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**EMBLICA OFFICINALIS (AMLA): PHYSICO-CHEMICAL AND FATTY ACID ANALYSIS FROM ARID ZONE OF RAJASTHAN**

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**ABSTRACT**

Fatty acid profiles of seed oil of *Embllica officinalis* species having 18% or more fixed oil (brownish-yellow) in colour in their seed/kernel was examined. Saponification number (SN), iodine value (IV) and acid value (AV) of was also determined and they varied from 155, 125.8 and 3.1 respectively. The fatty acids composition of *Embllica officinalis* have been analysed as their phenacyl ester (FAPE) by High Performance Liquid Chromatography (HPLC).The oil has been found rich in unsaturated acids. Most predominant polyunsaturated fatty acid (PUFA) was linoleic acid (18:2n-6) or omega-6. Oleic acid or omega-9 was also found as major fatty acid.

**Key Words:** *Physico-Chemical Characteristics, Fatty Acid Phenacyl Ester, Omega-6, Medicinal Drug*

**INTRODUCTION**

The *Embllica officinalis* is the medium to large deciduous plant of Euphorbiaceae family. Amla is a medium-sized deciduous tree (reaching 7 to 19 m in height) with gray bark and reddish wood which successfully grows in variable agro-climatic and soil conditions. Amla leaves are feathery, linear oblong in shape and smell like lemon. The flowers are greenish yellow in colour which starts appearing in the beginning of spring season.The *Embllica officinalis* fruit is light greenish yellow, spherical and appear to be much heard and the taste of fruit is sour or bitter. fruits globose, fleshy, pale yellow with six obscure vertical furrows enclosing six trigonous seeds in 2-seeded 3 crustaceous (Indian Medicinal Plants (1997)). It is a potential crop which grows in the marginal soils and various kinds of degraded lands such as salt-affected soils, salines and dry and semi-dry regions. It is common all over tropical and sub-tropical India and also found in Burma (Dey KL (1896)), it is abundant in deciduous forests of Madhya Pradesh also grows in tropical and subtropical parts of Ceylon, Malay Peninsula and China (Thakur et al., (1989)).

This plant was profound medicinal used in anaemia, jaundice, dyspepcia, haemorrhage disorders, diabetes, asthma and bronchitis (Tripathi KP (2003)). Medical studies conducted on Amla fruit suggest that it has antiviral properties (Udupa KN (1985) ) and also functions as an antibacterial and anti-fungal agent (Treadway L (1994)).

The fruit is occasionally pickled or preserved in sugar. When dry it is said to be gently laxative (Drury CH (1873)), according to some sources the fresh fruit is also laxative The bark partakes of the astringency of the fruit. A decoction and evaporation of the root solution produces an astringent extract equal to catechu (Nadkarni and Nadkarni, 1999). Photoaging of the skin is a complex biologic process affecting various layers of the skin with major changes seen in the connective tissue within the dermis. *Embllica* was shown to reduce UV-induced erythema and had excellent free-radical quenching ability, chelating ability to iron and copper as well as MMP-1 and MMP-3 inhibitory activity (Chaudhuri et al., (2003)).

Fatty acids are the basic building blocks of all lipids. They are generally constituted of a linear chain of 16 to 22 carbon atoms, with zero to six double bonds of *cis* or *trans* configuration. Omega-3 ( $\omega$ -3) and omega-6 ( $\omega$ -6) fatty acids are unsaturated "Essential Fatty Acids" (EFAs) that need to be included in the diet because the human metabolism cannot create them from other fatty acids. Fatty acids are traditionally analysed as methyl esters (FAME) by gas chromatography (GC), but it is usually considered that such derivatives are not suitable for locating double bonds or other centre of unsaturation. Thus the present study deals with the identification of fatty acid by HPLC which operate at ambient temperature so there is

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little risk to sensitive functional groups (Knothe G and Derksen JTP (1999) and Gunstone FD and Padley FB (1997)).

*Emblica Officinalis* is found natively in India. The literature survey revealed that there are no sufficient studies carried out regarding the fatty acid composition of Amla. So the present study was hence determined the physico-chemical examinations and fatty acid composition by standard TLC and HPLC methods.

