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FATTY ACID PROFILING IN DIPLOID (n = 12) AND TETRAPLOID CYTOTYPES (n = 24) OF *PHYSALIS ANGULATA* LINN. FROM RAJASTHAN BY GAS CHROMATOGRAPHY

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ABSTRACT: Present paper reports the chemical constituents in the fatty acids obtained from the whole plant of diploid and tetraploid *Physalis angulata* Linn. collected from hot desert areas of Rajasthan. The fatty acid analysis was carried out by gas chromatography-flame ionization detector (GC-FID). Meiotic studies confirm the ploidy level of the plant. Diploid (n = 12) cytotype is reported from Udaipur district and tetraploid (n = 24) cytotype from Sri Ganganagar. There is a considerable difference in the fatty acid constituents in the cytotypes. Major constituents of fatty acids are Oleic acid (24.93%, 2x; 18.71%, 4x); capric acid (13.4%, 2x; 0.14%, 4x); Palmitic acid (6.966%, 2x; 12.38%, 4x); Linoleic acid (5.5%, 2x; 9.76%, 4x) and Linolenic acid (6%, 2x; 11.67%, 4x). The main objective of the object work was to compare the fatty acid composition within two cytotypes. Such analysis of plants is very important on commercial level for the productions of cosmetic and has great interest in pharmaceutical companies for the production of drugs.

INTRODUCTION: *Physalis angulata* Linn. contains bioactive lipids which act as skin barriers, anti-irritant and anti-inflammatory agents. Fatty acids are required for normal well being of the body; these must be obtained from the diet as cannot be synthesized by the body. Fatty acids are the aliphatic monocarboxylic acids that work as the building blocks of lipids. Fatty acids can be classified into two main groups Saturated fatty acids (SFA, without any double bond) and unsaturated fatty acids (UFA, with double bonds). Unsaturated fatty acids are further divided into two a) monounsaturated fatty acids MUFA, with one double bond; b) polyunsaturated fatty acids PUFA, with more than one double bond.

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Each fatty acid has its own discrete biological activity [4, 14]. All higher organisms and plants contain lipids which are the chief source of substantial quantities of PUFA. The omega-6 (n-6) and omega-3 (n-3) fatty acids are the two principal group of PUFA derived from α-linolenic acids (18:3 n3) and linoleic acid (18:2 n6), respectively. These two parent fatty acids can only be naturally synthesized in plants and therefore, have become the essential dietary components for animal and human diet for maintaining optimum health [3, 5].

There are many methods to identify fatty acids and their derivatives (fatty acid methyl esters, FAME) like gas chromatography-mass spectrometry (GC-MS), gas chromatography-flame ionization detector (GC-FID), high performance chromatography (HPLC), nuclear magnetic resonance (NMR) spectroscopy and silver performance liquid chromatography. HPLC is the most commonly employed tool for the analysis of the pharmaceutical products but the method is not successful in the quantitative analysis of fatty acids

due to the absence of chromophores or fluorescent functional group [17]. Most of these techniques use derivatization reagents. GC is widely adopted as highly applicable tool in micro scale analytical work in analysis of fatty acids. GC analysis of fatty acids require derivatization as the boiling point of the fatty acids is very high which makes them difficult to evaporate and has low FID response [10]. Derivatization is time consuming process, laborious, tedious and lowers the accuracy and precision.

These derivatization reagents also sometimes interact with the active drug compound and cause changes in the results. So it is very important to use direct determination of the fatty acids without using any derivatization agents. Analysis of underivatized acids was first carried) by using Gas chromatography in 1956 [7, 8]. After three years, same authors published another article presenting the GC analysis of fatty acids in the form of methyl esters. In early days this technique has its own limitations in the form of large amount of sample requirement and sample wastage. But today the technique is much developed with fully automated computerized methods. A derivatization free GC-FID method was developed which resulted in good recoveries and reproducibility [11].

Plant Material: Plant material was collected from Udaipur and Sri Ganganagar districts of Rajasthan. Meiotic studies were carried out to confirm the ploidy level. Diploid cytotype was collected from Udaipur and tetrapliod cytotype from Sri Ganganagar. Plant materials were washed with water and shade dried at room temperature. The dried plants were crushed into powder for further analysis.

