

Full Length Research Paper

## High performance liquid chromatography (HPLC) analysis of embelin in different samples of *Embelia ribes* Burm. f. - a threatened medicinal plant of India

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***Embelia ribes* Burm.f. is one of the important medicinal plants of India. Six samples of *Embelia* were collected from different geographical regions of India. The seed extract was isolated and analyzed for the principle bioactive constituent, embelin, by high performance liquid chromatographic (HPLC) method. All the samples showed characteristic peaks of embelin at the same retention time as that of standard embelin. However, lot of variation in the percentage of embelin content was observed among the different samples. High percentage of embelin was observed in the samples collected from the Kerala (4.9%) followed by the Orrisa (4.6%). The percentage of embelin in Madhya Pradesh and Maharastra sample was 1.27 to 2.0%, respectively. The commercial samples collected from the local market showed low percentage of embelin (1.0 and 1.05%). The HPLC procedure has given the reliable quantification of embelin from different samples, which can be used for analytical work and also for selection of embelin rich plant for commercial exploitation.**

**Key words:** *Embelia ribes* (Myrsinaceae), medicinal plant, seed extract, high performance liquid chromatography (HPLC) analysis, embelin.

### INTRODUCTION

Plants are the source for important chemicals with wide range of bioactive properties. In recent years, there is a growing demand for natural products of commercial importance in both domestic and international market (Lewington, 1993). The current demand for the plant based products in medicine and industry has resulted in extensive investigation of the plant kingdom for useful chemicals (Srivastava et al., 1996; Varier, 2007).

*Embelia ribes* Burm.f. (Myrsinaceae) commonly known as *Vidang* or *Baibirang* is one of the important medicinal

plants widely used in several indigenous systems of medicine (Syed et al., 2011; Shailendra, 2012; Rama Shankar et al., 2012). It is distributed in some packets of Eastern and Western Ghats of India and one of the red listed species in India (Ravi and Ved, 2000). The plant is a climber with slender branches and long internodes. The leaves are elliptic, broad and covered with minute glands. The flowers are small, white racemes arranged in panicle inflorescence at the end of the branches. The fruits are berries, round, red to black color and tipped with style

(Chopra et al., 1992). The fruits look so much like pepper and are often referred to as false pepper.

The fruits are astringent, carminative and stimulants. Traditionally the seeds are employed as a remedy for toothache, headache and snakebite. The seeds are mainly used for maintaining healthy skin and to support the digestive function. It is also effective in the treatment of fevers and for the diseases of chest. The seeds are called *Krimighana* in Sanskrit, which literally means killer of worms. It is widely used as antihelminthic and to cure skin diseases (Girthori et al., 2003; Swamy et al., 2007). Seed extract possess antioestrogenic properties and specially used as contraceptive (Pandey et al., 1993).

*Embelia* is the most widely used species in Siddha as well as in Unani medicine and is used in more than 75 Ayurvedic commercial formulations. The seed extract is reported to be antidiabetic (Bhandari et al., 2007) anti-tumour, analgesic, anti-inflammatory (Handa et al., 1992; Chitra et al., 2004) antispermatogenic (Seth et al., 1982), chemo preventive (Sreepriya and Bali, 2005), free radical scavenging (Joshi et al., 2007), antifungal and antibacterial activities (Sanjesh et al., 2010; Radhakrishnan et al., 2011). Embelin is the principle chemical compound reported from the seeds. The other chemical constituents isolated from the seeds are quercitol, tannin, christembine, an alkaloid and a resinoid and a volatile oil (Sharma et al., 2002). Embelin (2,5-dihydroxy-3-undecyl-2,5-cyclohexadiene-1,4-benzoquinone) has been isolated from different species of *Embelia* and other members of the family Myrsinaceae (Chauhan et al., 1993).

