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ESTIMATION OF SCOPOLETIN CONTENT IN COMMERCIALLY EXTRACTED LEAVES OF MEDICINAL HERB *ARTEMISIA ANNUA* L. USING HPTLC

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ABSTRACT: Plants have a long history as therapeutic tools in the treatment of human diseases and have been used as a source of medicines for ages. In search of new biologically active natural products, many plants and herbs used in traditional medicine are screened for natural products with pharmacological activity. Qinghao is a traditional Chinese medicine prepared from the aerial parts of *Artemisia annua* L. which belongs to Compositae family. It is used as an anti-parasitic and fever relieving agent. Artemisinin was isolated from Qinghao as a major bioactive constituent and has been used to treat malaria. Besides artemisinin, the chemical constituents of Qinghao include volatile oil, terpenes, flavonoids, and coumarins. Scopoletin-derived sesquiterpene ethers Sesquiterpene derivatives of 7-hydroxy-6-methoxycoumarin (Scopoletin) are not so common. The aim of the study was to investigate chemical constituents present in the leaves of Artemisia annua after the commercial extraction process of the plant for artemisinin. From the preliminary studies, the compound identified was found to be a coumarin- Scopoletin. The residual leaves were used for the extraction of Scopoletin. The study estimated Scopoletin using thin-layer chromatography (TLC). The Scopoletin content of the leaves was calculated from the area calibration curve by this method was found to be 0.04477% w/w (plant dry weight basis). This HP-TLC procedure may be used effectively for identity, quality evaluation as well as quantitative determination for this plant or its derived products.

INTRODUCTION: Scopoletin (6-methoxy-7-hydroxycoumarin) is a coumarin compound with antifungal properties that have been isolated from several plant species. It is a biomarker widespread in plant kingdom, especially in the phytotherapic and nutraceutical ones.

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Scopoletin is derived from the phenylpropanoid pathway with strong blue fluorescence under UV light [1], can be isolated from many plant species [2], and was proposed as an important phytoalexin against microbial pathogens [3]. Scopoletin increases considerably after fungal infection exhibiting fungitoxicity *in-vitro* [4 - 8]. Scopoletin possesses antioxidant property, scavenged superoxide anion in the xanthine/xanthine oxidase reaction system in a concentration-dependent manner [9] Scopoletin obtained from fruits of *Tetrapleura tetraptera* (Mimosaceae) has hypertensive effect [10]. It also shows antidepressant activity, angiogenic activity

and antifungal activity [11, 12, 13]. Lee (2013) isolated Scopoletin from Artemisia iwayomogi, 14Hofer & Greger (1984) found scopoletin farnesyl ether scopofarnol and the new scopoletin drimenyl ether scopodrimol A from the roots of *Artemisia persica* [15]. Silva *et al.*, (2002) reported, Scopoletin inhibited the conidial germination of *Corynespora cassiicola* isolated from the uninfected mature leaves of *Hevea brasiliensis*.

The present study deals with the investigation of the compounds which are so tightly bound that even after the rigorous extraction process, they remain in the plant. Such compound should be extracted as they hold important medicinal value. With this view, the estimation was done in the commercial sample of *Artemisia annua* L. which were previously extracted (extracted for Artemisinin), again with solvent. This was an effort to extract such important compounds from the residue so that no compound goes wasted.

MATERIALS AND METHODS:

Thin Layer Chromatographic Study: Thin-layer chromatography (TLC) is an important analytical tool in separation, identification, and estimation of different classes of natural products. Comparative TLC (co-TLC) with marker compound/s can be used for identification of chemical constituents and to standardize the herbal raw materials.

High-Performance Thin Layer Chromatography: Camag TLC systems equipped with Camag Linomat V, an automatic TLC sample spotter, Camag glass twin trough chamber (20 × 10 cm) were used for the analysis. Chromatography was performed using pre-activated (60 °C for 5 min) silica gel 60F254 TLC plates (20 × 10 cm; layer thickness 250 µm) (Merck, Darmstadt, Germany). Samples and standards were applied on the plate as 6 mm wide bands with an automatic TLC sampler under a flow of N_2 gas, 10 mm from the bottom and 10 mm from the side and the space between two spots was 15 mm of the plate. The linear ascending development was carried out in a camag twin trough chamber saturated with 20 ml mobile phase (chloroform: methanol: toluene, 8:1:1, v/v/v) for 20 min at room temperature (25 ± 2 °C and 40% relative humidity). The plates were developed up to 8 cm under chamber saturation conditions. Subsequent to the development, TLC plates were dried in current air with the help of a hairdryer. Evaluations of the plates were performed with Camag scanner 3 (win CATS 4.0 integration software). Densitometric scanning was performed at 360 nm (absorption-reflection mode), using a slit width of 6 × 0.45 mm, data resolution 100 µm step and scanning speed 20 mm/s with a computerized Camag TLC scanner. Peak areas were recorded and the amount of scopoletin was calculated using the calibration curve. Quantification was done by using external standard method.

