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IDENTIFICATION OF LINOLENIC ACID FROM *PORTULACA OLERACEA* LINN., BY HPLTC

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ABSTRACT: Purslane *(Portulaca oleracea L.)* is an herbaceous weed belonging to the family Portulacaceae. It has high nutritional value and possesses potent pharmacological actions such as analgesic, hepatoprotective, anti-inflammatory, wound healing, bronchodilator, neuropharmacological, anti-diabetic, antioxidant and antihypertensive. It is listed in a World Health Organization database because of these many health benefits. From the phytochemical investigations it is revealed that Purslane has the presence of antioxidants (vitamins A and C, α- tocopherol, β-carotene, and glutathione), linolenic acid and omega-3 fatty acids. This study aimed was to identify the linolenic acid content of Purslane extracts using HPTLC. In this study, three different solvents (Petroleum ether, methanol, and water) were used for the extraction of dried leaves and were compared. Successive solvent extraction was carried out with increasing polarity of the solvents. The extracts were analyzed for the identification of linolenic acid, and its concentration was determined using HPTLC. For HPTLC analysis, the mobile phase comprising of chloroform: methanol: water: ammonia (65:24:4:0.4 v/v/v/v) was used and the Rf value for standard linolenic acid was found to be 0.499. Linolenic acid was detected in all three extracts, and the amount of linolenic acid in petroleum ether extract was less as compared to methanolic extract; however, in aqueous extract negligible amount was detected. From the results, it was revealed that the methanolic extract exhibited the highest linolenic acid content; hence methanolic extract of dried leaves serves as a better solvent for the identification of linolenic acid.

INTRODUCTION: Plants are an inexhaustible source of medicinal drugs. Several countries in the world use many distinct chemicals derived from plants as vital drugs [1]. Plants and plant communities give the required habitat for the wildlife and fish population. Therefore, plants and their products have continuously influenced human culture [2]. Medicinal plants play a significant role in the maintenance of human health throughout the world. They are of crucial importance in poor communities. Medicinal plants additionally play a cultural and further as imperative economic role. Knowledge of their usage is well-known and effectually is believed, supported a protracted history of use [3]. In Indian systems, plants and their extracts are an important part of the health care system. Pharmaceutical companies are investing enormously in research on the potential benefits of medicinal plants. Health care systems even today rely on plant-based ancient drugs [4]. The herbal industry shares about US$100 billion, with decent growth potential worldwide. The WHO has stated that trade in medicinal plants, herbal raw materials, and herbal drugs are growing at an annual growth rate of about 15% [5].

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Even today some novel molecules developed as drugs have natural products as the probable source.

Many herbal plants are also utilized as flavoring medicines, increasing the demand for such plants. To fulfill the increased demands and because of the commercialization of herbal medicines there, is a great decline in the quality of these medications. There are no stringent laws governing the quality, safety, and efficacy issues of herbal medicines. For standardization of herbal drugs, flavoring agents, well-designed strategies, methodologies need to be developed and adopted. Standardization plays a critical role in assuring that good quality products reach the market and for these, numerous methods are employed.

Preliminary phytochemical screening, marker compound quantification, fingerprint identification for plant extracts and polyherbal formulations are required for assuring good quality products with bonded constituents [6, 7].

Phytochemicals are naturally occurring chemical compounds in plants. Phytochemicals are usually used to refer to those chemicals which are of plant origin and which could have an effect on health, however are not established as essential nutrients. Whereas there is torrential scientific and government support for recommending diets wealthy in fruits and vegetables, there is solely restricted proof that health benefits are because of specific phytochemicals. Phytochemicals isolated from plants used as traditional medicines are considered good alternatives. Most plants are even currently being in use for their medicative therapeutic importance by numerous social group communities, and currently, it's been established that the medicative property of a plant is because of bound chemicals in it. It has been calculable that concerning 80% of modern medicines are derived from plants [8]. Phytochemicals are non-nutritive plant chemicals that have disease preventive properties. Plants produce these chemicals to shield themselves however, recent studies' research analysis demonstrates that several phytochemicals will protect humans against diseases [9, 10]. For the estimation of phytochemicals and biochemical markers, fingerprint analysis by High-performance thin layer chromatography (HPTLC) has become a good and powerful tool [11, 12-14].

