OPENACCESS

Quality and Potency Analysis of IVY Leaf Extract

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Authors' Contributions

1 conceived the idea, 1,2,3,4 participated in the design of the study and wrote the manuscript, 5-10 designed the project. 11,12 editing of the manuscript. All authors reviewed and approved the final manuscript.

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ABSTRACT

Background: Hedera helix generally recognized as Ivy or English *Ivy* and has been employed since a long time in conventional medication in management of respiratory problems.

Objective of the study: The current study was conducted with the aim to evaluate *lvy* leaf extract to authenticate the quality of extract.

Methods: Evaluation was conducted on different physicochemical factors of extract that include physical appearance, organoleptic properties and solubility. Loss on drying, microbial analysis and heavy metal analysis was also carried out to execute the safety of extract. High performance liquid chromatography was executed to evaluate the presence of Hederacoside C in *Ivy* leaf extract employing phosphoric acid 85 %, acetonitrile, water (2:140:860 v/v/v) as a mobile phase.

Results: Extract exhibited greenish brown color, distinctive odor and sweet taste. An *Ivy* extract was found in agreement of the allowable microbial limit as well as with the heavy metal contents limit. The presence of Hederacoside C in *Ivy* leaf extract was confirmed by HPLC.

Conclusion: The current evaluation reveals conformity with all the analytical procedures. Hence Ivy leaf extract is well standardized formulation at the base line consideration.

Keywords: Analysis; Evaluation; *Ivy* leaf extract; Hederacoside C

INTRODUCTION

Hedera helix L. belongs to a family Araliaceae and generally recognized as Ivy or English *Ivy*. The young leaves extract of the plant has been employed since a long time in conventional medication in management of respiratory problems owed to its broncho spasmolytic and expectorant effects [1]. At present a variety of pharmaceutical formulations of Ivy leaf extract including solid, liquid and semisolid dosage forms are accessible for public to cure the ailments [2,3]. There are several clinical trials conducted using *lvy* leaves that have verified the effectiveness and quality of such drugs. Due to the investigational and pragmatic proof on its efficiency and safe use, a noteworthy augmentation of its prescribing practices has been observed in several European countries includes Germany [4]. Mass percentage i.e. greater than 80% of plant based expectorants recommended in Germany In 2007, consisted of *lvy* leaf extract and accounted to almost two million prescriptions countrywide.

There is a vast variety of chemical constituents that have been isolated from *H. helix*, including polyacetylenes, triterpene, flavonoids, saponins and various phenolic compounds [5]. A triterpene saponin α -hederin, is known to be accountable for the beneficial consequences of *Ivy* leaf extract since it possess β_2 -adrenergic properties [6]. Hederacoside C, an important triterpene saponin, is recognized to be metabolized in the active form and generate the consequences of α -hederin in the body [6,7]. The current investigation was conducted with the plan to evaluate the quality and potency of *Ivy* leaf extract.

Experimental

Extract preparation

All the herbal drugs to be utilized in the research were sieved by using mesh #60 and grinded. Every herb was put in extractor; water was used as a solvent in the proportion of 1:10 with herbs. Then finally the decoction was obtained and filtered.

Organoleptic evaluation

Ivy leaf extract was evaluated for its organoleptic properties including color, odour and taste.

Loss on drying

Before testing, Petri dish was kept in the oven at 105°C for 30 minutes and cool the Petri dish by using desiccator and weigh it, the weight was noted as W1. After accurately weighing transfer about 1.0g of sample into Petri dish and note down the weight as W2. Petri dish was placed with sample into the oven at 105°C for approx 1.5hr. After drying the Petri dish was removed and kept into desiccator. After cooling, the Petri dish was weighed with sample and note down the weigh as W3.

Calculation

% of loss on drying = $\frac{(W2 - W3)}{(W2 - WI)} \times 100$

Where,

WI = Weight (g) of the empty weigh bottle

W2 = Weight (g) of the weigh bottle with sample before drying.

W3 = Weight (g) of the weigh bottle with sample after drying.

Assay using High Performance Liquid Chromatography

Sample preparation

In 10 volumetric flask, 100 mg of extract was dissolved and diluted using methanol to make up to mark with the same solvent. The solution was then pass through the process of sonification for 30 minutes using ultra sonic bath and filtered by means of a filter having a pore size of 0.45 μ m, proceed for HPLC analysis.

Standard solution preparation

In a 10 ml volumetric flask, 10 mg of Hederacoside C standard was taken and dissolved using methanol; make up the volume with methanol. The solution was sonicated in the ultra sonic bath. Filter the obtained solution by means of a filter having a pore size of 0.45 μ m and use filtrate for chromatography.

Analysis

20 μ L of sample and Hederacoside C standard solutions were injected and 3 chromatograms in the subsequent conditions were obtained:

Injection sequence was Diluent (blank): 01 followed by Standard: 05 Sample: 02 and Bracket standard: 01. System suitability was RSD NMT 2.0% (5 replicate injections).Column: Size: L = 250 mm, Ø = 4.6 mm at 40 °C column tremperature. Stationary phase: Octadecylsilyl silica gel (5 µm). Mobile phase were phosphoric acid 85 %, Acetonitrile, water (2:140:860 v/v/v) (Phase A) and phosphoric acid 85 %, Acetonitrile (2:998 v/v) (Phase A) respectively with 1.5 ml/min of flow rate at a wavelength of 205 nm.