In the earlier studies, embelin was isolated from the fruits of *Myrsine africana* L. (Myrsinaceae) using analytical methods like high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC) (Paul et al., 2007). Variation in phenolic content was analyzed among the different market samples of *E. ribes* (Sharadha et al., 2009). Some studies have been done in isolation of embelin from *E. ribes* and *Embelia robusta* (also known as *Embelia tsjerium-cottam*) using plant extract as standard. But there are no studies on analysis of embelin content in different samples of *Embelia* collected from various geographical regions. In the present work, embelin content was quantified by HPLC method in various samples of *Embelia* collected from different geographical regions.

## MATERIALS AND METHODS

### Collection of plant material

Authenticated sample of *E. ribes* fruits were collected from M.S Swaminathan Agro Biodiversity Research Centers, Kalpetta, Kerala. Dried seeds of *Embelia* were collected representing the

different geographical region from the traders of Orissa, Madhya Pradesh and Maharashtra. Two commercial samples were purchased from local market of Hyderabad and botanical authentication was performed at Botany Department, Osmania University, Hyderabad.

### Preparation of the extract

*E. ribes* fruits collected from different sources were shade dried and crushed to coarse powder. Three gram of coarse powder (seeds along with the pericarp) was mixed with 50 ml of methanol and refluxed for 3 h. The extracted methanol was filtered through 0.45 µm PVDF (Poly-Vinylidene-Di-Fluoride filters), transferred to standard volumetric flask and the extraction was repeated 3 to 4 times until the solution was colorless. This process was repeated for all the samples. Stock solution of standard embelin was prepared by dissolving 10 mg of standard embelin in 2.5 ml methanol and heated on a water bath to dissolve completely. The solution was cooled and filled up to 5 ml in a standard volumetric flask to obtain a concentration of 1 mg/ml.

### Chromatography

HPLC instrument employed in the study is Make waters Analytical system (USA) with alliance 2690 pump, automatic injector, UV-dual lambda observance detector and empower-2 software. The stationary phase used is C18 column. Calibration of the system was done by accurately weighing 0.01 g caffeine (Merck, Germany) dissolved 100 ml of HPLC grade water. 20 µl of different concentrations made from the caffeine stock solution were injected through a C18 Column. The mobile phase consisting of water: methanol (70:30 v/v) was degassed before use. Detection of caffeine was done at 273 nm and flow rate was maintained at 1 ml/min. All the chemicals used are HPLC grade (99.8% pure). The methanol was obtained from Merck and Tri fluoro acetic acid from Finar. De-ionized water was obtained from Milli-Q (millipore, USA). Standard embelin (98% pure) was purchased from Natural Remedies Pvt. Ltd (Bangalore, India). 20 µl of standard embelin was injected using methanol: water: acetic acid: tetra hydrofuran (Make: Thomas Baker) (85: 15: 3: 0.1v/v/v/v) as mobile phase, at a flow rate of 1 ml/min and the standard peak was obtained. Fruit extract was made from different sample *Embelia* and subjected to HPLC analysis. The percentage of the major constituent, embelin, in different samples was calculated based on standard embelin concentration.

## RESULTS AND DISCUSSION

Initially, various solvent systems and mobile phase with different combinations were tried to standardize the best combination for the embelin extract. Finally, the mobile phase consisting of methanol: water: acetic acid: tetrahydrofuran (85: 15: 3: 0.1v/v/v/v) was optimized. The six different samples of embelia fruit extracts were eluted at 288 nm at the retention time (tR) of 20 min. All the samples showed characteristic peaks of embelin at the same retention time as that of standard embelin, indicating the presence of embelin in all the samples (Figures 1 to 4). A single peak at retention time of 20 min

