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**Ivy Herbal Products for Cough and its Validation for Hederacoside C Biomarker
Determination with HPLC _PAD Techniques**

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ABSTRACT

Different pharmaceutical companies in Pakistan have started production of high quality Ivy natural medicines for the cure of cough. Three different brands of Ivy syrup were evaluated for Hederacosides C biomarker with high-performance liquid chromatographic method coupled with a photodiode array detector. Good linear relation was observed through regression analysis (correlation coefficient 0.9994) between peak concentration the peak area. and limits of detection (LOD) and quantitation (LOQ) were 0.011 and 0.032 mg/mL, respectively, and percentage recovery was in the range of 99.69% to 100.90% (RSD < 2%).

Keywords: Ivy, Hedera helix, cough, hederacosides C, HPLC

INTRODUCTION

A cough usually brings reflex activity the to clear the throat of mucus or foreign irritants. Coughing in a way to direct the throat is representative of an infrequent action, although there are different conditions that can produce frequently cessation or attack of coughing. In general, a cough that persists for less than three weeks is referred to as an acute cough. A cough that remains ultimately during three weeks and eight weeks, and finally improves in this period, is designated as a sub-acute cough. While a persistent cough that goes beyond eight weeks is well known as a chronic cough. The cough medicines are prescribed to treat the medical conditions as nasal

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decongestants (unclog a stuffy nose) ,cough suppressants (quiet a cough), and expectorant (loosen mucus) However, common cough symptoms are caused by acute viral upper respiratory tract infections but generally the course of the cough is mostly benign and self-limiting. The chronic cough causes are diagnosed as chronic obstructive pulmonary disease and asthma and these in turn block the airway and increase hypersecretion of mucus, due to which sign and symptoms such as wheezing or dyspnoea ensues. Inappropriate use of antibiotics for viral respiratory tract infections poses significant problem causing resistance of pathogenicity [1]. Therefore, alternative treatment options are now a day's more popular and efficacious

for the mucolative and antitussive activity and these are widely prescribed in primary healthcare settings. Among these non-antibiotic cough remedies, herbal medicine containing extracts from the leaves of ivy (*Hedera helix* L.) are very popular and effective to combat cough in European countries as well as in developing and developed countries of the world [2]. It has been cited that in 2007, more than 80% of herbal expectorants prescribed in Germany consists of ivy extract to ward off cough malaise. The medical prescription written were over 2 million both in hospital and clinic settings and a volume of sales of Ivy cough syrups exceeded the amount 13 million Euro. Ivy leaf contains saponins which are considered to have mucolytic, spasmolytic, bronchodilatory and antibacterial effects [3-4]. Cough is a type of defensive reflex of the respiratory tract that is important to clear the upper airways and should not be suppressed indiscriminately. Cough is thought to be caused by a reflex and it occurs because of mechano-or chemoreceptor stimulator in throat, respiratory passage or stretch receptor in the lungs. The sensitive receptors are located in the bronchial tree, particularly in the junction of the trachea. These receptors can be stimulated mechanically or chemically, for example, by inhalation of various irritants, than nerve impulses activate the cough center in the brain.[5]. In a post marketing study 9657 patients (5181 children) with bronchitis (acute or chronic bronchial inflammatory disease) were treated with ivy leaf syrup and after 7 days of therapy, 95% of the patients showed improvement in their symptoms [6]. A study has been undertaken to validate the Ivy syrup for the determination of biomarker hederacoside C for consistency of manufacturing. The biomarker is indicative of the drug development process for the purpose of quality control.

MATERIALS AND METHOD

The spray dried standardized extract of *Hedera helix*, *Glycyrrhiza glabra*, and *Thymus vulgaris* were obtained from M/S Martin Bauer Group, Germany. UNIT COMPOSITION – Ivy leaf or Ivy leaf Plus

Syrup

Each 10 ml syrup contains: Herbion Cofnoves and Scilife Nokuff contains Ivy Leaf powder extract 70.0 mg, where as Genics Ileaf consists of Ivy Leaf powder extract 70.0 mg, Thyme Powder Extract 43.0 mg, Licorice Powder Extract 43.4 mg, and it Sorbitol, Glycerin, Sweeteners, citric acid, sodium benzoate, potassium sorbate, cellulose sodium, honey flavor, Purified water q.s. are added.

Table 1: Composition of Ivy Leaf syrup

Herb Composition	Herbion Cofnoves	Scilife Nokuff	Genics Ileaf
<i>Hedera helix</i> (Ivy Leaf)	70mg	70mg	70mg
<i>Glycyrrhiza glabra</i> (Licorice rhizomes)	Nil	Nil	43.4mg
<i>Thymus vulgaris</i> (Thyme leaf)	Nil	Nil	43mg

Ivy Syrup was successfully developed for cough and cold. The different formulations by different companies are as Herbion_Cofnoves, Scilife_Nokuff, Genics_Ileaf, (additional Licorices and Thyme).

