



Estimation of Epicatechin from the ethanolic extract of *Aesculus hippocastanum* using HPTLC technique

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ABSTRACT

Aesculus hippocastanum (family Hippocastanaceae) is commonly known as Horse chestnut is rich in many phytochemicals and has many potential medicinal properties. The seeds have been used as an analgesic, antipyretic, narcotic, tonic, and vasoconstrictor. They have been used to treat backache, sunburn, neuralgia, rheumatism, whooping cough and hemorrhoids. They have pharmacological effects such as anti-hyperlipidemic, anti-inflammatory, antioxidative effects, anticarcinogenic, and cytoprotective. In this study, we have made an attempt to estimate Epicatechin present in the Horse Chest Nut (HCN) extract by HPTLC technique as it is known for its therapeutic benefits so that further exploration of the *Aesculus hippocastanum* can serve millions in a variety of disease conditions. The ethanolic extract of *Aesculus hippocastanum* was chromatographed on silica gel GF254 plates with Toluene: EA: FA: MeOH (3: 3: 0.4: 0.1) as mobile phase. Detection and quantification were performed by densitometric scanning, at 280 nm. The average recovery of Epicatechin was found to be 0.81 %. The HPTLC technique has provided a good resolution of Epicatechin from other constituents present in the ethanolic extract.

Keywords: Horse chest nut, Epicatechin, Ethanolic, Extract, HPTLC

INTRODUCTION

Aesculus hippocastanum (family Hippocastanaceae) is commonly known as Horse chestnut is native to Western Asia. Horse chestnut is a deciduous tree up to 35 meters high with a large regular crown, five to seven digitate leaves and erect racemes of flowers with a yellow or reddish spot at the base of the white petals. It is indigenous to the mountains of Greece, Bulgaria, the Caucasus, northern Iran and the Himalayas. It is widely cultivated as an ornamental tree, especially in northern Europe and North America.^{1,2} The plant consists of Constituents like Aescin, aesculin, fraxin, scopolin, quercetin, kaempferol, astragalol, isoquercetrin, rutin, leucocyanidine oleic acid, linoleic acid, amino acids, allantoin, argyrol, carotin, choline, citric acid, epicatechin, leucodelphinidin, phyosterol, resin, scopoletin, tannin, and uric acid^{3,4,5}

The seeds have been used as an analgesic, antipyretic, narcotic, tonic, and vasoconstrictor. They have been used to treat backache, sunburn, neuralgia, rheumatism, whooping cough and hemorrhoids.^{4,6} The Aescin from Horse chest nut seed extract is a triterpenoid saponin glycoside is very beneficial in strengthening varicose and in chronic venous insufficiency. It has diuretic effect as well. It can be used in case of cerebral tumours, meningitis, encephalitis and cerebral edema due to cerebral trauma and other brain fluid problems.

Horse chest nut seed extract is found to be active against oral microbes.⁷ It also has antioxidant properties. Antioxidant potential of the ethanolic extract of *Aesculus hippocastanum* was studied using different in vitro free radical scavenging models like DPPH and Hydrogen Peroxide. This study demonstrates the significant antioxidant activity of the ethanolic extract of *Aesculus hippocastanum* in both the models utilized for the free radical scavenging activity.⁸ The present study has carried out to estimate epicatechin present in the seed extract by HPTLC technique as Epicatechin is a powerful flavonoid that falls under the polyphenol group. It is a major constituent in tea, fruits and chocolate. Epicatechin has been extensively researched for its diverse actions on human health. It has potent antioxidant, antiviral, antimalarial and anti carcinogenesis properties. Epicatechins have been proven to have diverse benefits to human health. It reduces the risks of diabetes mellitus and cardiovascular diseases. They have pharmacological effects such as anti-hyperlipidemic, anti-inflammatory, antioxidative effects, anti carcinogenic, and cytoprotective. These flavonoids can be used as therapeutic agents individually or in combination with other synthetic drugs and antibiotics to produce a new generation of phytopharmaceuticals.^{9,10} Epicatechin is radioprotective, especially to patients undergoing radiotherapy.¹¹

EXPERIMENTAL SECTION

Plant material

The ethanolic extract of *Aesculus hippocastanum* (HCN) was obtained from Green Chem Herbal Extract & Formulations, Bangalore.

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HPTLC FINGERPRINTING¹²⁻¹⁴

The finger printing has been done using the following chromatographic conditions.