HPTLC is a routine analytical technique for herbal drug standardization due to its advantages of low disbursement, high sample outturn, speed, simplicity, and need for minimum sample clean up, reproducible, reliable, accurate, and robust [10, 14]. It includes the little quantity of mobile phase, the possibility of the study of many samples simultaneously, and cloudy samples and suspensions can also be analyzed directly by HPTLC [7, 15].

Purslane is a weed belonging to the Portulacaceae family. The leaves are an upscale source of antioxidants, Linolenic acid, beta-carotene, omega-3 fatty acids. The plant has analgesic, hepatoprotective, anti-inflammatory; wound healing, bronchodilator, neuropharmacological, antidiabetic, antioxidant, and antihypertensive activity [16]. Omega-3 fatty acids belong to a group of polyunsaturated fatty acids essential for human growth, development, prevention of numerous cardiovascular diseases, and maintenance of a healthy immune system [17]. Our bodies do not synthesize omega-3 fatty acids. So, omega-3 fatty acids need to be consumed from dietary sources. Omega-3 fatty acids have 18 to 24 chains of carbon atoms and contain three or additional double bonds at intervals in their fatty acid chain. Fish is the richest source of omega-3 fatty acids. Health authorities extremely advocate consuming fish often to fulfill our bodies' necessities of omega-3 fatty acids, as different sources are restricted and do not offer nearly the maximum amount of omega-3 fatty acids [18]. Purslane as an herbaceous plant has recently been known because of the richest vegetable source of alpha-linolenic acid, an essential omega-3 fatty acid. From the phytochemical investigations, it is revealed that Purslane has the presence of the antioxidants (vitamins A and C, α- tocopherol, β-carotene, and glutathione), linolenic acid, and omega-3 fatty acids [19].

The present study aims to identify and compare the HPTLC fingerprinting profile of linolenic acid for the extracts from three different solvents (Petroleum ether, methanol, and water) of plant Portulacaorelacea. There are papers involving the identification and determination of linolenic acids by oxidations with alkaline permanganate solutions [20], TLC [21], GC-MS [22,] and HPLC [23-25], but none of

the paper reports the extraction of linolenic acid using different solvents and its identification by HPTLC.

MATERIALS AND METHODS:

Collection of the Plant Material: The leaves of *Portulaca oleracea* Linn., were collected from Saswad, Pune, Maharashtra, India. The plant was authenticated by A. S. Upadhye, Agharkar Research Institute, Pune.

Solvent Extract Preparation: The leaves were dried, powdered, and successively extracted by Soxhlet extraction with solvents of increasing polarity. The solvents beginning with petroleum ether, methanol, and water were chosen for extraction. The solvents were removed under reduced pressure on a rotary evaporator for complete drying. All the extracts were subjected to HPTLC analysis.

Preparation of Standard: Determination of the content of the Linolenic acid in plant material was performed by the external standard, using pure Linolenic acid procured from Sigma-Aldrich as standard. About 1 mg of pure Linolenic acid was taken and diluted with 1 ml of methanol (1mg/ml). This solution was used as a reference standard for HPTLC analysis.

Preparation of Sample: The obtained dried extracts 100 mg each were weighed accurately on Contech electronic balance and dissolved in 1 mL of appropriate solvent based on the extracted solvent and centrifuged at 3000 rpm for 5 min. These solutions were used as test solutions for HPTLC analysis.

HPTLC Analysis of Plant Extracts for Linolenic Acid: The optimized chromatographic conditions are reported in **Table 1**.

TABLE 1: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Stationary phase	Silica gel 60F_{254} TLC plate
Mobile phase	Chloroform: methanol: water: ammonia (65:24:4:0.4 v/v/v/v)
Plate Development Technique	Ascending development technique
Chamber Saturation Time	20 min
Temperature	Ambient Room temperature
Run distance	90 mm
Detection wavelength	Visible light, 254 nm and 366 nm
Derivatization	Sprayed with iodine

Table 2 represents the area and R_f value for linolenic acid obtained from leaf extracts of *Portulaca oleracea* and **Fig. 1a** – Represents the Densitogram of Reference Standard Linolenic acid, **b**- Densitogram of petroleum ether extract, **c**- Densitogram of methanolic acid extract, **d**- Densitogram of water extract.