Preparation of 7 Litre Ivy leaves syrup: 1000 ml de-ionized water was taken in manufacturing tank. Heated and boiled for 40 to 45 minutes. When boiling was added, preservatives were added, then citric acid was added. In 750 ml deionized water, Ivy extract 49g, (additional in case of Thyme and Licorices 30g each) was added, with constant stirring, and transfere Comparative d to previously warm water. Then sorbitol was added into it, allowed to cool, and the flavor added The heating was considered with constant stirring till it became a homogenize syrup.Sorbitol 3. 5kg, glycerin 1.0g, sweetener 95 (Stevia) 10.5g, , citric acid 7.0 g, sodium benzoate 7.0g, potassium sorbate 7.0g were separately dissolved, honey flavor mixed, heated and boiled thoroughly. This solution was mixed with the homogenized syrup and stirred for 20 minutes. It was heated till fully dissolved and then filtered the solution. Afterward final mixing was allowed by stirring for half an hour. .

Phytochemical Evaluations

Brown color syrup was prepared with characteristic odor, having pH between 3.0 - 6.0, and density 1.25 - 1.35 g/cm³. Identification of polysaccharides, tanning agent, and ascorbic acid were performed by test tubes method. While the identification of Hederacoside C was carried out by HPLC.

Physical Evaluation

Appearance

Brown color viscous syrup with characteristic odor, ?? Determination 10 ml of syrup was dissolved in 100 ml of water. Filtered and pH potentiometrically Standard: pH 3.0 - 6.0 determined. Density Determination: It was conducted by SP XII method 3 and it was according to Standard: 1.25 to 1.35 g/cm³.

Qualitative Identification Reactions: 5 ml of the preparation was placed in a flat-bottomed 50 ml flask. 20 ml of 96% spirit was added and mixed; precipitated suspension was formed (polysaccharides). The solution was left for 1 hour for layer separation and filtered carefully through a filter paper (Solution ?). 3 drops of ferric chloride was added solution to 3 ml of solution A. After shaking greenish-yellow coloring appeared (tanning agents). To 3 ml of A solution 3 drops 0.1% alcoholic solution of sodium 2.6-dichlorfeno-lindofenol solution was added, a reddish-violet coloring at mixing (ascorbic acid).

Determination of Hederacoside C by HPLC

Sample preparation

Place about 10 mL of Ivy leaf syrup was placed in a 25 ml volumetric flask, made up the volume with methanol R. The solution sonicated for about 30 minutes in a ultrasonic bath. The solution was filtered through a filter paper with pore size 0.45 µm, the filtrate was used for chromatography.

Standard solution preparation

10mg of Hederacoside C standard, accurately weighed, was placed in 10 ml volumetric flask and made up to volume with methanol R. The solution

was sonicated for about 30 minutes in the ultrasonic bath. The solution was filtered through a filter paper of pore size 0.45 µm and the filtrate used for chromatography.

Chromatographic analysis

20 µL of test solution and Hederacoside C standard solutions were alternately injected into a liquid chromatograph with UV detector obtain not less than 3 chromatograms of sample solution in the following conditions:

Column: Size: L = 250 mm, Ø = 4.6 mm.
Stationary phase: octadecylsilyl silica gel for chromatography R (5 µm).

Mobile phase: Mobile phase A: phosphoric acid R, Acetonitrile R, water R (2: 860: 140 v/v/v).
Mobile phase B: phosphoric acid R, Acetonitrile R (2:998 v/v).

Time (min)	Mobile Phase A (% v/v)	Mobile Phase B (%v/v)
0-40	100 → 60	100 → 40
40-41	0	100
41-55	0	100
55-56	100	0

Ivy Syrup was successfully developed for cough and cold. The different formulations by different companies are as Herbion_Cofnoves, Scilife_Nokuff, Genics_ileaf, (additional Liquorices and Thyme).

Flow rate: 1.5 ml/min
Wavelength: 205 nm
Column temperature: 40 °C.

RESULTS AND DISCUSSION

Hederacoside C content, in Ivy leaf syrup should not be less than 4 mg per 10 mL, calculated by the following formula:

$$ASMP \times WSTD \times \text{Sample dilution} \times P \times 10$$

$$X =$$

ASTD × Standard dilution × WSMP × 100
Where,

ASMP – Mean value of peak area of tested solution samples.

ASTD – Mean value of peak area of standard solution samples.