Chromatography was performed on a 10x10 cm pre-activated HPTLC silica gel 60F 254 plate. Samples were applied to the plate as 6mm wide band with an automatic TLC applicator Linomat 5 with N₂ flow (CAMAG, Switzerland), 8mm from the bottom. Densitometric scanning was performed on CAMAG scanner III. The plates were prewashed by methanol and activated at 600 C for 5 minutes prior to chromatography. The slit dimension was kept at 5 minutes x 0.45 minutes and 20 minutes scanning speed was employed. The mobile phase was chosen after running extract in different mobile phases of varying polarity (Toluene, Toluene: Ethyl acetate and Ethyl acetate: Methanol etc :). Linear ascending development was carried out in 20cm x 10cm twin glass chamber saturated with the mobile phase and 10 ml of mobile phase was used per chromatography

CHROMATOGRAPHIC ANALYSIS

The plant extract and standard of required concentration have been prepared in methanol and were spotted using CAMAG applicator. The method was optimized by selecting appropriate mobile phase for the plant extract and respective compounds and developed in a twin trough chamber, 10 x 10cm at 25°C. The plates were dried by hair dryer. The developed plates were scanned at appropriate wavelength using CAMAG TLC scanner 3 and photo-documented using CAMAG REPROSTAR 3. (figure 1,2)

Preparation of stock solutions:

Preparation of Epicatechin standard solution:

Quantification of the standard Epicatechin (100 mcg/ml) and the sample (50mg / ml) HCN extract were prepared in methanol and estimated with the help of Toluene: EA: FA: MeOH (3: 3: 0.4: 0.1) as the mobile phase. Silica gel GF254 was used as the stationary phase. 2 µl, 4 µl, 6 µl, 8 µl and 10µl of the standard Epicatechin were run in track 1-5 respectively and sample HCN extract in track 6,7 and 8 having 2 µl, 4 µl, 6 µl, using ascending development mode and the developed plates were scanned at 280 nm using CAMAG TLC scanner 3 and photo-documented using CAMAG REPROSTAR 3 and the result is given in the figure 3 and 10 and the peak area obtained from different tracks.

Quantification of Epicatechin

Chromatographic condition

Sample : Horse Chest Nut (HCN extract)
Standard : Epicatechin
Sample & Standard prepared in : Methanol

Stationary phase	: Silica gel GF ₂₅₄
Mobile phase	: Toluene: EA: FA: MeOH (3:3:0.4:0.1)
Scanning wavelength	: 280nm
Sample concentration	: Extract (50mg/ml), Std (100µg/ml)
Applied volume	: Standard Track – 1-5- (2µl - 10µl) Sample Track – 6 – 8 – (2µl - 6µl)
Development mode	: Ascending mode

RESULTS AND DISCUSSION

The high performance thin layer chromatography seems to be a highly reliable technique for isolation and estimation of plant components.^{12,13,14} The finger printing following the chromatographic condition showed a comparable peak of epicatechin in the extract as that of the standard epicatechin in every track **6,7,8 (Figure 4,5,6)**. The epicatechin percentage was calculated from the peak area and was found to be 0.81 % with an Rf value of 0.34 (Table 1)

Photodocument

Figure: 1 HCN extract at 254nm

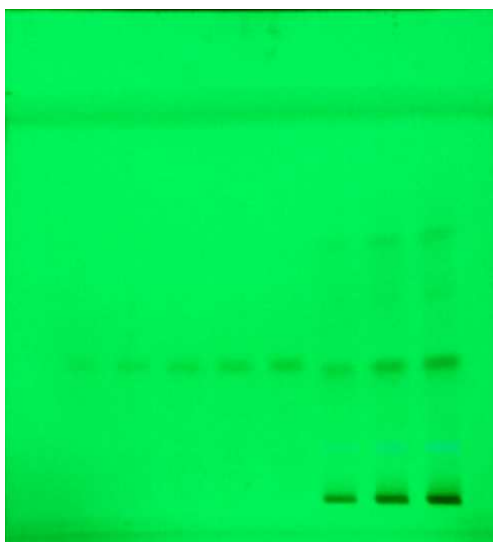


Figure: 2 HCN extract at 366nm



Peak area

Figure 3: Track 5
Graph 1: Estimation of standard (epicatechin)
Injection volume : 10 µl

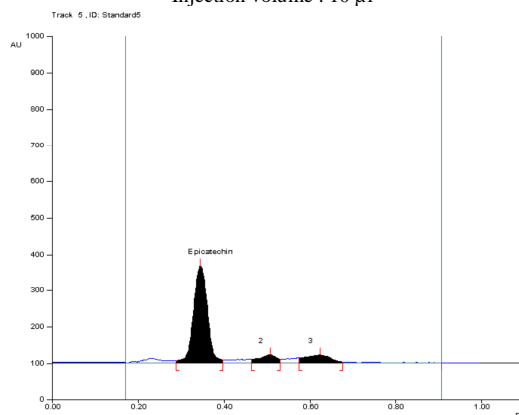


Figure 4: Track 6
 Estimation of epicatechin content in ethanolic extract of *Aesculus hippocastanum*
 Injection Volume : 2µl

