

# EXTRACTION AND TLC-BASED IDENTIFICATION OF VITAMIN E FROM *TERMINALIA CATAPPA* HULL: EVALUATION METHOD FOR A COST-EFFECTIVE APPLICATION

<sup>1</sup>Vaisakh. R, <sup>2</sup>Ramya. K, <sup>3</sup>Suja. G, <sup>4</sup>Nithyadevi. M, <sup>5</sup>Vidhya. V

<sup>1</sup>PG Student, <sup>2,3,4,5</sup>Assistant Professor

<sup>1,2,3</sup>Department of Biotechnology, <sup>4</sup> Department of Biochemistry, <sup>5</sup>Department of Microbiology

CMS College of Science and Commerce, Coimbatore, India

**Abstract:** Vitamin E is a commonly found antioxidant usually distributed in plant seeds and oils. However, the attention directed towards the Agricultural byproducts like a seed hull as a potential source of bioactive compounds is relatively limited. In this study, *Terminalia catappa* seeds hull was evaluated as an alternative Phytochemical source of Tocopherols and Tocotrienols using saponification assisted solvent extraction followed with qualitative screening by Thin Layer Chromatography (TLC) using two solvents and also comparing their effectiveness in separation. The aim of the study, is to emphasize the viable method of utilizing *Terminalia catappa* hull as a Vitamin E source and also to demonstrate the applicability of a cost-effective preliminary screening method.

**Index Terms:** Vitamin E, Solvent Extraction, *Terminalia catappa*, Saponification.

## 1. INTRODUCTION

Vitamins are indispensable micronutrients mandatory to the body in small amounts for appropriate growth, progress, and maintenance of healthiness. They do not offer energy directly, but they support the body utilize carbohydrates, proteins, and fats competently. The vitamins are the foundation of our life. These are low molecular weight organic compounds that come under the Xenobiotic compounds for many living beings like humans and other animals [1].

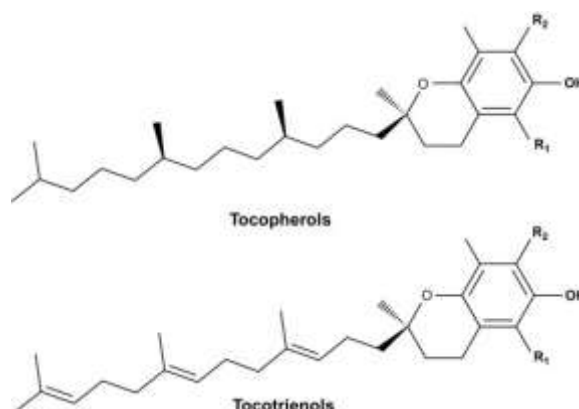


Figure 1 Chemical structure of Vitamin E

Evans and Contemporaries acknowledged the compounds within the vitamin E group and mentioned to them as Tocopherols (from Greek: Tocos—childbirth; pheros—to carry; ol—alcohol). Casimir Funk introduced the term “Vitamine” (from Greek, vita-life) for the accessory aspects under the postulation that they were all amines. The term, vitamin, continues to be used, although without the

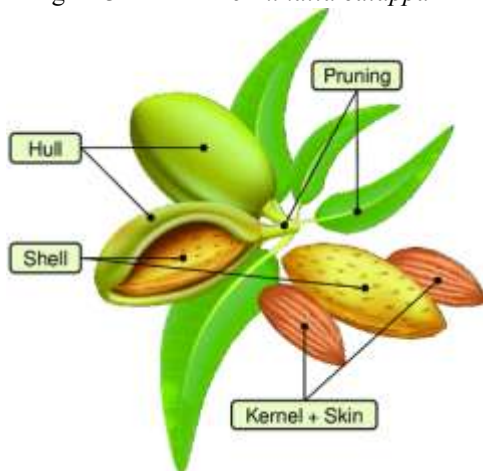
final letter “e.” Vitamins are categorized based on their biological and chemical activity, rather than their structural composition, which are shown in Figure 1 [2]. The term vitamin E refers to a collection of eight naturally occurring, fat-soluble compounds, which include four tocopherols and four tocotrienols. These are categorized as  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -. Notably, almonds and hazelnuts are rich in  $\alpha$ -tocopherol [1]. Alpha-tocopherol was the first vitamin E variant identified, and there are now eight unique variants known, composed of alpha ( $\alpha$ ), beta ( $\beta$ ), gamma ( $\gamma$ ), and delta ( $\delta$ )-tocopherols (T) as well as  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ -tocotrienols (T3), all of which are grouped under the umbrella of vitamin E [2]. Vitamin E is highly receptive to temperature, light, and alkaline surroundings. It is characterized as a biological antioxidant. By inhibiting lipid peroxidation, it defends against the influence of Polyunsaturated Fatty Acids(PUFA), reduces Platelet Hyper-aggregation, reinforces the walls of blood vessels, shields the body from Atherosclerosis, Coronary Artery disease, and various Cardiovascular conditions, and plays a pivotal role in hormone synthesis [1]. Nuts, seeds, and vegetable oils are rich in alpha-tocopherol, while green leafy vegetables and fortified cereals also provide substantial amounts [3].



