6) seeds are small, plump, ovoid, pale, and smooth with white embryo. The width of the mesocarp decreases as less in aurantifolia than in jambira and medica. Juice sacs of aurantifolia are very thin and with more juice as compared with jambira and medica, the exocarp is also thin in case of aurantifolia than jambira and medica.

**Table 2:** Results of different test performed on fruit Juices of *Matulung*, *Jambira* and *Nimubaka*

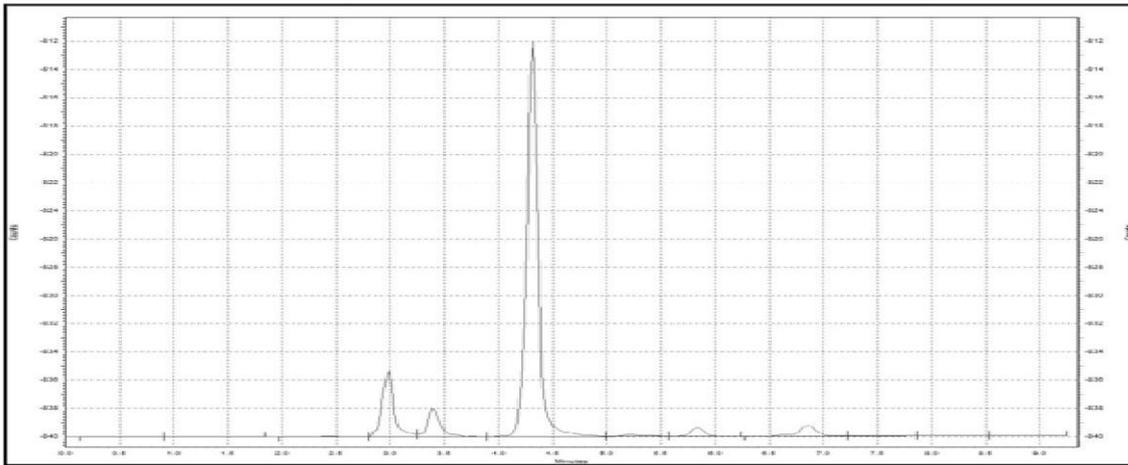
Test performed	<i>Mahalung</i>	<i>Jambira</i>	<i>Nimbuka</i>
Size of the Fruit in cm	11*5.5/20*7.2/ 7.2*7.8	7*5/10*5/9*6	3.5*3.6/3*3.1/ 3.5*3.4
Weight in gram	132/841/240	267/347/274	23.87/23.33/24.13
Obtained Swarasa	10 ml /75 ml /21ml	20 ml/30 ml/ 20ml	10 ml/ 11ml/ 11ml
Color	Off white	Off white	Off white
pH	3.5/3.7/4.1	3.0/3.1/3.2	2.3/2.4/2.2
Sp. Gravity	1.097/1.077/1.090	1.057/1.060/1.055	1.080/1.084/1.089
Total solid content	25.22/20.00/20.02	14.82/15.60/14.3	20.68/21.24/23.14

Citrus aurantifolia yields more percentage of juice than medica and jambiri even though having smallest diameter among the citrus fruits. Juice of aurantifolia was more acidic in nature than medica and jambiri. *Tikshna* [14] and *Bhedan* property of aurantifolia may be due to strong acidic nature. Maximum total solid content of Medica may be due to presence of non acidic substances like ions which makes it less acidic. Fruit decoction has alkaloids, flavonoids, phenols, carbohydrates and mucilage. The flavonoids reported from the fruits are hesperidin: 3, 5, 6- trihydroxyl- 4, 7 -dimethoxy