WSMP – Preparation weight, g.

WSTD – Standard weight, mg.

P – Percent Purity of standard sample, %.

Note: Hederacoside C contents should not be less than 4 mg/ 10 mL.

Structure of Hederacoside C

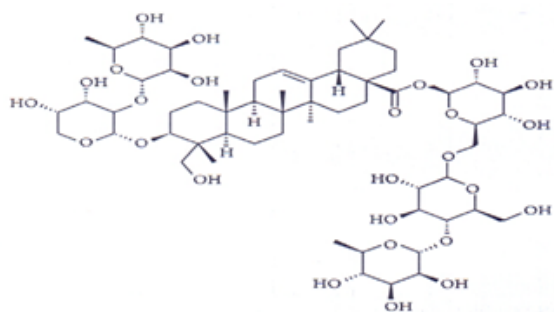


Fig. 1: Hederacoside C Standard

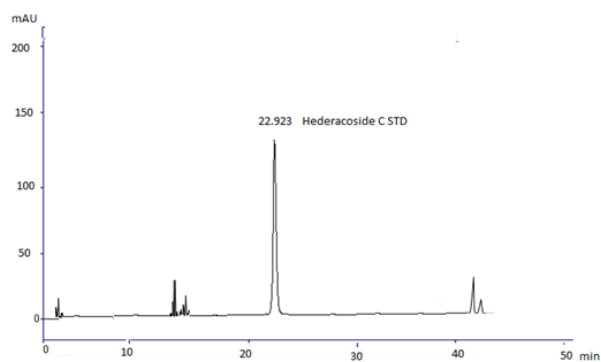


Fig 2: Genics Ileaf

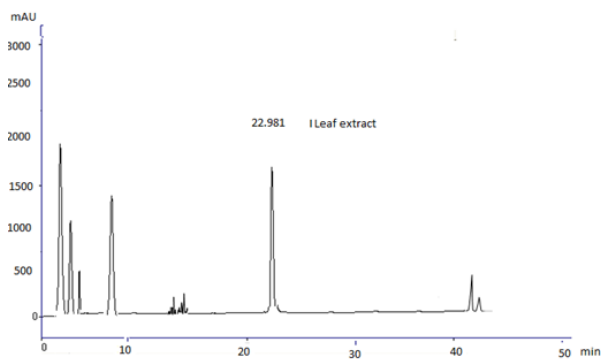


Fig 3: Nokuff syrup

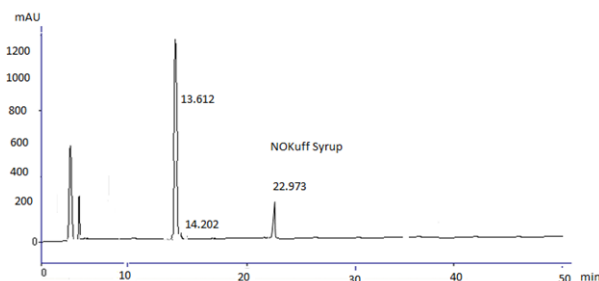
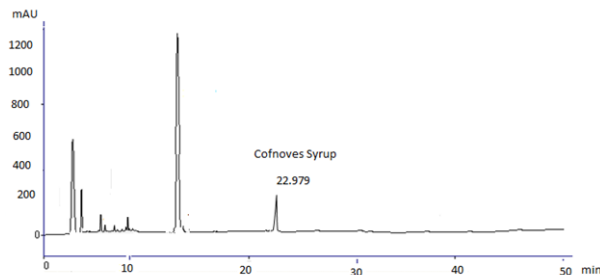


Fig 4: Herbion Cofnoves



Hedera helix is widely used to treat bronchial asthma for many years, however, the effects this herb on lung histopathology is still far from clear. An experimental investigation was conducted to determine the effect, oral administration of Hedera helix lung histopathology in a chronic mouse model Asthma. The BALB / c mice were divided into four groups; I (placebo), II (Hedera helix), III Dexamethasone) and IV (control). All mice were sensitized with the exception of checks and challenged with ovalbumin. Then, the mice in Group I received saline, Group II 100 mg / kg and Hedera helix Group III 1 mg / kg dexamethasone against orogastric gavage once a day for a week. The histopathology was evaluated by optical and electronic microscopy in all groups. The number and thickness of the basement membrane found significant goblet cells lower in Group II, but there was no statistically significant difference in the number of Mast cells. The thickness of the epithelium and smooth muscle layers subepithelial between Group I and II were compared to each other. The thickness of the epithelium, muscle subepithelial layers, the number of mast cells and goblet cells Group III were significantly improved compared to group II. Although Hedera helix administration only

reduces the number of goblet cells and the thickness of the basement membrane in asthmatic airways, improved all parameters except dexamethasone histopathological thickness of the basal membrane better than *Hedera helix* [7].