Figure 2 Indian almond (*Terminalia catappa*)

*Terminalia catappa L.*, known as Indian Almond (Figure 2), is a member of the *Combretaceae* family. It is an underappreciated fruit that is abundant in antioxidants, vitamins, and pigments. The kernels/seeds are high in nutritional protein and essential amino acids, and their excellent digestibility could aid in endorsing growth and addressing undernourishment in impoverished and developing Nations [4]. The Indian almond is commonly enjoyed for its nuts, which are rich in Vitamin E [5]. Typically, the edible parts, such as the nuts and fruit, are treasured for their nutritional benefits, while components like the hull and shell are often deemed as waste, the parts of the fruit are mentioned in the Figure 3 [6] . This study presents an upfront and operative method for extracting Vitamin E from the fruit's hull and separating it through TLC. This approach may reduce waste from the fruit and create economic advantage.

Figure 3 Parts of *Terminalia catappa* fruit



## 2. MATERIALS AND METHODS

### 2.1 PLANT MATERIAL

*Terminalia catappa* fruit samples were collected from the premises of CMS College of Science and Commerce, Coimbatore to serve as the raw material for the extraction of Vitamin. The nut and hull were removed from the fruit, the hull was further shadow dried, finely ground to fine powder (Figure 4).



Figure 4 Powdered sample of *T. catappa* Hull

### 2.2 REAGENTS

L-Ascorbic acid, Sodium Sulphate anhydrous and Silica gel G, Potassium Hydroxide, Ethanol were purchased from Himedia. Hexane, Benzene, Chloroform, Diethyl ether of high purity was used for the extraction procedure. Mobile phases were prepared as hexane: Diethyl Ether and Benzene: Chloroform each in the ratio of 9:1.

### 2.3 EXTRACTION PROCEDURE

#### 2.3.1 Saponification

Saponification is a chemical process in which a triglyceride (such as those found in plant oils) reacts with a strong base to form glycerol and the salt of a fatty acid, known as soap [7]. To a saponification flask, 5grams of the powdered sample was transferred along with 1g of L-Ascorbic acid, 50ml of 100% ethanol and 15ml of 50% KOH was added. The flask was placed in a water bath at 74°C for 90 minutes with periodic shaking for the process of saponification. The flask was then cooled immediately using ice cold water [8].

#### 2.3.2 Solvent Extraction Method

Solvent extraction is a technique used to separate compounds based on their solubility properties. In this study, hexane was chosen as the solvent due to its ability to dissolve non-polar compounds such as Vitamin E. The saponified sample was transferred to a separating funnel along with 50 ml of 100% ethanol and 100 ml of distilled water. The funnel was closed and shaken vigorously for 2 minutes. The heat produced due to the shaking was then allowed to cool down to room temperature. A volume of 50 ml hexane was added and shaken well; the flask was let to stand until the hexane layer separated completely. The lower aqueous layer was discarded and 50 ml of hexane was again used for washing. The lower sample residues were discarded and 50ml of hexane was used for washing again. The separated hexane layer was washed with 100 ml of distilled water to remove the KOH from the funnel and hexane two more times. To check for the presence of KOH in the washed water, Phenolphthalein indicator was used. It was washed until no pink colour is observed. The hexane layer was filtered drop wise through a filter paper and funnel with 10 grams of Disodium sulphate, this is to remove water content completely from the hexane layer. The hexane extract was kept at 37°C to 40°C until the solvent evaporated completely [8].

