Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(2): 192-196
Received: 16-01-2017
Accepted: 17-02-2017

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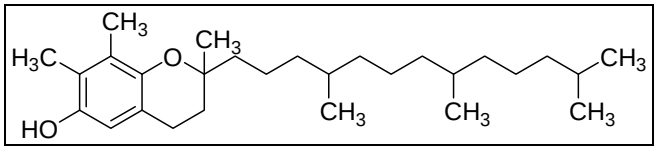
Identification and quantification of γ-tocopherol in *Cucurbita pepo, Cucumis melo* and *Cucumis sativus* seeds extracts by high performance liquid chromatography

Gaurav M Doshi and Preeti P Kanade

Abstract
The present research study focused on identification and quantification of γ-tocopherol in *n*-hexane and petroleum ether seed extracts of *Cucurbita pepo* (CP), *Cucumis melo* (CM), *Cucumis sativus* (CS) belonging to family Cucurbitaceae by High Performance Liquid Chromatography (HPLC). *n*-Hexane and petroleum ether extracts were prepared using soxhlet extraction and γ-tocopherol was identified and quantified using HPLC analysis with ultraviolet/visible (UV–vis) detection. The chromatographic conditions for HPLC analysis followed were: column, reverse-phase C-18, 5µ, 250x4.6mm; column temperature, 25°C; mobile phase, methanol: acetonitrile 60:40(v/v); flow rate, 1.0ml/min; detection wavelength, 292nm; run time, 15minutes. *n*-Hexane and petroleum ether seed extracts of *Cucurbita pepo*, *Cucumis melo* and *Cucumis sativus* were determined to contain 0.2900%w/v, 0.6666%w/v, 0.9162%w/v and 0.5093%w/v, 0.7781%w/v, 0.5564%w/v γ-tocopherol respectively. From the results obtained it can be concluded that seeds of *Cucurbita pepo*, *Cucumis melo* and *Cucumis sativus* are good sources of γ-tocopherol which justifies their medicinal and commercial uses.

Keywords: *Cucurbita pepo, Cucumis melo, Cucumis sativus*, High Performance Liquid Chromatography, γ-Tocopherol

Introduction
Vitamin E is the most potent and abundant lipid-soluble antioxidant *in vivo*. Vitamin E exists in eight major different isomeric forms- α-, β-, γ-and δ-tocopherols and α-, β-, γ-and δ-tocotrienols. Vitamin E functions by inhibiting lipid peroxidation reactions, oxidative reactions of other biomolecules thus, preventing the formation of reactive oxygen / nitrogen species that induce oxidative stress. Oxidative damage leads to the development of cancer, cardiovascular disease, and neurodegenerative disorders. Tocopherols are compounds with a chromanol ring and a hydrophobic side chain and exist in four different isomers called alpha, beta, gamma and delta, which differ in the methylation pattern of the benzopyran ring. γ-Tocopherol has two methyl groups [1, 2].



γ-Tocopherol is the primary form of vitamin E which is ultimately converted to α-tocopherol once inside the body. Among the isomers of vitamin E, γ-tocopherol is the most potent free-radical remover. γ-Tocopherol and its water soluble metabolite inhibit cyclooxygenase (COX-2) activity, inhibit production of pro-inflammatory prostaglandin E2 and forms a better trap for lipophilic electrophiles such as reactive nitrogen oxide species. This results in prevention of chronic inflammation-related diseases such as cancer, cardiovascular disease and neurodegenerative disorders [3].

Vegetable oils are perhaps the major dietary source of vitamin E as vitamin E is synthesized only in plants. The seeds of *Cucurbita pepo, Cucumis melo* and *Cucumis sativus*, family: Cucurbitaceae are enriched with minerals, vitamins, flavonoids, lignans, triterpenes, phytosterols, carotenoids, proteins, tocopherols and polyunsaturated fatty acids. *Cucurbita pepo, Cucumis melo* and *Cucumis sativus* seeds and seed oil possess anti-inflammatory, antiulcer, anthelmintic, anti-fungal, anti-bacterial, anti-viral, anti-diabetic and anti-tumor activity.

