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PHYSICO-CHEMICAL, PHYTOCHEMICAL AND HPTLC FINGER PRINTING ANALYSIS OF ALLIUM CEPA: A REPUTED HERBAL DRUG

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Abstract
Allium cepa is a biosynthetic laboratory of multitude of compounds that exerts physiological and therapeutic effect. Onions can act as a guard against many chronic diseases and are highly valued for their therapeutic properties in which the quality control of crude drug and its bio constituents is of paramount importance in ensuring its efficacy. The present work aims to develop quality control parameters of Allium cepa in terms of physico-chemical and phytochemical properties. Physico-chemical parameters such as volatile oil content, moisture content, ash content, fibre content, sugar content and solubility analysis were carried out. Qualitative analysis was also carried out along with Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) fingerprinting. Physico-chemical parameters of Allium cepa were established and HPTLC chromatogram was developed with alcoholic extract of Allium cepa using the mobile phase Ethyl acetate: methanol: water in the ratio 7.4: 1: 0.7. The present study on physico-chemical and phyto-chemical evaluation and HPTLC fingerprinting of Allium cepa provides useful information regarding quality control parameters and identifying parameters to substantiate and authenticate the drug. A specific and accurate HPTLC method was validated for its fingerprint analysis.

Keywords: Allium Cepa; Phsico-Chemical Analysis; Phytochemical, Analysis, HPTLC, Ingerprinting.

Introduction

Allium cepa isoneofleadingvegetablecropsintheworldandthephytochemicalandbiological characteristics of this species have been deeply investigated [1]. The traditional systems of medicine have become significantly more popular all over the globe because of the curative property, less toxic and minimal side effects. It is more widely used for the human ailments from time immemorial [2].Allium cepa has been traditionally used for its remedial characteristics in the management of various ailments [3]. Allium cepa is highly valued for its therapeutic properties. The onions ability to relieve congestions, especially in the lungs and bronchial tract is well-documented [4]. Onions contain large amounts of flavonoids such as quercetin, which is abundant in onions and protects against cataracts, cardiovascular diseases and cancer [5, 6]. In addition, compounds from dry outer scales of yellow onion bulbs were shown to have antioxidative and antibacterial activities [7, 8]. Many epidemiological studies confirmed that dietary consumption of onions is associated with a reduced risk of developing many forms of cancer and cardiovascular and neurodegenerative diseases [9, 10]. Its beneficial effect on health is attributed to high contents of biologically active phytomolecules, such as phenolic compounds, especially flavonoids, and several organosulfur compounds [11] possessing pre-biotic, anticarcinogenic, antithrombotic and antiinflammatory activities[12].The World Health Organization supports the use of fresh onions for the treatment of coughs, colds, asthma, bronchitis, appetite loss, relieving hoarseness and preventing atherosclerosis [13].

Allium cepa can be considered as a source of critical phytopharmaceutical agents with potential applications in emerging fields of interest. A profusion of literature has been revealed and published on

onion dealing with its chemical analysis. A study was conducted on four varieties of Allium cepa (red, violet, white, green) for their respective phenolic composition through high performance liquid chromatography (HPLC). Ferulic acid, gallic acid, protocatechuic acid, quercetin, and kaempferol were identified [14].Vazquez-Armenta et al. identified dipropyl disulfide and dipropyl trisulfide as the main constituents in onion oil [15]. Additionally, Dhumal et al. confirmed the presence of pyruvic acid, reducing, and non-reducing sugars in both red and white onion. The amounts (g/100 g FW) of reducing, non-reducing, and total sugars (6.69, 9.56 and 16.1 respectively) were higher in red onion compared to that of white onion (3.17, 7.17 and 10.4, respectively) [16].