Chemicals and Reagents: HPLC grade n-Hexane, Toluene, activated Sodium Sulfate and GR grade Petroleum ether were purchased from Merck. Suprapure Sulfuric acid and Pestanal Grade Metanol from Sigma. Trans Methylene mixture (15ml Methanol, 7ml Toluene and 0.75ml Sulfuric Acid) was used. Certified reference material FAME-37 MIX SUPELCO was purchased from Sigma Aldrich Chemical Company, containing methyl esters of fatty acids including key monounsaturated and polyunsaturated fatty acids.

Preparation of Methy-esters of Fatty Acids: Trans-methy mixture was prepared by mixing 150ml of methanol and 70ml of toluene and adding 7.5 ml of concentrated sulphuric acid. 10-12mg of fat was added to 10ml of transmethylation mixture. This mixture was heated in water bath under reflux for 90mins. After cooling, 10ml of petroleum ether (40-60 °C) and 10ml of water was added. The mixture was shaken well and the layers were allowed to separate. The aqueous layer was pipetted out using fine pipette. The washing was repeated using 10ml of water. Afterwards, 2-3g of anhydrous sodium sulphate was added to remove the moisture. Decanted the clear ether layer and evaporated to dryness. Residue was dissolved the residue in 0.3ml of petroleum ether for gas chromatography.

GC Analysis of Flames: Gas chromatography coupled with flame ionization detection (GC-FID) is widely used, as it is rapid and efficient method. Moreover, separation, quantification and identification of long chain fatty acids mixture, which is done by GC-FID, are utilized to acquire the information about various biological functions. Determination of fatty acid profiles in nutritional and clinical research with precision and fastness has become popular for human health and basic research.

The operating conditions used to examine methyl esters of fatty acids are as follows. Fatty acids were converted into methyl esters (FAMEs) before gas chromatography (GC) analysis according to the standard methods by Ranganna (1986). Quantitative determinations of FAMEs were conducted by using Gas Chromatograph with flame ion detector (GC-FID) and capillary column HP-88 agilent technologies (100×0.25mm×0.20µ). The injection volume was 1.0µL, with split mode of injection, oven temperature was kept at 250 °C and helium was used as carrier gas. The temperature programme increased from 40 °C to 140 °C. The retention time of the FAMEs was compared with that of the standards (FAME-37 MIX SUPELCO) for the identification and quantification.

Fatty Acid Analysis: The current study evaluated the Fatty acid composition of diploid [18] (n = 12) and tetraploid (n = 24) cytotypes of *Physalis angulata* Linn. Saturated fatty acids (SFAs),

monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) were analyzed separately (**Table 1**). The amount of SFAs and MUFAs were higher in diploid cytotype but the amount of PUFAs was higher in tetraploid cytotype. The chromatogram of fatty acid mixture displays number of peaks. The resolution of the peaks is very high, the peaks of saturated and unsaturated fatty acids do not coincide and full picture of analysis of fatty acids is clear (**Fig. 1A** and **1B**). The fatty acid composition of *P. angulata* contains a healthy mixture of all type of fatty acids, saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids. The principle fatty acid among saturated fatty acid was Capric acid (13.38%) in diploid cytotype and Palmitic acid (12.38%) in tetraploid cytotype; among monounsaturated fatty acids Oleic acid in both diploid (24.93%) tetraploid cytotypes (18.71%). Among polyunsaturated fatty acids linolenic acid in both diploid (6%) tetraploid cytotypes (11.67%).

TABLE 1: DIVERSITY IN FATTY ACID PROFILE OF TWO CYTOTYPES (DIPLOID AND TETRAPLOID) OF *PHYSALIS ANGULATA* LINN. FROM RAJASTHAN