TABLE 2: HPTLC ANALYSIS OF LEAF EXTRACTS OF *PORTULACA OLERACEA* FOR LINOLENIC ACID

Track	Peak	R_f	Area
Standard	1	0.499	0.00070
Petroleum extract	1	0.464	0.00001
Methanolic extract	1	0.448	0.00040

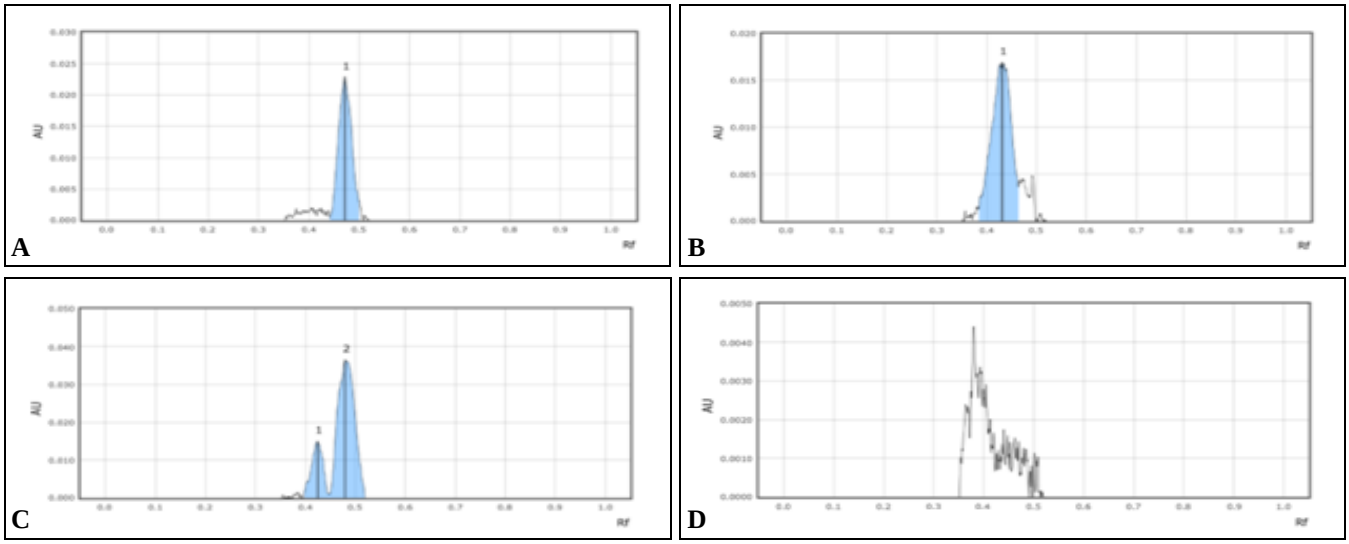

FIG. 1: A – REPRESENTS THE DENSITOGRAM OF REFERENCE STANDARD LINOLENIC ACID, B- DENSITOGRAM OF PETROLEUM ETHER EXTRACT, C- DENSITOGRAM OF METHANOLIC ACID EXTRACT, D- DENSITOGRAM OF WATER EXTRACT

RESULTS: The method developed was a normal phase HPTLC method using silica gel 60 F254 stationary phases precoated on the aluminium sheet for the analysis [26]. The mobile phase used Chloroform: methanol: water: ammonia in the proportion of (65:24:4:0.4 v/v/v/v), which gave good separation and identification of Linoleic acid (R_f=0.45) in methanolic and petroleum ether extracts of *Portulaca oleracea* shown in **Table 2**. The identity of Linoleic acid was confirmed by an overlay of spectrum chromatograms obtained with the Camag TLC scanner. Derivatization was performed in the visible and UV 254nm region as shown in **Fig. 2** and **3,** respectively, indicating the presence of linolenic acid in methanolic and petroleum extract.