Several herbal drugs in the market still cannot be identified or authenticated based on their morphological or histological characteristics. Use of wrong drugs may be ineffective or it may worsen the condition [17].The mode of preparation and plant used in traditional medicine varies from place to place. Climatic, geographic and varietal differences might also play an important role in the composition of phytochemical components of onions. Therefore, there is a need of authentication for successful and reliable clinical applications on drugs. The objective of the present study is to develop the quality control parameters of Allium cepa in terms of physico-chemical and phytochemical properties for establishing its therapeutic properties and efficacy.

Materials and methods

Raw material

The specimen used in the present study was bulb of onion collected from Drug Standardization unit, Government Ayurveda College, Thiruvananthapuram.Theonion bulb was washed with freshly

Prepared sterile distilled water and the outer covering of the bulb was peeled off. The fleshy part of the onion was rewashed with freshly prepared sterile distilled water and weighed.

Chemicals

All the chemical reagents used in the study were purchased from Sigma Aldrich and were ofHPLC chromatographic grade. $60F_{254}$precoated silica gel TLC plates were purchased from Merck, Darmstedt (Germany).

Experimental design

Volatile oil content, moisture content, ash content, fibre content, sugar content and solubility were determined using standard methods as per API [18](Ayurveda Pharmacopoeia of India) guidelines and was carried out at Drug Standardization unit, Government Ayurveda College, Thiruvananthapuram.Qualitative tests for the presence of alkaloids, tannin, saponin, proteins, steroids, flavonoids, phenol, and sugar were also carried out [19].Along with these investigations,methanolic extract of Allium cepa was used for spotting in TLC. Two types of solvent systems were used in the study and they are ethylacetate: methanol: water in the ratio 50: 6.7: 5 and ethylacetate: methanol: chloroform in the ratio 25: 15: 20. The spots were detected in UV and in Iodine chamber.

In HPTLC fingerprinting,alcoholic extract of Allium cepa was prepared. HPTLC was done (CAMAG, Switzerland) using $60F_{254}$ TLC plate, keeping in TLC twin trough developing chamber with respective mobile phase Ethyl acetate: methanol: water in the ratio of 7.4: 1: 0.7. The developed plate was dried and kept in Photo-documentation chamber (CAMAG REPROSTAR 3). The plate was fixed in scanner

stage (CAMAG TLC SCANNER 3) and scanning was done at 254 nm in the scanner range of 190-800 nm. The software used was WinCATS 1.3.4 version.

Table 1. Physico-Chemical parameters of Allium cepa.

Physico-chemical parameters	Results
Moisture content	81.92%
Volatile oil content	Trace
Total ash	0.73%
Acid insoluble ash	0.19%
Water insoluble ash	0.15%
Fibre content	0.12%
Total sugar	8.50%
Reducing sugar	0.15%
Non reducing sugar	7.99%
Cold water extractive	16.67%
Cold alcohol extractive	7.84%

Table 2. Qualitative Chemical evaluation of Allium cepa.

Chemical constituents	Name of the test or Reagents	Inference
Alkaloid	Dragendroff's reagent	Present
Tannin	Lead acetate	Present
Saponin	Foam test	Present
Protein	Xanthoprotein test	Present
Steroid	Libermann Burchard's test	Present
Flavonoid	Shinoda test	Present
Phenol	Ferric chloride test	Present
Sugar	Benedict's reagent	Present

Table 3. Results of TLC

Solvent system	Detection	R_f value
Ethylacetate: methanol: water (50: 6.7: 5)	UV Iodine	0.69 0.65
Ethylacetate: methanol: chloroform (25: 15: 20)	UV Iodine	0.59 0.35