Fatty Acid	*Physalis angulata* Linn. (Diploid %)	*Physalis angulata* Linn. (Tetraploid %)
	Saturated Fatty Acids	
Butyric Acid	0.606	0.088
Caproic Acid	0.265	0.058
Caprylic Acid	0.480	0.115
Capric Acid	13.380	0.140
Undecanoic Acid	Nd	Nd
Lauric Acid	11.257	10.212
Tridecanoic Acid	Nd	0.143
Myristic Acid	4.460	7.066
Pentadecanoic Acid	0.913	1.289
Palmitic Acid	6.696	12.382
Heptadecanoic Acid	1.340	Nd
Stearic Acid	0.468	0.557
Arachidic Acid	Nd	Nd
Heneicosanoic Acid	0.669	0.340
Behenic Acid	4.986	4.122
Tricosancic Acid	1.273	1.164
Lignoceric Acid	2.696	6.023
	Monounsaturated Fatty Acids	
Myristoleic Acid	0.080	0.128
Cis-10 pentadecanoic Acid	Nd	Nd
Palmetoleic Acid	2.231	Nd
Cis-10 heptadecanoic Acid	Nd	Nd
Oliec Acid	24.935	18.710
Cis-11-Eicosanoic Acid	5.082	5.841
Erucic Acid	Nd	Nd
Nervonic Acid	2.292	2.148
	Polyunsaturated fatty acids	
Linoleic Acid	5.503	9.760
Y-linolenic Acid	3.508	5.548
Linolenic Acid	6.004	11.676
Cis-11,14-Eicosadenoic Acid	Nd	0.332
Cis-8,11,14-Eicosatrinoic Acid	Nd	0.172
Cis-11,14,17- Eicosatrinoic Acid	0.140	Nd
Arachidonic Acid	0.369	1.384
Cis-13,16-Docosadenoic Acid	Nd	Nd
Cis-5,8,11,14,17-Ecosapentenoic Acid	Nd	Nd
Cis-4,7,10,13,16,19-Docosahexanoic Acid	0.429	0.594
	Trans fatty acids	
Trans Elaidic Acid	Nd	Nd
Trans Linolelaidic Acid	Nd	Nd

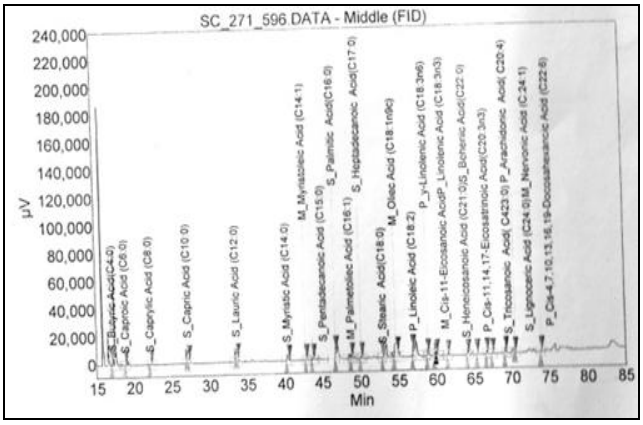

FIG. 1A: GC-FID CHROMATOGRAM OF FATTY ACID PROFILING IN DIPLOID CYTOTYPE OF *PHYSALIS ANGULATA* LINN.

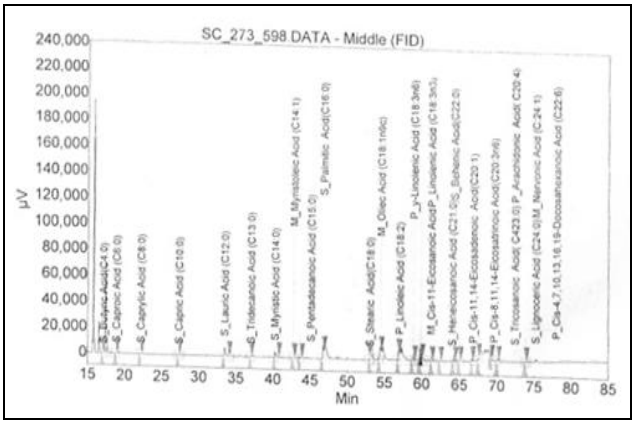

FIG. 1B: GC-FID CHROMATOGRAM SHOWING FATTY ACID PROFILING OF TETRAPLOID CYTOTYPE OF *PHYSALIS ANGULATA* LINN.