Gradient system

Time (min)	Mobile Phase A (% v/v)	Mobile Phase B (%v/v)
0	100	0
40	60	40
41	0	100
55	0	100
56	100	0
70	100	0

Formula:

% of Hederacoside C =
$$\begin{array}{c} A_{SPL} \times W_{STD} \times dilution \ of \ spl \times P \\ \hline A_{STD} \times dilution \ of \ std \times W_{SPL} \times 100 \end{array}$$

Where,

As_{PL} – Average Area of sample

Ast - average area of standard

- W_{SPL} Sample weight, mg
- WSTD Standard weight, mg
- P Percent Purity of standard sample, %

Note: Quantity of Hederacoside C contents in *Ivy* leaf extract should NLT 3.0%

Microbial contamination:

Microbiological Examination of *Ivy* leaf extract was carried out as per the of herbal medicinal products for oral use [8].

Determination of heavy metal contents:

Evaluation of trace elements was carried out by atomic absorption spectrometry flame (FAAS) at the Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratory at Karachi, Pakistan.

RESULTS

Ivy leaf extract exhibited greenish brown color, typical odor and sweet taste. A vital practice of flavor masking is executed to remove repulsive drugs from getting contact with the taste buds [9]. (Table 1). Determination of physicochemical properties plays a significant part in the quality measurement [10].

Table	1.	Organoleptic	evaluation	of	lvy	leaf
extract						

Items	Specification	Test Results	
Physical & Chemical Data			
Appearance	Fine Powder	Complies	
Color	Greenish brown powder	Complies	
Odour	Characteristic	Complies	
Solubility	Soluble in hydro, Hydro –alcoholic solutions	Complies	

Table 2.	Specifications	of Iv	y leaf	extract

S.NO	TEST	SPECIFICATIONS
01	Appearance	Greenish brown powder.
02	Identification	The retention time for the major peak in chromatogram of the Assay preparation match to that of the Standard preparation.
03	Loss on drying	NMT 5.0 %
04	Assay (Hederacoside C)	NLT 10.0%



Figure 1. Structure of Hederacoside C found in Ivy leaf extract.



Figure 2. Chromatogram of Hederacoside C.

Microbial Analysis	Limit CFU/g	Observation	
Aerobic viable count up	NMT 10⁴ CFU/g	conform	
Salmonella	not present	not present	
E. coli	not present	not present	
S. aureus	not present	not present	
P. aeruginosa	not present	not present	
Entire fungal count	NMT 10 ² CFU/g	conform	

Table 3.Acceptablecontentsfor1gofpreparation.

Figure 1 represents the structure of Hederacoside C.Loss on drying and assay results are given in Table 2. Figure 2 represents the structure of Hederacoside C. Massive bacteria may be frequently viewed in soil or derived fertilizers [11,12]. Hence there is enhanced probability for the occurrence of microbial pollution in plant based drugs. An Ivy extract was found concord of the permissible microbial confines. Table 3 Plant based drugs are typically accessible as a blend of more than one plant component and its medicinal action relies on its phytochemical components [13]. Precise recognition and quality assurance is an essential prerequisite to make certain reproducible quality of such drugs [14]. Phytochemical evaluation indicates the quality dimension; include preliminary phytochemical analysis, chemoprofiling, and biomarker examination using novel analytical methods. HPLC is a considerable means for the quantitative phytochemical scrutiny of the plant based drugs. In current study Hederacoside C an active biomarker of *Ivy* leaf was detected and the peak of Hederacoside C complies with standard.

DISCUSSION

The toxic effects of trace metals on human welfare and the atmosphere have fascinated major contemplation in recent years. The heavy metal contents have small excretion rates that may result in unfavorable consequences on human being still at very low amount. Metals for instance copper, manganese, zinc, iron, and chromium are basic nutrients; required for the basic physiological and natural functions of the human being. On the other hand, an increase in their ingestion over definite acceptable confines can turned out to be noxious. There are numerals of healthiness issues linked to excessive uptake of dietary heavy metals [15]. The elements that have been revealed to be noxious are precisely Arsenic (As), mercury (Hg), Cadmium (Cd) and lead (Pb). The concentration of As, Cd, Pb and Hg were examined in *Ivy* leaf extract and they were not detected in extract (Table **4**).

Plant	Specifications	Heavy Metals	
Hedera helix L.	Arsenic	Not found	
	(NMT 10 ppm)	Notiouna	
	Cadmium	Not found	
	(NMT 0.3 ppm)	Notiouna	
	Lead (NMT 10 ppm)	Not found	
	Mercury	Not found	
	(NMT 1 ppm)	Notiouna	

Table 4. Heavy metal analysis of extract.

CONCLUSIONS

The current evaluation reveals conformity with all the analytical procedures. The evaluation provides precise and accurate means to develop qualifications for identity, precision and reproducibility of biomarkers in *Ivy* leaf extract. Hence the extract is well standardized formulation at the base line consideration.

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