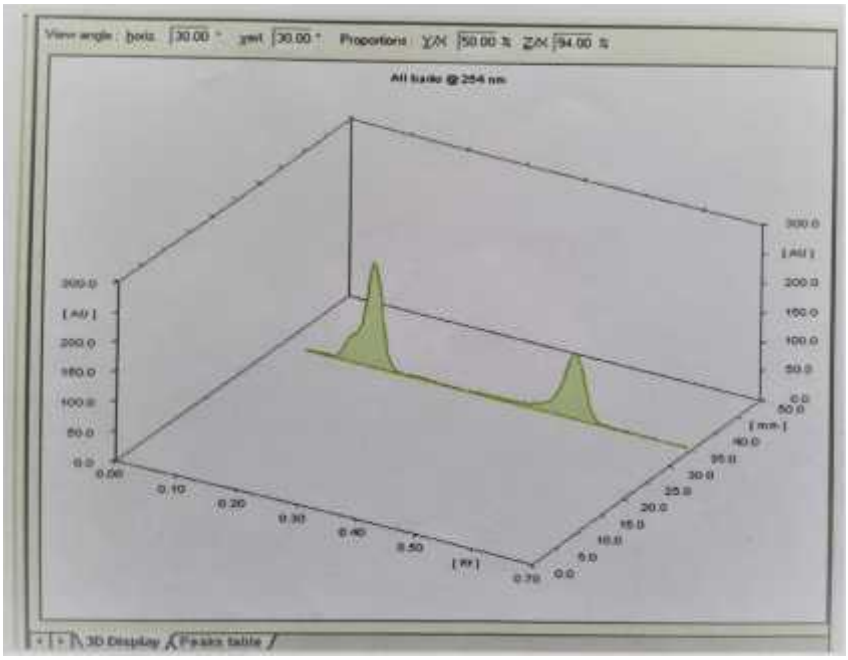

Figure 1. 3D display of HPTLC chromatogram @254nm.

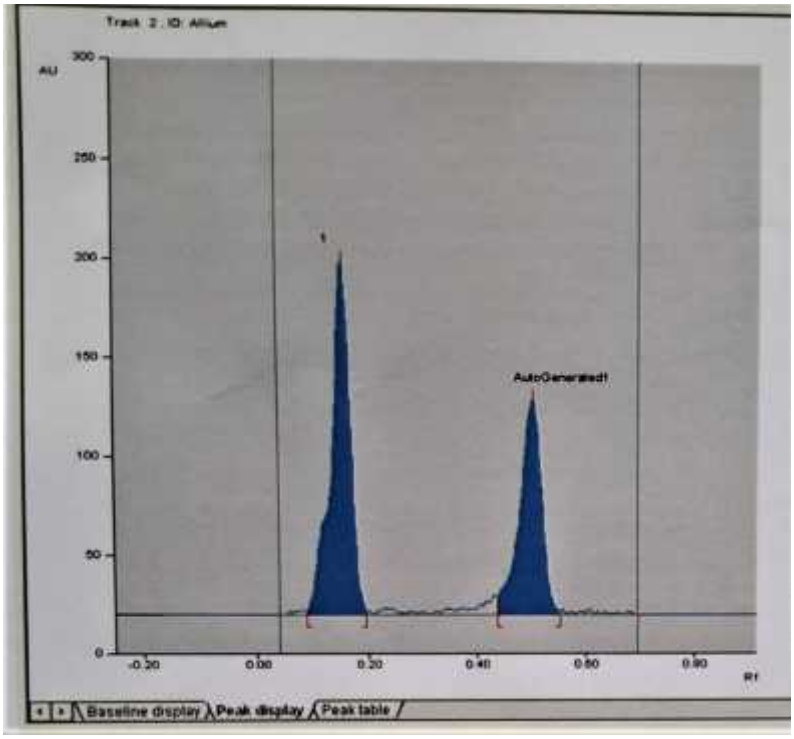

Figure 2. Peak Densitogram of Allium cepa.