Preparation of Extract: Accurately weighed 5 g of previously extracted *Artemisia annua* sample was again extracted with methanol (4 × 50 ml) under reflux (30 min each time). The combined extracts were filtered, concentrated and transferred to a 50 ml volumetric flask and the volume was made up with the same solvent.

Preparation of Standard Scopoletin Solution: A stock solution of scopoletin **Fig. 1** (Himedia Laboratories Pvt. Ltd., Mumbai, India) (5 mg/50 ml) was prepared in methanol. Working solutions were prepared by appropriate dilution of the stock solution with the same mixture of solvents.

Calibration Curve for Scopoletin: Standard scopoletin solution in the range of 200 to 700 ng spot-1 was applied on TLC plate for preparation of the calibration curve of peak area versus concentration.

RESULT AND DISCUSSION: Plants use an intricate defense system against pests and pathogens, including the production of low molecular mass secondary metabolites with antimicrobial activity, which are synthesized de novo after stress and are collectively known as phytoalexins. Phytoalexins are antimicrobial secondary metabolites produced de novo by plants in response to stress, including microbial attack. Scopoletin, a derivative of coumarin, is a benzopyrone in nature and found in the root of plants in the genus Scopolia like *Scopolia japonica*, *Artemisia scoparia*, *Kleinhovia hospita*, Stevia, *Agle marmelos etc.* [16] Coumarins, flavonol glycosides and a group of unidentified aglycones have been reported from the inflorescence of *Artemisa scoparia* [17]. Several species of *Artemisia annua* recorded the presence of Scopoletin like

Artemisia capillaries [18], *Artemisia campestris* [19], *Artemisia feddei* [20], *Artemisia argyi* [21], *Artemisia annua* [22] and *Artemisia iwayomogi* [13]. The sample plant taken for the study was examined for Scopoletin and the results were satisfactory.

TLC Separation Optimization: The *A. annua* extract (5 µl), when subjected to TLC showed the presence of scopoletin **Fig. 2**. A comparison of the spectral characteristics of the peak for standard compound and that of the sample further confirmed the presence of scopoletin in the sample **Fig. 3**. Good resolution with a symmetrical and reproducible peak was obtained.