The potential of *Cucurbita pepo, Cucumis melo* and *Cucumis sativus* seeds to inhibit breast, colon, lung and prostate cancers and anti-inflammatory potential may be due to the presence of γ-tocopherol [4-8].

High Performance Liquid Chromatography is an analytical technique used to separate, identify, and quantify each component in a mixture. In the present work, we have identified and quantified γ-tocopherol by HPLC from the *n*-hexane and petroleum ether extracts of CP, CM and CS seeds following the method of Grilo *et al*.

Materials and Methods

Reagents and standard

The standard, γ-Tocopherol was purchased from Sigma-Aldrich (India); product number: T1782; CAS-No.: 54-28-4. HPLC-grade methanol, hexane and acetonitrile and ultrapure water were used.

Standard preparation

The standard solution of γ-Tocopherol was prepared to a concentration of 500 µg/ml in hexane and kept at 2-8°C, protected from light. Before injecting 30µl solution on to the HPLC column, it was passed through 0.45 µm filter.

Sample preparation

The seeds of *Cucurbita pepo, Cucumis melo and Cucumis sativus* were purchased from local market and were authenticated at the Agharkar Research Institute, Pune with voucher specimen no.3/187/2016/Adm-2472/143/144/145 respectively. Soxhlet extraction of the seeds was done using *n*-hexane and petroleum ether as solvent at 55-60°C for 24-48 h. Sample solution of each seeds extract was prepared by diluting with hexane. The sample solutions were then vortexed and centrifuged at 3000 rpm for five minutes, passed through 0.45-µm pore size syringe filter and were placed into individually labeled HPLC vials.

Instrumentation

HPLC system (Shimadzu-HPLC LC2010CHT), equipped with an auto sampler and UV-Visible detector was used for the analysis of standard and seed extracts. The data was recorded using LC solutions software.

Chromatographic Conditions

The HPLC analysis was performed on reversed phase C-18 column (5 µ, 250 x 4.6 mm). The mobile phase was a mixture of methanol and acetonitrile (60: 40, v/v) and eluted at a flow rate of 1.0 ml/min. The analytical column was kept at 25°C. The UV-Visible detector was set at 292 nm wavelength. The total run time was 15 min. The injection volume was 30 µl.

Data Analysis

Chromatograms for the samples and standard were collected. γ- Tocopherol in seed extracts was identified by comparison of retention times with standard of γ- tocopherol. Results were calculated by following formula,

$$\text{Percentage of } \gamma\text{- Tocopherol} = \frac{\text{sample area x standard dilution x purity}}{\text{Standard area x sample dilution x 100}} \text{ x 100}$$

Results and Discussion

In the present study, based on the chromatograms obtained γ-Tocopherol was found to be present in both the *n*-hexane and petroleum ether seed extracts of *Cucurbita pepo, Cucumis melo and Cucumis sativus*. Good separation of γ-tocopherol was achieved in all the seed extracts by use of the chromatographic conditions described. The retention time for γ- Tocopherol standard was 8.399min. Retention times for *n*-hexane seed extracts of *Cucurbita pepo, Cucumis melo and Cucumis sativus* were 8.305, 8.427 and 8.208min respectively. Retention times for petroleum ether seed extracts of *Cucurbita pepo, Cucumis melo* and *Cucumis sativus* were 8.491, 8.499 and 8.493min respectively. According to the formula, the amount of γ- Tocopherol present in *n*-hexane seed extracts of *Cucurbita pepo, Cucumis melo* and *Cucumis sativus* was 0.2900% w/v, 0.6666% w/v, 0.9162% w/v respectively and in petroleum ether seed extracts of *Cucurbita pepo, Cucumis melo* and *Cucumis sativus* it was 0.5093% w/v, 0.7781% w/v, 0.5564% w/v respectively.