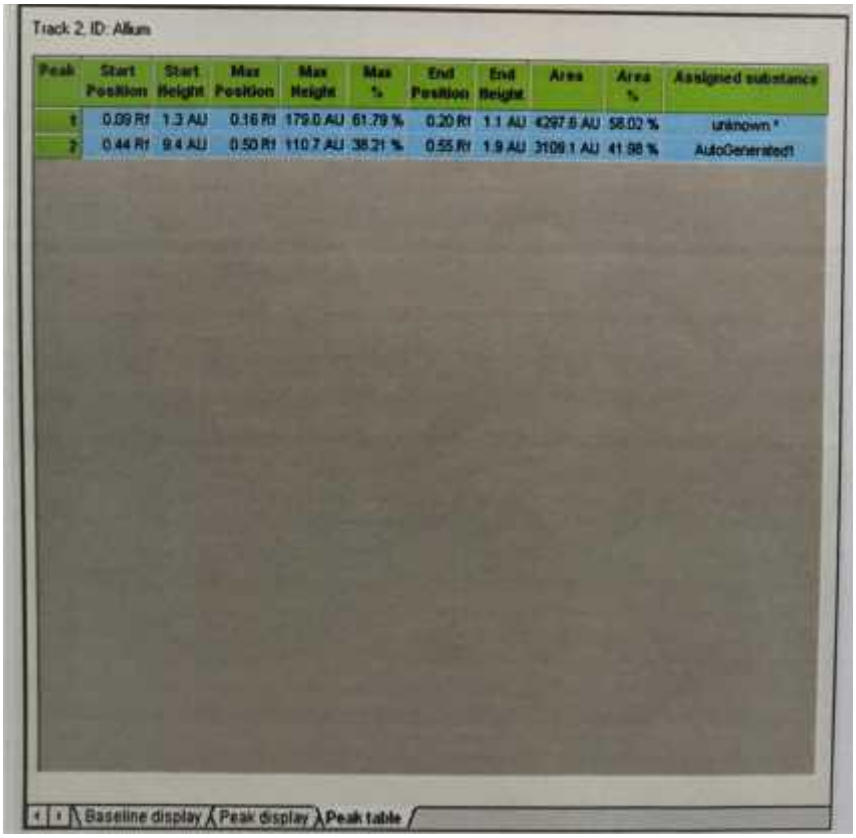

Figure 3. Peak table of tracks.

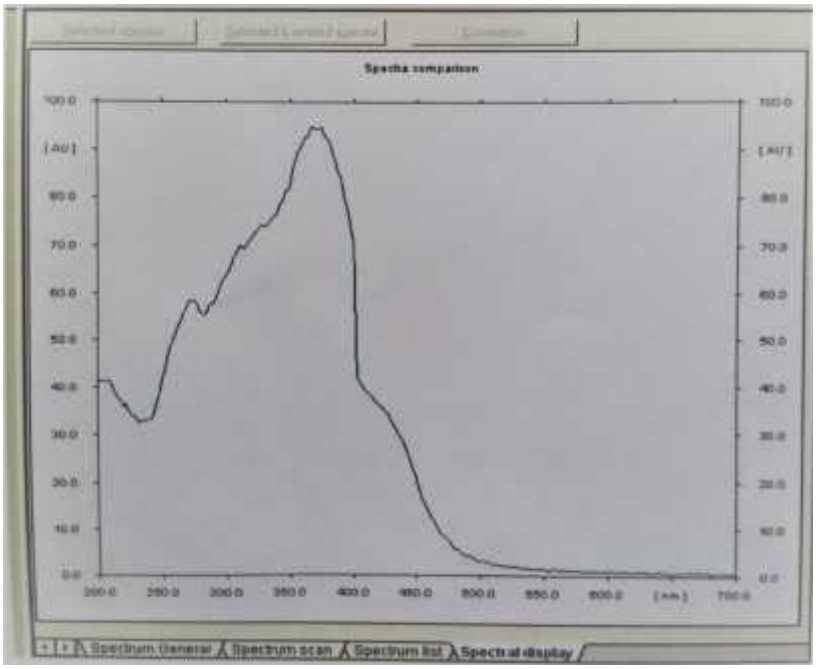

Figure 4. Spectral display of the peak.

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