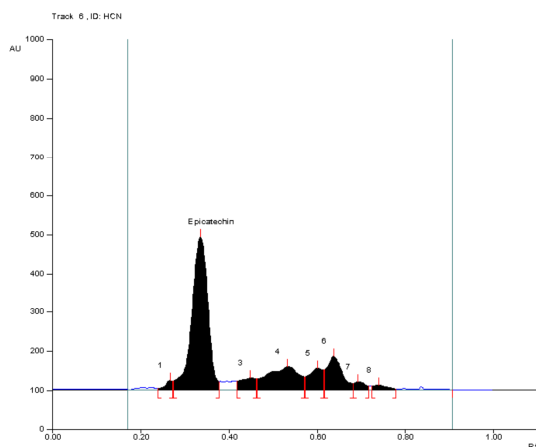


Figure 5: Track 7
 Injection Volume : 4µl

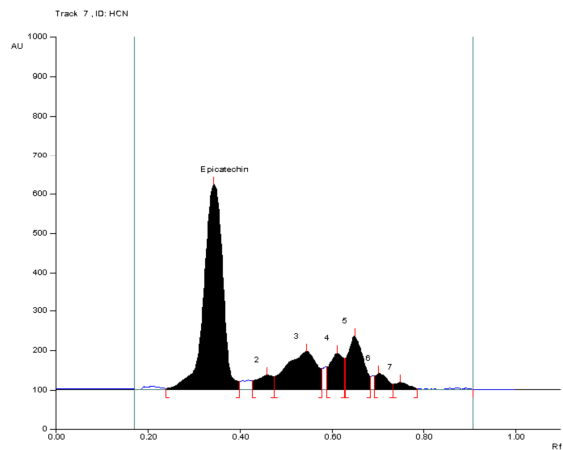


Figure 6: Track 8
 Injection volume : 6µl

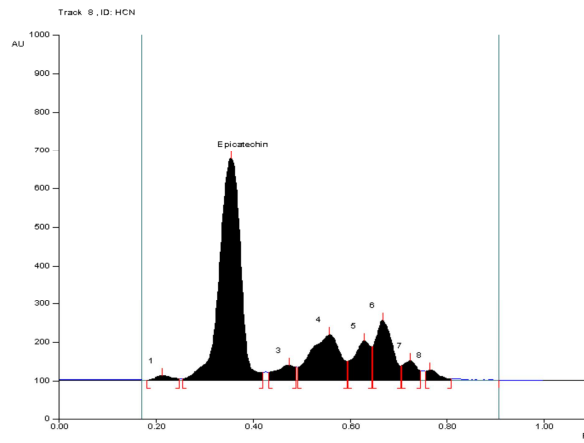


Figure: 7 Standard Calibration Curve

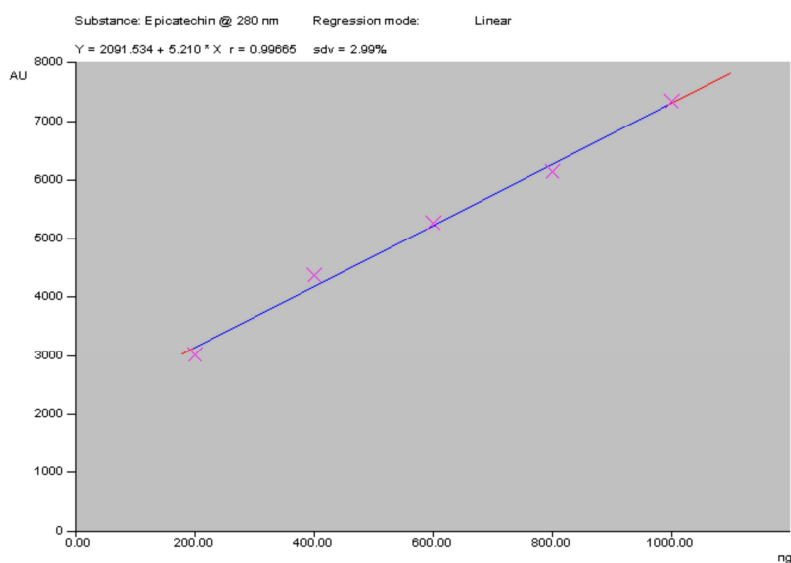


Table:1 Quantification of Epicatechin

Sample ID	R _f	%
Horsechest nut	0.34	0.81%

CONCLUSION

Estimation of Epicatechin present in the Horse Chest Nut (HCN) extract was done by HPTLC technique. The method was found to be simple, precise, specific, sensitive and accurate and can also be used for the quantification of various compounds present in herbal raw materials. From our study we conclude that the percentage of epicatechin in ethanolic extract of *Aesculus hippocastanum* was found to be 0.81.

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