The cough preparations containing herbal extracts of ivy leaves (*Hedera helix*) are very popular. Target. A systematic review to assess the effectiveness and tolerability ivy tolerability in acute infections of the upper respiratory tract is the subject matter. The search for randomized controlled trials (RCTs), controlled clinical non-randomized trials and observational cited were evaluated and ivy preparations for acute upper respiratory Urtis was assessed using the Jadad score or EPHPP tool. Results displayed that 10 eligible studies were identified reports on 17,463 subjects. The studies were heterogeneous design and leadership; Two were RCTs. Three studies examined a combination of ivy and thyme, reviewed seven studies monopreparations ivy. Only one RCT (n = 360) examined a combination of ivy / thyme used placebos, and showed a statistically significant superiority in reducing the frequency and duration of cough. All other studies did not show a placebo control and AWS serious methodological flaw. It was in agreement that ivy extracts are is effective in reducing the symptoms of URTI. It was deduced that all studies indicate that ivy extracts were effective in reducing symptoms of URTI, there is no convincing evidence because of serious methodological fl AWS and the lack of placebo controls. The combination of ivy and thyme can be more efficacious [8].

In this post-marketing study on 9657 patients were treated (5181 children) with bronchitis (acute or chronic airway inflammatory disease) with a syrup, dry extract of ivy leaves. After 7 days of treatment, 95% of patients showed improvement or cure their symptoms. The safety of the therapy was very good with an overall incidence of side effects of 2.1% (mainly gastrointestinal diseases associated with 1.5%). In patients, concomitant medication and got, it was demonstrated that the use of additional

antibiotics does not increase benefits to each of finess but the relative risk of occurrence of side effects by 26%. The conclusion drawn that the extract dried and effective ivy leaf is well tolerated in patients with bronchitis [9].

Hederacoside C, R-hederin hederagenin and saponins are found in ivy leaves and as such influenced this receptor regulation was the target of the study. The adrenergic receptor internalization - GFP fusion proteins after stimulation with 1 μ M terbutaline was by pre-incubation of the transfected HEK293 cells stably with 1 μ M R-hederin for 24 hours while locked or C, or hederacoside hederagenin (1 μ M respectively). Alexa532-NA complexes by fluorescence correlation spectroscopy. However after incubation of A549 cells with 5 nM Alexa532-NA two different time constants for the distribution of two AR were found. The evaluation of the autocorrelation curve showed diffusion constants in time bound1 t) 1.4 (1.1 ms (n) 6) available to the receiver - ligand complexes with free lateral mobility and t bound2) 34.7 (14 1 ms (n) 6) receptor - ligand complexes with reduced mobility. The distribution of diffusion time constants was 24.3 (2.5% for t bound1 and 8.7 (4.3% bound2 t (s) 6) A549 cells with 1 micron. R-hederin pretreated for 24 hours showed a dose-dependent relationship of changes in this distribution 37.1 (5.5% and 4.1 bound1 t (t bound2 1.1%. At the same time the level of Alexa532-NA-binding was significantly increased from 33 0 (6.8 to 41.2 (4.6%. In saturation experiments, R-hederin influence of the density of adrenergic receptors steps (B max), while the KD value for Alexa532-NA-binding decreased from 36.1 (9.2 to 24.3 (11.1 nM pretreatment of cells with R HASM-hederin. (1 μ M, 24 h) led to an increase in intracellular cAMP levels of 13.5 (7.0% in stimulating saponins conditions is related to the structure as remarkable. hederacoside C ederagenin affect either 2GB binding behavior or the level of intracellular cAMP. [10]

All these work on different parameter conclusively prove that Ivy leave for cough is the drug of choice

that is why in the western countries this is rather more popular to combat different forms of respiratory malaise. In order to formulate the Ivy cough syrup, the most important factor is to determine the biomarker for the standardization. All the three brand of Ivy syrup were evaluated for Hederacosides C biomarker with high-performance liquid chromatographic method coupled with a photodiode array detector and furnished the good linear relation. The limits of detection (LOD) and quantitation (LOQ) were 0.011 and 0.032 mg/mL, respectively, and percentage recovery was between 99.69% and 100.90% (RSD < 2%).

CONCLUSION

A HPLC method to determine three different Ivy syrup for the quantification of hederacoside C, present in cough Ivy syrup has been applied for its linearity and accuracy for quality control analyses. It was found that hederacoside C is present in all the Ivy cough syrup estimated by HPLC PAD techniques. The assessment compared well with authentic hederacoside C reference standard.

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