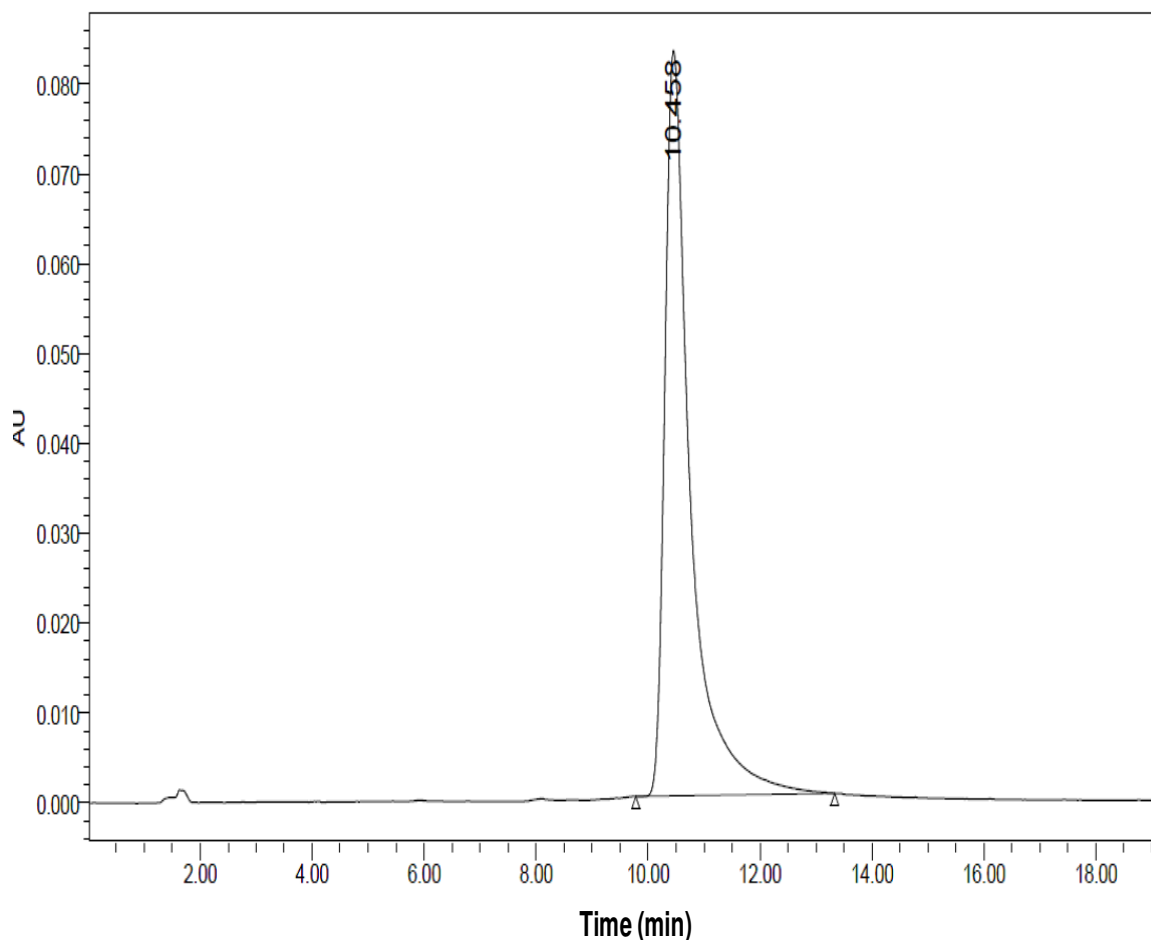


Figure 1. Embelin standard.

was observed in the chromatogram of the seed extract of different samples of *Embelia*. There was not much interference from the other components present in the samples. The concentration of embelin in different samples was calculated by measuring their peak area and comparing their peak areas of standard.

The HPLC analysis of *E. ribes* and different market samples representing the different region of India showed some interesting results. There is a lot of variation in the percentage of embelin content among the different samples. Fruits collected from the Kerala showed the presence of high amount of embelin (4.9%), which was followed by Orissa samples (4.6%). The percentage embelin in Madhya Pradesh and Maharashtra sample is 1.27 and 1.20%, respectively. The two commercial samples collected from the local market showed less percentage of embelin that is, 1.0 and 1.05%.