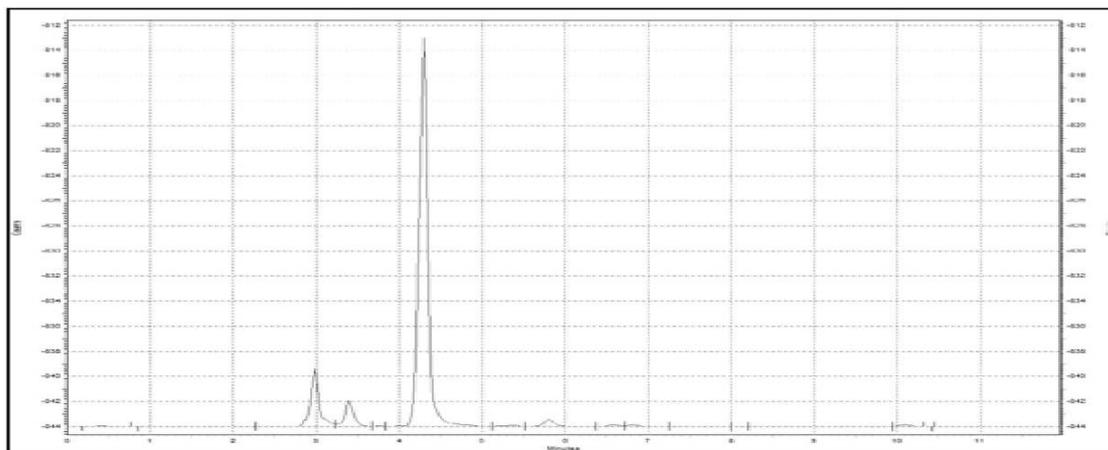
flavone; 3, 5, 6-trihydroxy-3', 4', 7- trimethoxy flavones [15, 16]. Jambiri (rough lemon) are mostly low in soluble solids, acid, and ascorbic acid content i.e. it has high percentage of fibers [17]. Its alcoholic extract is found to have polymethoxyflavones, Flavonoid, glycosides and Limonoids [18]. Nimbuka (*Citrus aurantifolia*)-It mainly contains carbohydrates, fiber, and proteins. It is also rich in Vitamin C, beta-carotene, organic acids like citric, malic, oxalic, quinic, malonic. It contains essential oils, flavonoids and coumarins [19].



**Fig. 4:** HPLC of Juice of *Nimbuka* Fruit for Ascorbic acid and Citric Acid Percentage



**Fig. 5:** HPLC of Juice of *Jambhira* Fruit for Ascorbic acid and Citric Acid Percentage



**Fig 6:** HPLC of Juice of *Matulung* Fruit for Ascorbic acid and Citric Acid Percentage

**Table 3:** Percentage of Ascorbic Acid and Citric Acid Juice in Different Citrus Fruit

Test performed	<i>Mahalung</i> Fruit Juice	<i>Jambira</i> Fruit Juice	<i>Nimbuka</i> Fruit Juice
Ascorbic Acid	0.84 ppm / 0.00084 %	1.26 ppm / 0.000126 %	1.20 ppm/ 0.000120 %
Citric Acid	13926.91ppm/ 1.3926%	14983.96 ppm/1.4983 %	14085.71 ppm/1.4085 %

Lime fruit juice content more percentage of citric acid than *jambira* and *matulung* which is also supported by more acidic pH of Lime fruit juice. Lime fruit content many organic acids like citric, malic, oxalic, quinic, malonic. *Jambira* fruit juice content more percentage of ascorbic acid than *nimbuka* and *matulung* fruit juice. Due to less acidic nature and easy availability of *matulung* and *jambira*, it was commonly used during the *Samhita* period. Later easy availability, large scale production with maximum yield of juice may lead to use of *Nimbuka* from 8<sup>th</sup> century.

There is need to study the effect of acidic nature of different fruit juices on physicochemical parameters of metal, minerals to ascertain difference caused due to change in juice of citrus fruit variant.

### Conclusion

Drugs mentioned under *amla varga* were used to carry out *shodhana*, *bhavana* and *jarana* like procedure on metal, minerals to make them bioassimable. Citrus fruits were commonly used herbs from *amla varga*. Ancient scholars also included other new variant in various pharmaceutical procedures depend upon availability, large scale production with maximum yield of required product as seen in case of Citrus fruits. There is need to study the effect of acidic nature of different fruit juices on physicochemical parameters of

metal, minerals to ascertain difference caused due to change in juice of citrus fruit variant.

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