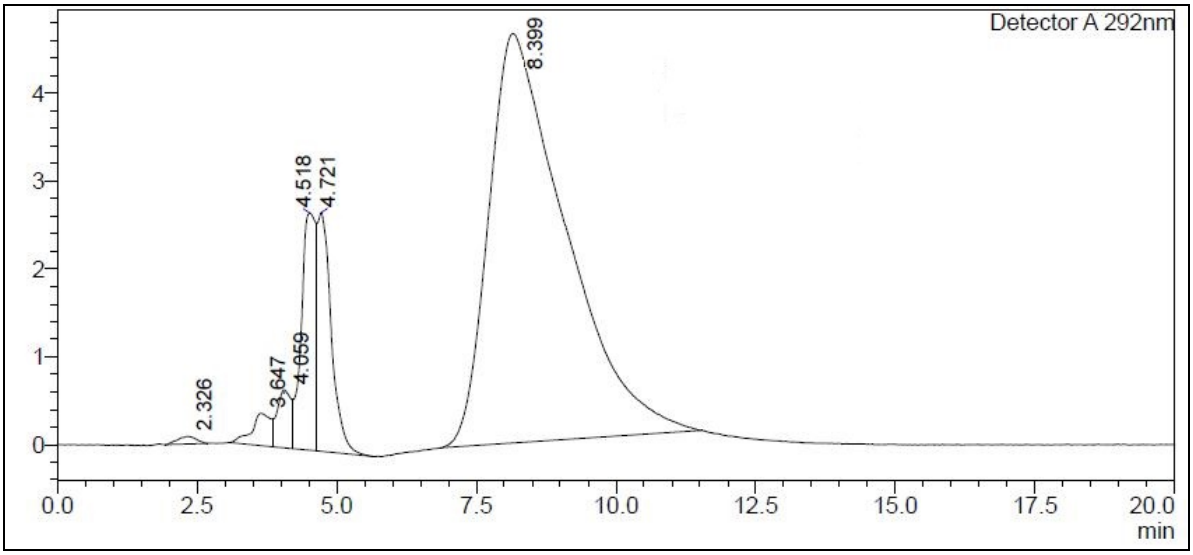


Fig 1: Chromatogram of γ- Tocopherol standard

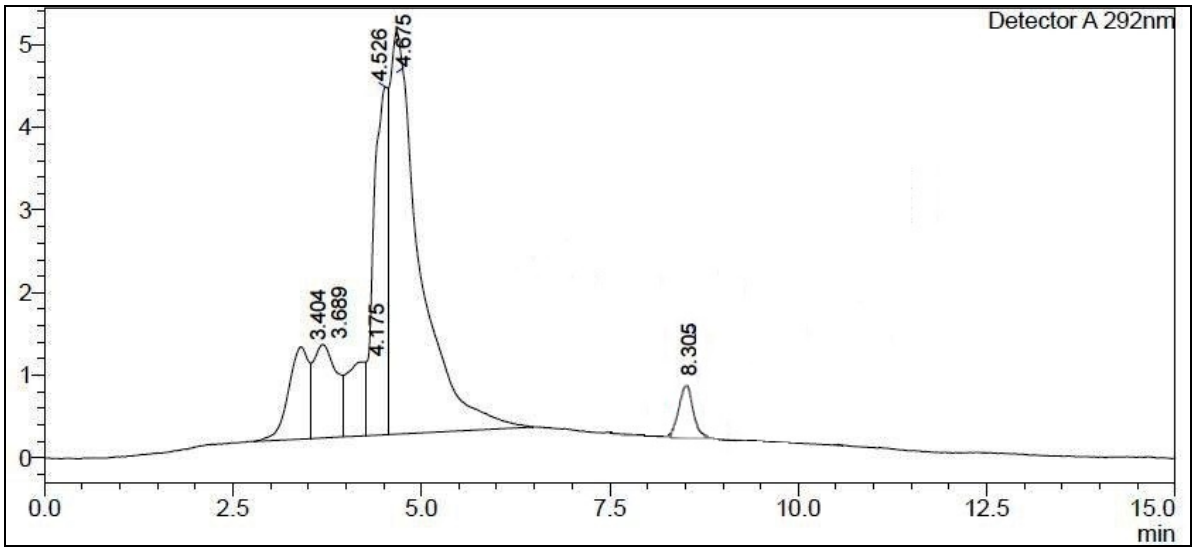


Fig 2: Chromatogram of *n*-hexane *Cucurbita pepo* extract

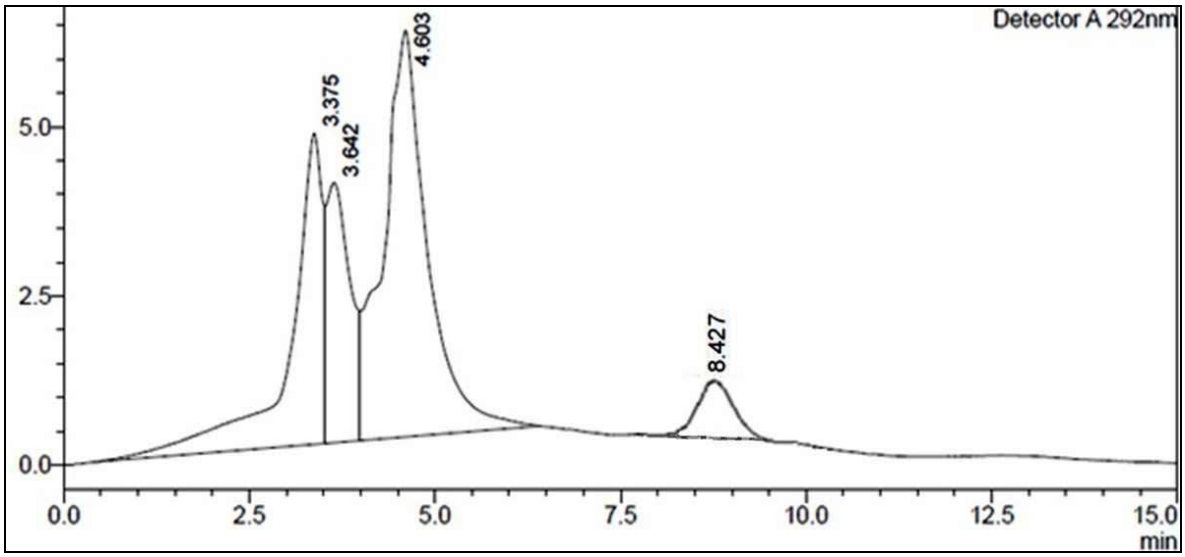


Fig 3: Chromatogram of *n*-hexane *Cucumis melo* extract

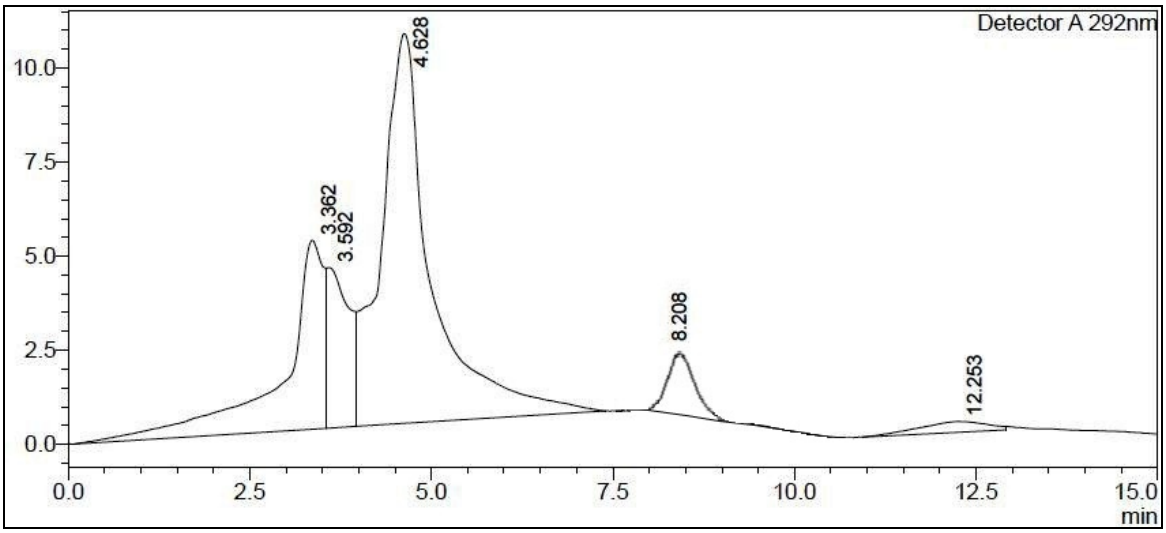


Fig 4: Chromatogram of *n*-hexane *Cucumis sativus* extract

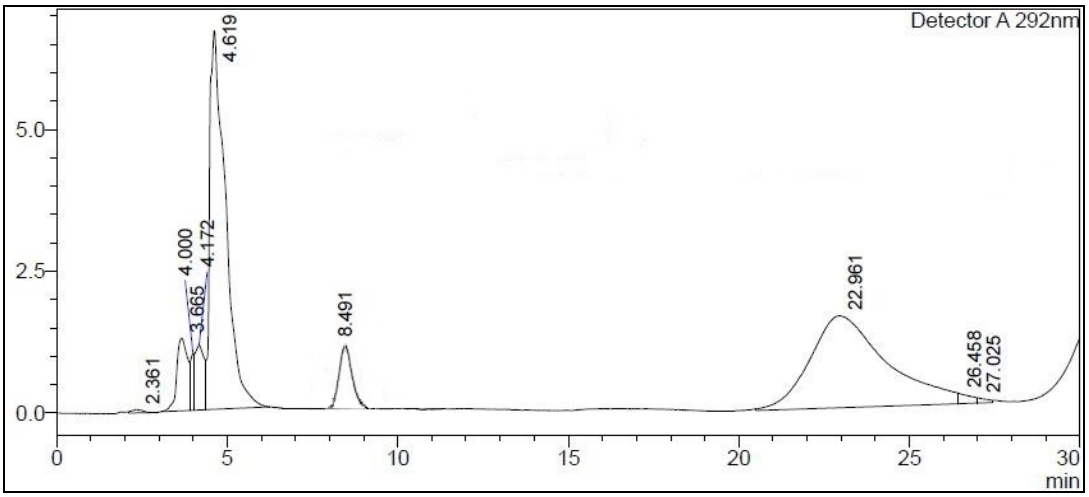


Fig 5: Chromatogram of petroleum ether *Cucurbita pepo* extract

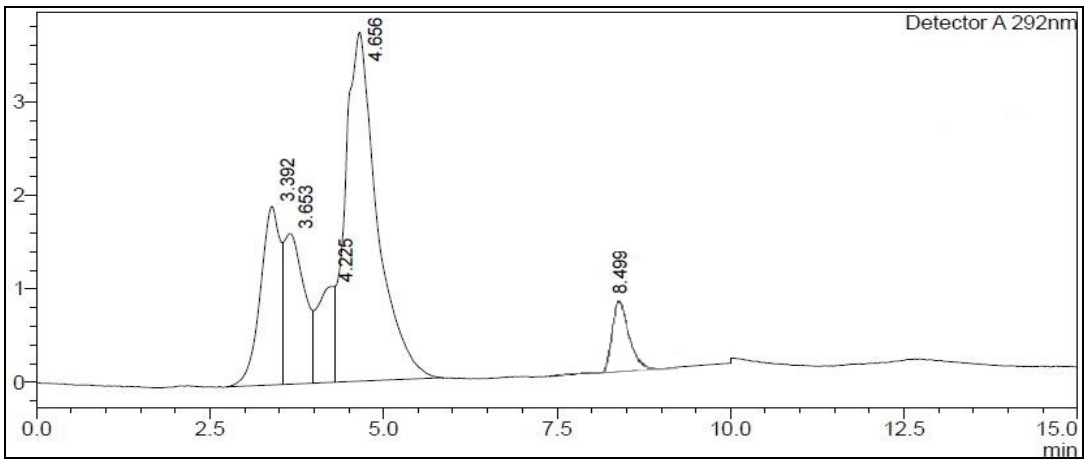


Fig 6: Chromatogram of petroleum ether *Cucumis melo* extract

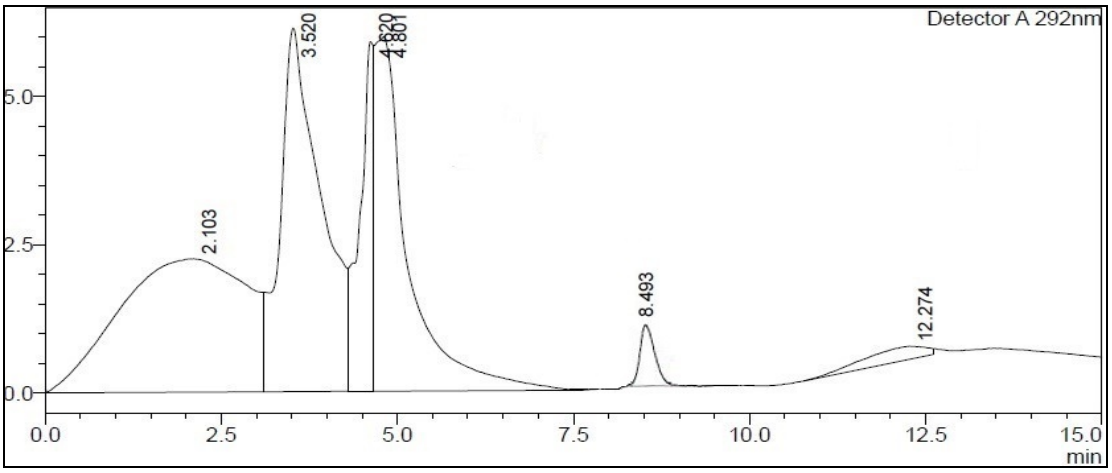


Fig 7: Chromatogram of petroleum ether *Cucumis sativus* extract

Table 1: HPLC analysis of *n*-hexane and petroleum ether seed extracts of CP, CM and CS and standard γ- Tocopherol