In the present study, fruits collected from the Kerala and Orissa showed highest percentage of embelin that is,

4.9 and 4.6%, respectively. The high content of embelin in these samples clearly indicates that the fruits belong to the original *E. ribes*, which is botanically accepted species of *vidanga*. All other samples comparatively showed less embelin content. This may be due to the fact that the samples may belong to the *Embelia robusta* commonly sold as *vidanga* instead of *E. ribes*, because of its restricted distribution and seed set. This is also supported with morphology of the fruits. The *Embelia* fruits collected from the Kerala are black in color, whereas other seed samples are reddish brown in color.

According to Pharmacopeia, the embelin content reported in *Embelia* is 1.85 to 2.15% (Anonymous, 2001). In another study, the embelin content up to 4.33% in *E. ribes* fruits and 3.96% in *E. robusta* was reported (Sudhakar et al., 2005). The present study of HPLC analysis of *E. ribes* has shown the presence of high amount of embelin that is, 4.9% which is significantly higher than the earlier reports.

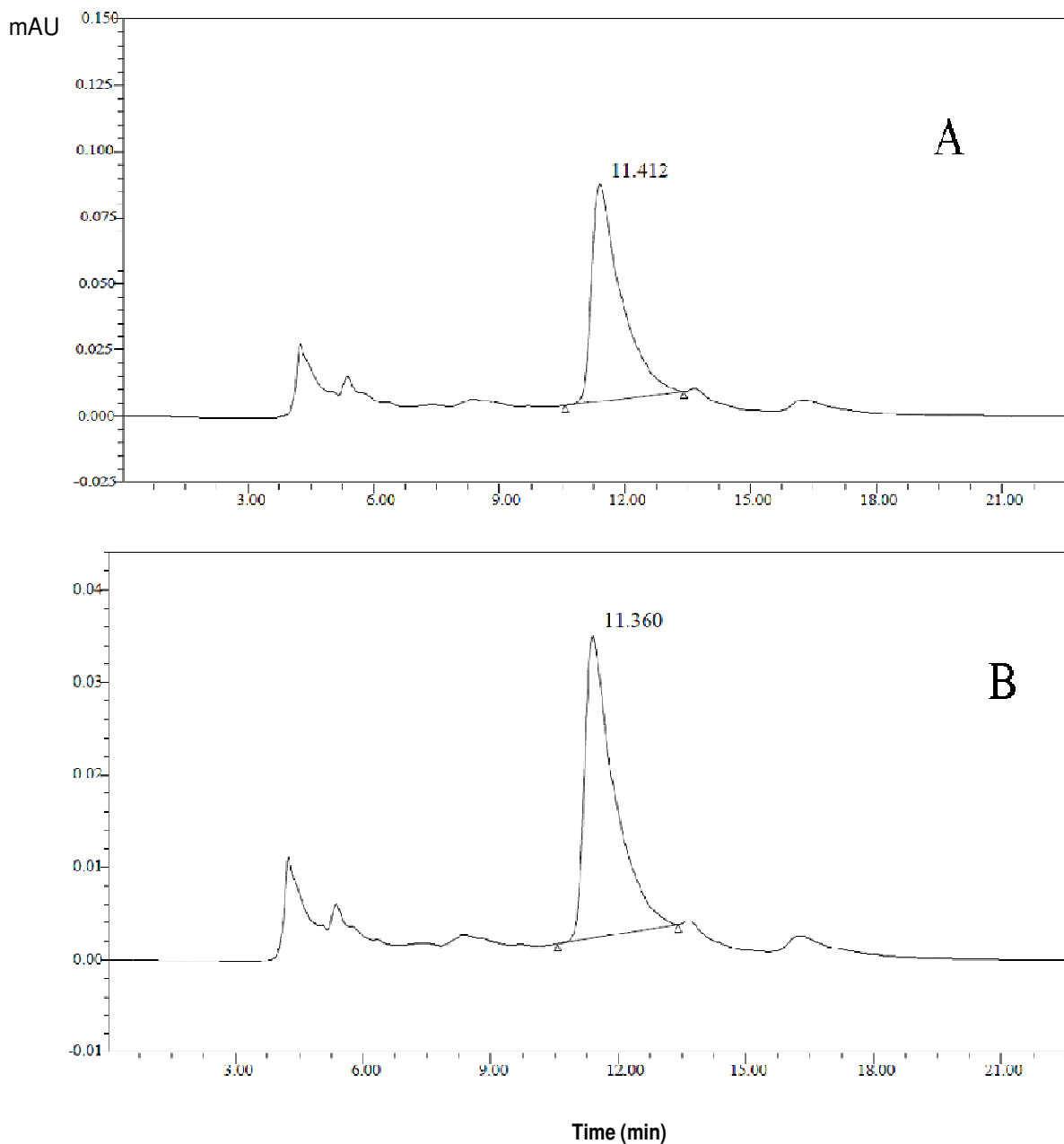


Figure 2. (A) Kerala sample; (B) Orrisa sample.

### Conclusion

The present method of HPLC has given the reliable quantification of embelin from different samples, which can be used for the routine analytical work. This procedure can also be used for checking the status of the adulteration in marketed sample of *Embelia* by comparing the embelin contents. The samples that are having high

percentage of embelin can be exploited for commercial purpose and industrial utilization.