## 2.4 ANALYTICAL PROCESS

### 2.4.1 Thin Layer Chromatography

Thin Layer Chromatography (TLC) is a chromatographic technique used to separate and analyse compounds based on their differential partitioning between a stationary phase and a mobile phase [9]. Silica gel G was prepared as a slurry. Hexane was added to the beaker to dissolve the extracted compounds to use as the samples for TLC. The hexane diluted samples were placed as spots in the Silica gel stationary phase using a capillary tube, as shown in the Figure 5. The freshly prepared mobile phases (hexane: Diethyl Ether and Benzene: Chloroform each in the ratio of 9:1) were taken in the coupling jar. The jar along with the TLC plates are left undisturbed for incubation until the mobile phase had completely run, after which the slides are shown in Figure 6. The plates are then carefully taken out, let to dry [10]. These plates were visualized under UV light to detect the presence of the target compound.



Figure 5 TLC slides with sample before run



Figure 6 TLC Slides after run with two different solvent systems

### 3. RESULTS AND DISCUSSIONS

#### 3.1 TLC Result Interpretation

The TLC of the obtained fractions revealed distinct bands under UV in both the Standard and sample tracks (Figure 7). The Vitamin E standard exhibited an R<sub>f</sub> value of 0.58 in the Benzene: Chloroform (B:C) and 0.66 in the Hexane: Diethyl ether (H: E) system. The test samples showed bands with the R<sub>f</sub> value in the range of 0.55 – 0.57 in B:C and 0.63 – 0.65 in Hexane: Diethyl ether. This close range between the standards and samples indicates the presence of Vitamin E compounds in the isolated extract.

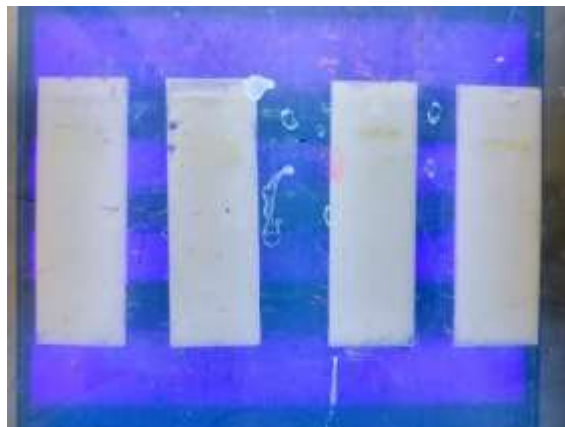


Figure 7 TLC plates under UV light

#### 3.2 Solvent System Comparison

Comparatively the Hexane: Diethyl ether system has moved the sample and standard compounds better than the Benzene: Chloroform which is confirmed by the relatively higher R<sub>f</sub> value. The enhanced migration of the compounds in the Hexane: Diethyl ether system was supported by the literature, that the migration is facilitated by the non-polar nature of the solvent which improved the solubility and reduced the interaction of the sample with the stationary phase (silica gel)[11]. Inclusive, the profiling by TLC after saponification and solvent extraction confirms that the extraction and speculative identification of Vitamin E in the sample is efficacious.

### CONCLUSION

The study demonstrated the effective extraction of Vitamin E from the unsaponifiable fraction of the sample through alkaline saponification which was followed by solvent extraction. The Thin Layer Chromatography analysis shows UV active bands along with R<sub>f</sub> values closer to the standard Vitamin E. Among the two mobile phase systems employed, which were Hexane: Diethyl Ether and Benzene: Chloroform both in 9:1 ratio, the Hexane: Diethyl Ether system divulged the better migration of the analyte, indicating enhanced chromatographic resolution of non-polar compound. This perceives migration of sample and standards with similar R<sub>f</sub> indicates the presence of Vitamin E in the extract. With this, the employed method extraction and analysis might be considered as suitable for qualitative identification of Vitamin E compound in the plant hull material.