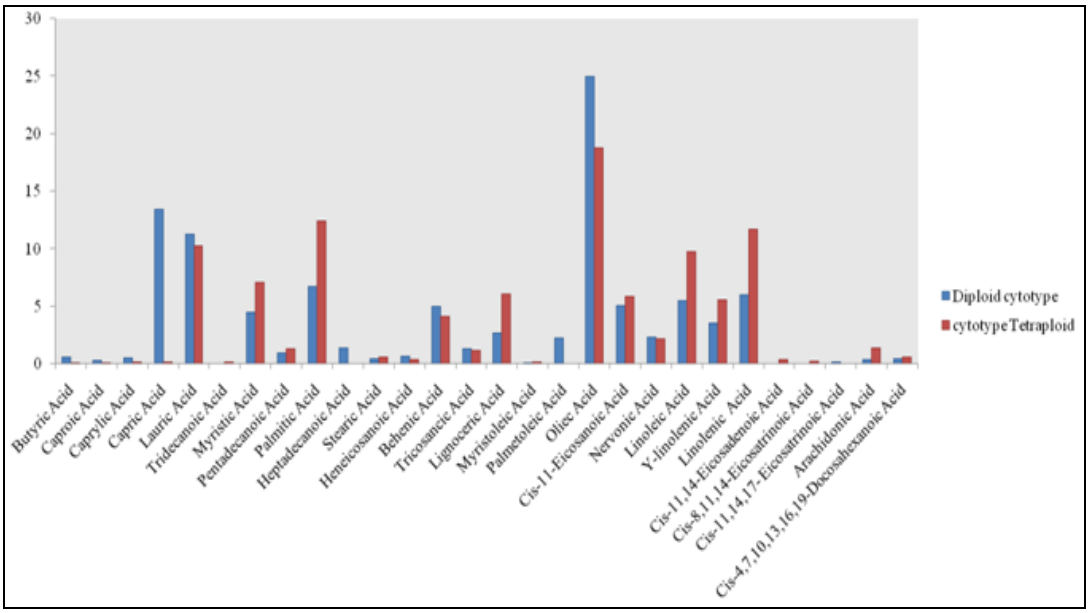

FIG. 1C: BAR GRAPH SHOWING COMPARISON OF FATTY ACID PROFILING IN DIPLOID AND TETRAPLOID CYTOTYPES OF *PHYSALIS ANGULATA* LINN.

Earlier there are few reports of fatty acid profiling of *Physalis* species. Quresh studied the quantification of methyl esters of fatty acids of *Physalis minima* by GC-MS [13]. Rodrigues *et al*., 2009 studied essential fatty acids from fruits *of Physalis peruviana* Linn. [15] Fatty acids composition during natural aging of seeds of *Physalis philadelphica* Lam. was also studied [9]. The present research is under taken to study the total fatty acid profiling of *Physalis angulata* Linn. as there is no report on the fatty acid composition of *Physalis angulata* Linn. Both diploid and tetraploid cytotypes are compared for useful for their exploitation. The results suggest the utilization of both the cytotypes as new commercial source of fatty acids with important medicinal and nutritional properties.

Saturated fatty acids with less than 12 carbon atoms are found in both the cytotypes. The dominating SFA were Capric acid (13.38%) in diploid cytotype and Palmitic acid (12.38%) in tetraploid cytotype. MUFAs are naturally present in whole fat milk products, red meat, nuts and dry fruits, olives and avocados. The amount of Oleic acid is higher in diploid cytotype (24.93%) than the tetraploid cytotype (18.7%). PUFAs are found in grapes, almonds, oils (coconut, safflower, olive, rice brain oil, hemp oil), pumpkin seeds and wheat germ. The most abundant PUFAs were Linolenic acid and Linoleic acid. The amount of Linolenic acid and Linoleic acid was reported to be more in the tetraploid cytotype (11.67% and 9.76% respectively) as compared to diploid cytotype (6% and 5.5% respectively).

PUFAs have a very diverse effect on human health. These help in the prevention of cardiovascular diseases, cancer and coronary heart diseases. Their non-substitutable roles in many biological pathways are crucial [1, 3]. These are also very helpful in treatment of diabetes type two, hypertension, autoimmune diseases, renal diseases and rheumatoid arthritis.

CONCLUSION: Both the cytotypes of *P. angulata* Linn. are source of important fatty acids including all the groups of SFAs, MUFAs and PUFAs. However the composition of varies in the two cytotypes of the plant. From the results it is concluded that the analyzed tetraploid cytotype is characterized by higher amount PUFAs.

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CONFLICT OF INTEREST: Authors declare no conflict of interest.

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