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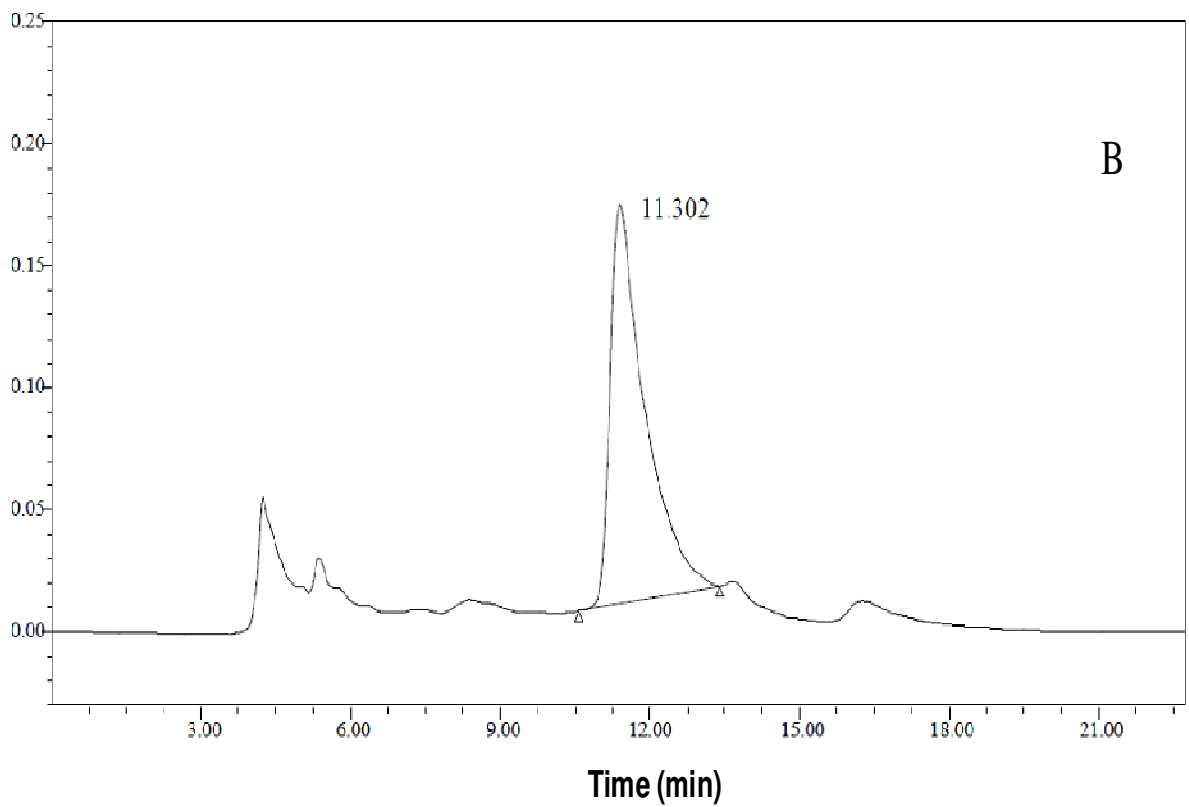