Sample Name	Value (retention time) peak area	% of γ- Tocopherol
Petroleum ether CP extract	(8.491) 19156	0.5093% w/v
Petroleum ether CM extract	(8.499) 29268	0.7781% w/v
Petroleum ether CS extract	(8.493) 20931	0.5564% w/v
n-Hexane CP extract	(8.305) 10910	0.2900% w/v
n-Hexane CM extract	(8.427) 25073	0.6666% w/v
n-Hexane CS extract	(8.208) 34464	0.9162% w/v
γ- Tocopherol	(8.399) 451346	

n-Hexane and petroleum ether seed extracts of *Cucurbita pepo, Cucumis melo* and *Cucumis sativus* have shown good peak at 292nm. The comparison of the retention times of extracts with the standard confirmed the presence of γ-tocopherol in the extracts. An adjustment was made to the method of Grilo *et al*. After several trials, we determined that the mobile phase did not give better peak separation as stated in Grilo *et al*. Rather than 100% methanol of Grilo *et al*, a 60:40 (v/v) mixture of methanol: acetonitrile was used as mobile phase and identifiable, separated, and symmetric peaks were observed.

γ-Tocopherol, a natural antioxidant has been reported to play a significant therapeutic ethno-pharmacological role as reported by various researchers from different plant extracts. The compound γ-Tocopherol is having anti-inflammatory, anti-cancer, antioxidant properties and protects against cancer, cardiovascular and neurodegenerative disorders. The anti-inflammatory, antiulcer, anthelmintic, anti-fungal, anti-bacterial, anti-viral, anti-diabetic and anti-tumor potential of seeds of *Cucurbita pepo, Cucumis melo* and *Cucumis sativus* is a combined therapeutic effect of other compounds along with γ-tocopherol.

Conclusion

Our study draws the readers towards the γ-tocopherol constituent isolated in the n-hexane and petroleum ether seed extracts of *Cucurbita pepo, Cucumis melo* and *Cucumis sativus* which was found to be in minute quantities as confirmed by HPLC. From the results obtained it can be concluded that the seeds of *Cucurbita pepo, Cucumis melo* and *Cucumis sativus* have great phytopharmaceutical importance and justifies their use for various human ailments.

Acknowledgement

We would like to acknowledge the college management who provided us all the facilities to do this work. We would also like to acknowledge Agharkar Research Institute, Pune for their help in plant authentication.

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