The Linolenic acid present in petroleum ether, methanol, and water extracts of Purslane was compared with the Linolenic acid standard. The petroleum ether and methanol extracts show the presence and identity of Linolenic acid while it was absent in the water extract. As compared to petroleum extracts, methanolic extracts show better concentrations of Linolenic depending upon the area shown in **Table 2**.

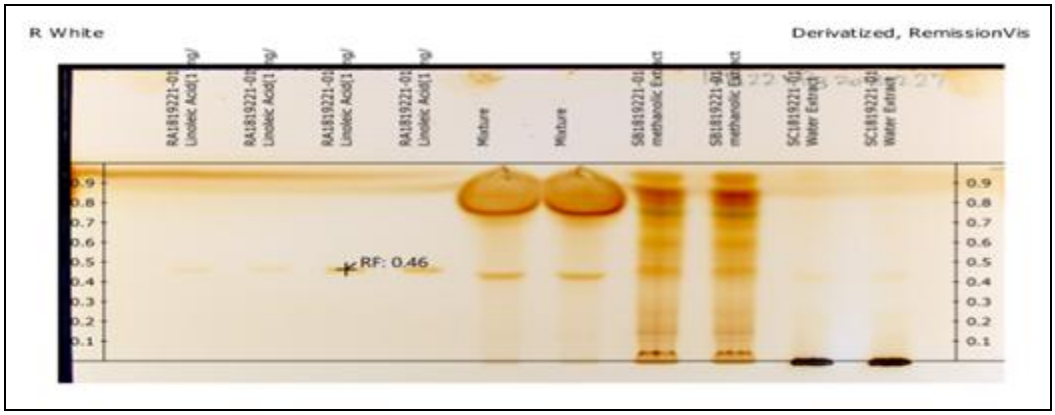

FIG. 2: CHROMATOGRAM OF DERIVATIZATION AT VISIBLE

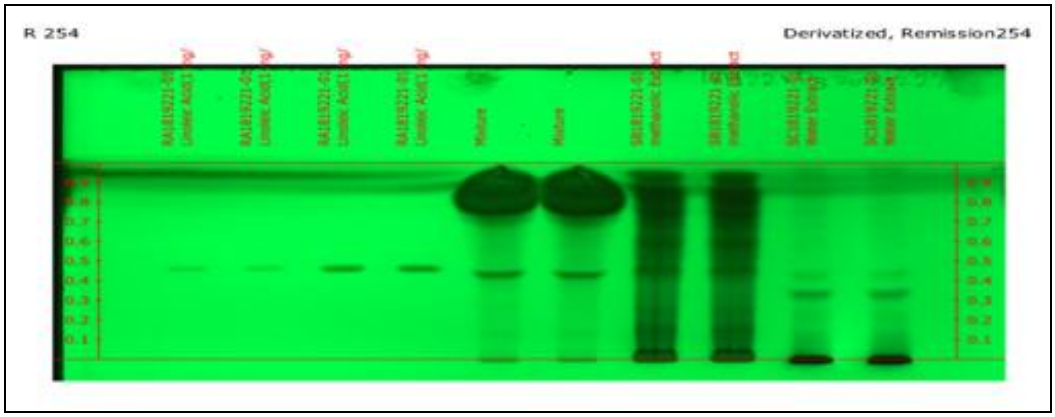

FIG. 3: CHROMATOGRAM OF DERIVATIZATION AT 254 nm

DISCUSSION: HPTLC analysis was carried out for the three different solvent extracts of *Portulaca oleracea* to investigate linolenic acid. Reference standard Linolenic acid was used to identify and compare the linolenic acid present in petroleum ether, methanol, and water extracts of Purslane. Scanning and analysis of samples after derivatization at different wavelengths (visible, 254, and 366 nm) is shown in **Fig. 1, 2, 3, 4,** and **5**. The optimized chromatographic conditions and R_f values obtained during this analysis is presented in **Table 1** and **2**. The presence of linolenic acid in all three solvent extract samples was confirmed based on the chromatogram obtained.

CONCLUSION: From the results, it was revealed that the methanolic extract exhibited the highest linolenic acid content; hence methanolic extract of dried leaves serves as a better solvent for identification and quantification of linolenic acid.

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