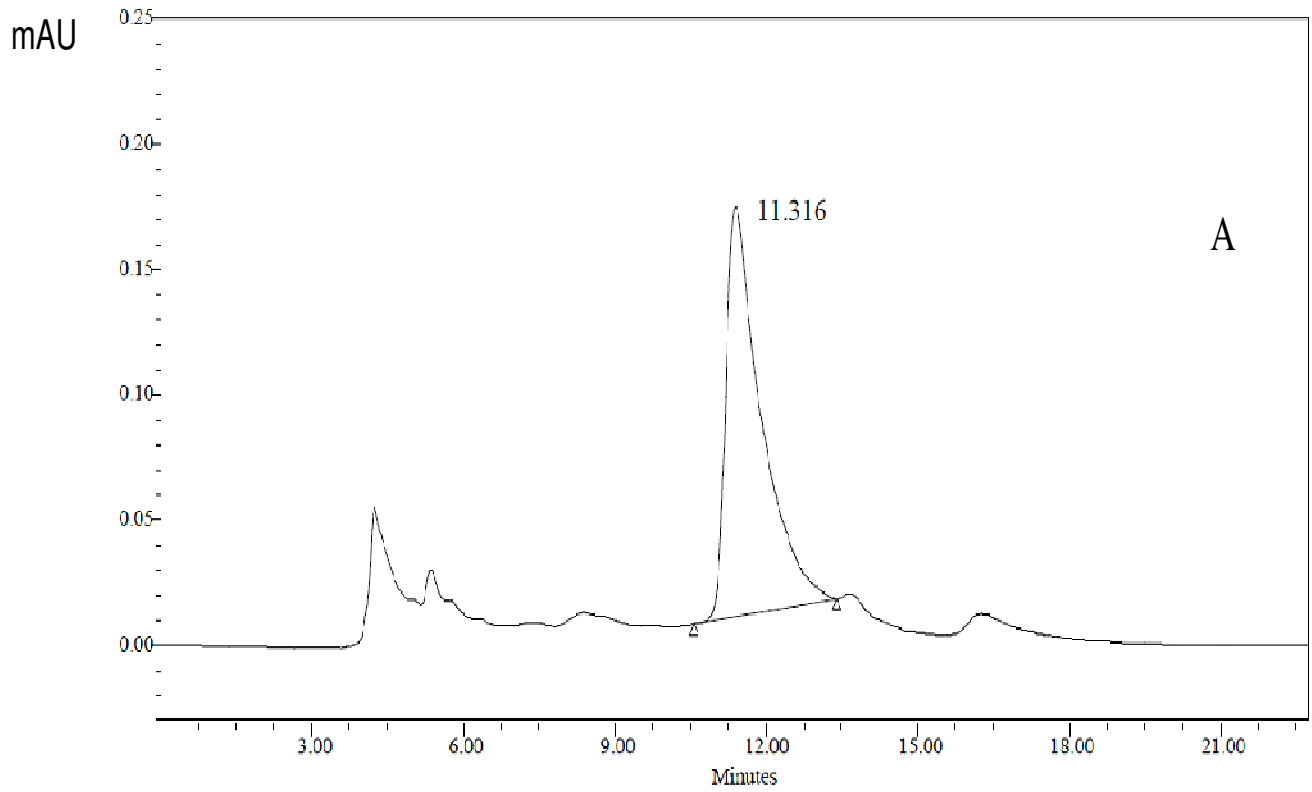


Figure 3. (A) Madhya Pradesh sample, (B) Maharashtra sample.