### FUTURE SCOPE

Further studies can be tracked by experimenting the extraction processes like Soxhlet extraction for obtaining the compounds more precisely. Apart from the basic qualitative techniques like Thin Layer Chromatography (TLC), advanced techniques like High Performance Liquid Chromatography (HPLC) can be utilized. Additionally, the solvent systems can also be optimized and the usage of densitometric analysis can enhance the resolution and accuracy of such chromatographic identification. Such approaches can improve the validation of Vitamin E content in surplus plants. The utilization of the compounds obtained from such sources into extraction procedures can deal with the economic contests connected with the convention of commercially available products.

### BIBLIOGRAPHY

- 1) Słowik-Borowiec, M., Głąb, N., Stach, S., & Szpyrka, E. (2023). A Miniaturized Sample Preparation Method for the Determination of Vitamins A and E in Food Products. *Molecules*, 28(8), 3449. <https://doi.org/10.3390/molecules28083449>
- 2) Ahsan, H., Ahad, A., & Siddiqui, W. A. (2015). A review of characterization of tocotrienols from plant oils and foods. *Journal of Chemical Biology*, 8(2), 45–59. <https://doi.org/10.1007/s12154-014-0127-8>
- 3) Rizvi, S., Raza, S. T., Ahmed, F., Ahmad, A., Abbas, S., & Mahdi, F. (2014). The Role of Vitamin E in Human Health and Some Diseases, *Sultan Qaboos University Medical Journal*, 14, e157-e165.
- 4) Ramanan S, S., Arunachalam, A., Singh, R., & Verdiya, A. (2025). Tropical almond (*Terminalia catappa*): A holistic review. *Heliyon*, 11(1), e41115. <https://doi.org/10.1016/j.heliyon.2024.e41115>

- 5) Etienne, D., Christelle, K., Yves, N., Constant, K., Adama, C., Daouda, S., Ysidor, K., & Marius, B. (2017). Antioxidants Contents of Terminalia catappa (Combretaceae) Almonds Grown in Côte d'Ivoire. *Archives of Current Research International*, 10(3), 1–12. <https://doi.org/10.9734/ACRI/2017/37125>
- 6) Zannou, O., Awad, N. M. H., Ballogou, V. Y., Mohammed, S., Miassi, Y. E., Hounghédji, M., Dossa, K. F., Abdoulaye, A., Ghellam, M., Kpoclou, Y. E., Aïssi, M. V., Goksen, G., Koca, I., & Tahergorabi, R. (2026). Tropical Almond Tree (*Terminalia catappa* L.): A Comprehensive Review of the Phytochemical Composition, Bioactivities and Economic Potential. *Pharmaceuticals*, 19(1), 99. <https://doi.org/10.3390/ph19010099>
- 7) Delgado-Zamarreno, M., Bustamante-Rangel, M., Sanchez-Perez, A., & Hernandez-Mendez, J. (2001). Analysis of vitamin E isomers in seeds and nuts with and without coupled hydrolysis by liquid chromatography and coulometric detection. In *Journal of Chromatography A*. [www.elsevier.com/locate/chroma](http://www.elsevier.com/locate/chroma)
- 8) Souza, A. H. P., Gohara, A. K., Rodrigues, Â. C., Ströher, G. L., Silva, D. C., Visentainer, J. V., Souza, N. E., & Matsushita, M. (2014). Optimization conditions of samples saponification for tocopherol analysis. *Food Chemistry*, 158, 315–318. <https://doi.org/10.1016/j.foodchem.2014.02.008>
- 9) Kowalska, T., & Sajewicz, M. (2022). Thin-Layer Chromatography (TLC) in the Screening of Botanicals—Its Versatile Potential and Selected Applications. In *Molecules* (Vol. 27, Number 19). MDPI. <https://doi.org/10.3390/molecules27196607>
- 10) Santiago, M., & Strobel, S. (2013). Thin layer chromatography. In *Methods in Enzymology* (Vol. 533, pp. 303–324). Academic Press Inc. <https://doi.org/10.1016/B978-0-12-420067-8.00024-6>
- 11) Shormanov, V. K., Chernova, A. P., Shakina, A. C., Davydkina, A. E., & Elizarova, M. K. (2023). Study of 2,6-di(propan-2-yl)phenol stability in biomaterial. *Forensic Medical Expertise*, 66(3), 46. <https://doi.org/10.17116/sudmed20236603146>

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