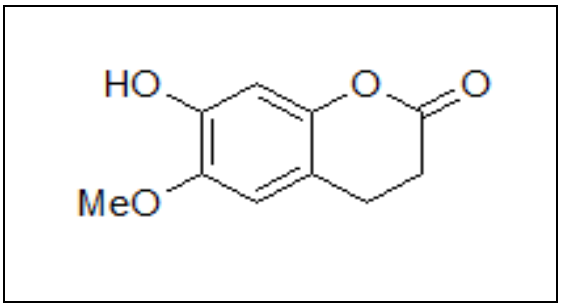

FIG. 1: CHEMICAL STRUCTURE OF SCOPOLETIN

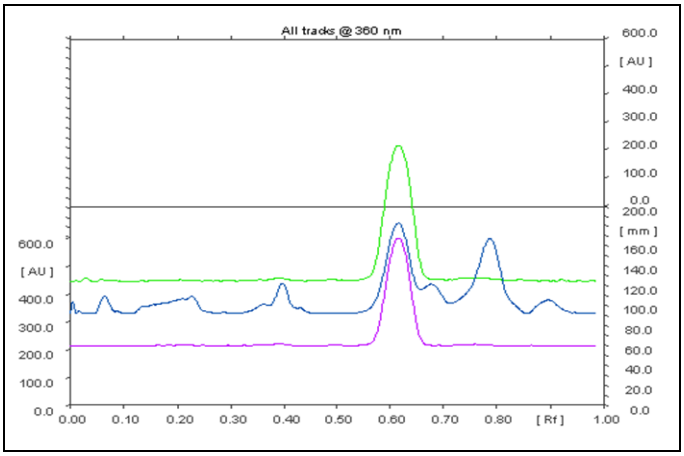

FIG. 2: *A. ANNUA* EXTRACT SHOWING IDENTICAL PEAK WITH STANDARD SCOPOLETIN OF

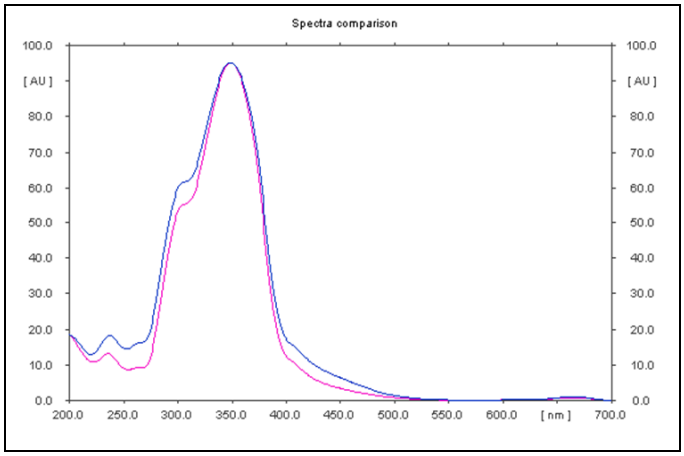

FIG. 3: SPECTRAL COMPARISON FOR THE PEAKS STANDARD SCOPOLETIN AND *A. ANNUA* EXTRACT

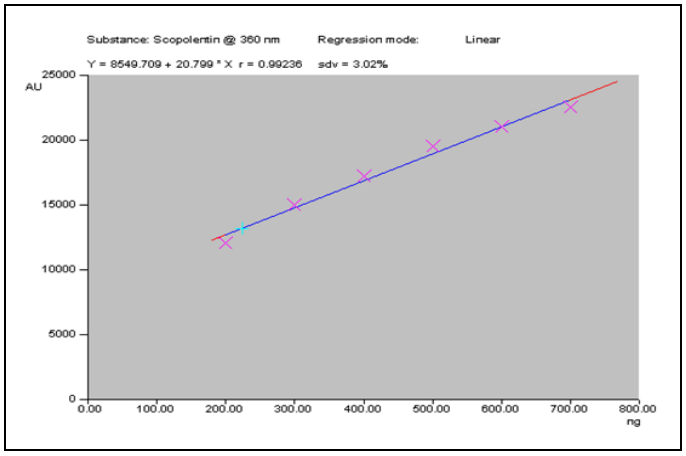

FIG. 4: CALIBRATION CURVE OF PEAK AREA VERSUS CONCENTRATION FOR SCOPOLETIN

Linearity: The peak area versus concentration plot was found to be linear in the range of 200-700 ng spot-1 for scopoletin **Fig. 4**. The regression equation and correlation coefficient for scopoletin indicated good linearity **Table 1**.

Sample Analysis: The scopoletin content of the leaves calculated from the area calibration curve **Table 2** by this method was found to be 0.04477% w/w (plant dry weight basis).

TABLE 1: VALIDATION PARAMETERS FOR QUANTIFICATION OF SCOPOLETIN

S. no.	Parameter	Results
1	R_f	0.62
2	Dynamic range (ng spot^{-1})	200 - 700
3	Equation	Y=8549.709+20.799x
4	Slope	20.799
5	Intercept	8549.709
6	Linearity (correlation coefficient)	0.99236

TABLE 2: CALIBRATION CURVE PARAMETERS FOR QUANTIFICATION OF SCOPOLETIN IN *A. ANNUA* EXTRACT

R_f	Amount/Fraction	Area	X(calc)
0.63	200 ng	12091.80	-
0.62	300 ng	15005.72	-
0.61	400 ng	17225.91	-
0.62	500 ng	19514.45	-
0.61	600 ng	21051.78	-
0.63	700 ng	22565.71	
0.62	-	13205.57	223.85 ng

CONCLUSION: From the above spectroscopic studies, it was concluded that this TLC procedure may be used effectively for identity, quality evaluation as well as quantitative determination for this plant or its derived products. Also, the residual leaves can be reused for the isolation of Scopoletin.

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CONFLICT OF INTEREST: Nil

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