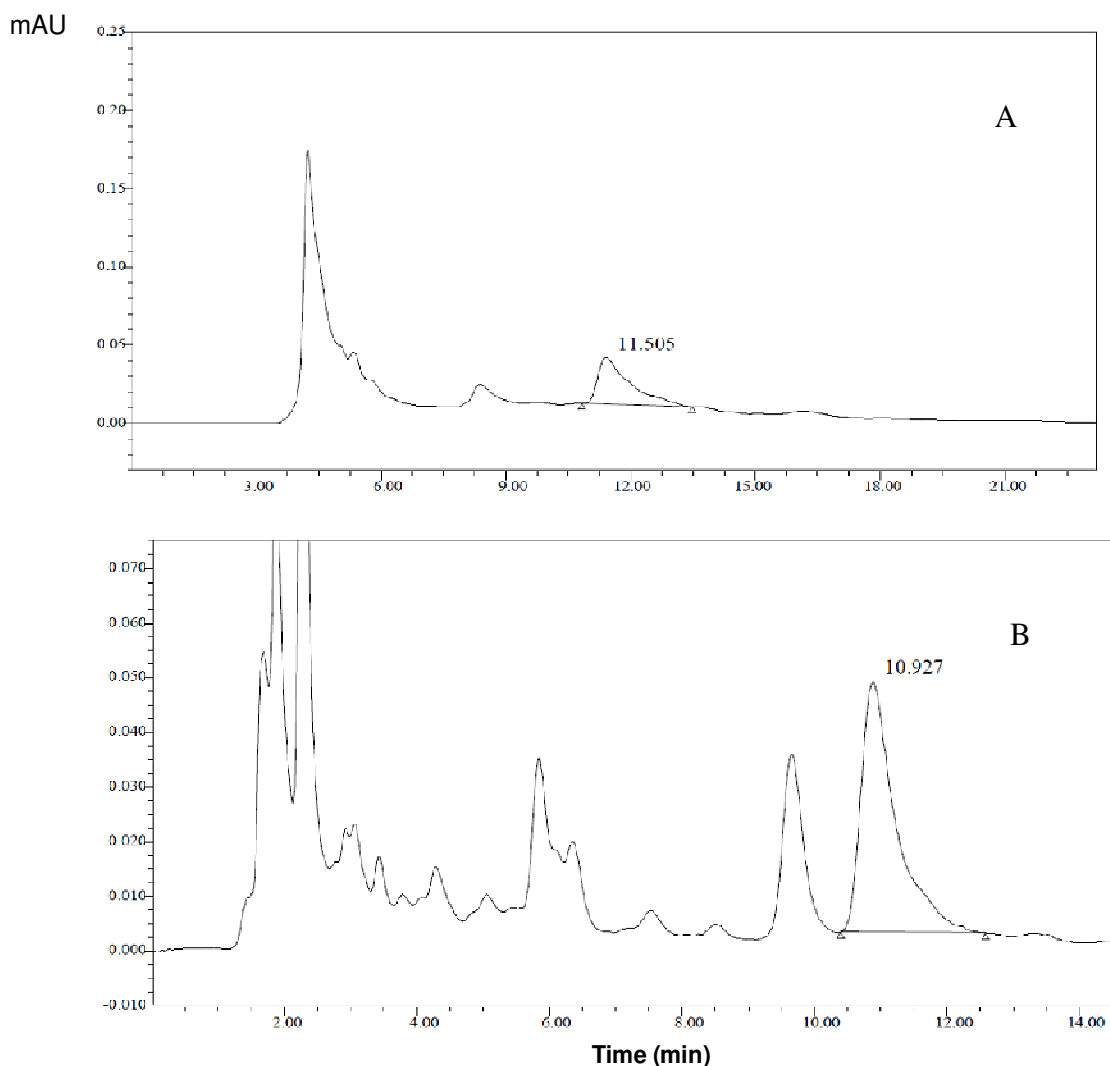


Figure 4. (A) Commercial sample 1, (B) commercial sample 2.

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