**MATERIALS AND METHODS**

The essential oil of The *Emblica officinalis* (Amla) fruit used in this study was brought from local place. The impurities were removed from the fruits and the cleaned, dried and crushed seeds were extracted with petroleum ether (40-60°C) in a Soxhlet extractor for 6-7 hours and the seed oil was weighed after evaporation. Fatty acid phenacyl esters were prepared by dissolving sample of free fatty acids in methanol and neutral it with KOH solution (with the help of phenolphthalein as an indicator). The mixture is dried under nitrogen on a rotary evaporator under reduced pressure. Then it is mixed with a mixture of 0.1 ml of 2mM 18-crown-6 in acetonitrile and 0.1 ml 4-bromophenacyl bromide. The mixture is heated at 80°C for 15 minutes in mixing gently several times and then mixture is cooled, diluted with acetonitrile. Analytical values of the oil and seed were determined using standard American Oil Chemist’s Society (AOCS) methods (Official Methods and Recommended Practices of the American Oil Chemists’ Society (1998)). The results are shown in Table 1.

**TLC analysis**

Thin Layer Chromatographic (TLC) investigation of the fatty acids present in the oil of was done in various solvent systems by converting the acids into their corresponding phenacyl esters.

**Analysis of Fatty Acid phenacyl Esters by HPLC**

For the analysis, a modified HPLC method (with gradient elution) was used. The equipment included Gilson HPLC with a degasser, a binary pump and a column (900×6.4 mm) was packed with  $\mu$ Bondapack C-18 and was eluted with acetonitrile-water in the proportions 67:33 (by volume) initially and is gradually increased to 74:26 in 10 minutes then gradually increased in another 15 minutes which is also changed to 97:3 in another 15 minutes at flow rate of 2ml/minute, and detection was completed with UV/VIS detector.

Standard phenacyl esters of caprylic, nonanoic, capric, undecanoic, lauric, myristic, palmitic, stearic, oleic, arachidic and behenic acids (Sigma Chemical Company, USA) were used for identification of the peaks under the same set of conditions and by peak enhancement method. The peak area percentages were taken as weight percentages. The fatty acids present in the lipid under investigation were thus identified by comparison of relative retention time and peak position. The percentage of the acids was computer estimated from the HPLC peaks. The fatty acid composition is given in Table 2.

**Table 1:** Physico-chemical characteristics of the oil of *Emblica officinalis*.

Seed properties		Oil properties	
Moisture content (% by w)	75	Melting point (°C)	36
		Refractive index (40°C)	1.11
Oil content (% by w)	18	Saponification Value (mg/g KOH)	155
		Un-saponified matter (% w/w)	2.99
Protein content (% by w)	9.8	Acid value (mg/g KOH)	3.1
		Iodine Value (g I <sub>2</sub> /100 g)	125.8

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### RESULTS AND DISCUSSION

The *Embllica officinalis* (Amla) fruit contain a fairly good amount of oil (yield 18 %). The oil is of brownish yellow color and is liquid at room temperature. The oil contents and physico-chemical properties of the Amla oil are presented in **Table 1**.

**Table 2:** Fatty acid composition of the *Embllica officinalis* Seed oil by HPLC.

Fatty Acid	Obtained % by weight
Lauric (C12:0)	nil
Myristic (C14:0)	3.6
Palmitic (C16:0)	2.3
Stearic (C18:0)	3.1
Oleic (C18:1)	<b>26.4</b>
Linoleic (C18:2)	<b>51.0</b>
Linolenic(C18:3)	11.8
Total saturated FA (TSFA)	<b>9.0</b>
Total unsaturated FA (TUSFA)	<b>89.2</b>

Values in bold indicate high values

### Chromatographic Examinations

**TLC analysis:** The fatty acid phenacyl esters mixture obtained from the oil was subjected to TLC examination and the fatty acid composition was identified by comparing the  $R_f$  values of phenacyl esters of standard fatty acids in different solvent systems. From the results, it may be suggested that the oil contains myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1).

**HPLC analysis:** The HPLC analysis of the seeds collected from arid zone of Rajasthan state shows that the plant seed oil has different type variable fatty acid composition. The total percentage (9.0%) of saturated fatty acids was found in which myristic, palmitic and stearic acids were in the range of 3.6%, 2.3% & 3.1% respectively. Although its higher amount in some species (Pathak et al., (2003), Singh V and Singh HK (2002) and Srivastava RP (1964)). The major unsaturated fatty acids like oleic, linoleic acid ( $\omega$ -6) were found. Regarding the monounsaturated fatty acid oleic acid (26.4%) was dominant fatty acid in this species. In the polyunsaturated fatty acids (PUFAs), C18:2 and C18:3 acids level was in the range of 51.0% and 11.8% respectively. In this study linoleic acid ( $\omega$ -6) was found in high percentage.

*Embllica officinalis* (Amla) fruit is the potential source of linoleic acid ( $\omega$ -6). The objective of this research is the determination of fatty acids composition (major + minor) in Amla fruit oil of arid or semi arid zone of Rajasthan. In outcomes of this study reported here will be a beneficial point for future scientific basis for use of the seeds, both in the human and commercial or industrial purposes.

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