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QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF HYDRO-ALCOHOLIC EXTRACT OF NEPHROPROTECTIVE PROPRIETARY POLYHERBAL FORMULATION - URIZONE

Ayurveda

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ABSTRACT

In the present study nephroprotective proprietary polyherbal formulation Urizone was chosen for screening the phytochemical constituents by qualitative and quantitative methods. The results indicates hydro-alcoholic extract of Urizone contains alkaloids, flavonoids, terpenoids, carbohydrates, protein and amino acids. Quantitative analysis were also conducted to determine the amount of primary and secondary metabolites present in the hydroalcoholic extract of Urizone. The amount of primary metabolites such as Total carbohydrates 11.10 %, total proteins 2.05 % and total lipids was found 0.17 %. The amount of secondary metabolites found in the hydroalcoholic extract are Alkaloid content was found 79.68 mg/gm, Phenoilc content was 282 mg/gm, tannin content was 125.5 mg/gm, flavonoid content was 0.784 mg/gm, Phytosterols was 0.11 mg/gm and Saponins 0.245 mg/gm. The results concludes that the formulation is potential source of various phytoconstituents.

KEYWORDS

Polyherbal formulation, hydro-alcoholic extract, Urizone, Phytochemicals

INTRODUCTION

The World Health Organization supports the use traditional medicine provided they are proven to be efficacious and safe [1]. Phytochemicals are the chemicals produces by various parts of the plant. Steroids, terpenoids, flavonoids, carotenoids, alkaloids, tannins and glycosides are the constituents found bioactive. Various environmental factors such as climate, altitude, rainfall and other conditions may affects the growth of plants which in turn affect the quality of herbal ingredients present in particular species even when it is produced in the same country. The conditions may cause variation in the bioactive compounds [2].

Qualitative phytochemical screening will help to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds for useful aspects to human beings. Plants have limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives [3]. Several bioactive compounds have been isolated based on quantity and studied for its pharmacological activity. Polyherbal formulation is a way of combination drug in which the two or more herbal ingredients combined together to form single drug. Urizone is a proprietary polyherbal formulation containing whole plant of *Aerva lanata*, fruits of *Tribulus terrestris*, bark of *Crataeva nurvala* and root of *Hemidesmus indicus* in different ratio. The present study is focusing on qualitative and quantitative analysis phytoconstituents present in formulation.

MATERIALS AND METHODS

Preparation of Hydro-alcoholic extract of Urizone (HAEU)

The contents of Urizone were used for the prepare the hydro-alcoholic extract by cold maceration method. Water and alcohol (7:3) was removed by distillation and dried under vacuum evaporator less than 50^0C. The resulted product is a greenish black sticky material. The hydro-alcoholic extract of Urizone was utilized to perform qualitative and quantitative estimation of phytoconstituents.

Preliminary phytochemical Screening[3]

The Hydro-alcoholic extract of Urizone was subjected to preliminary phytochemical screening to identify the active chemical constituents.

1. Test for carbohydrate: A small quantity of the extract was dissolved in 4 ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrate and glycosides.

a. Molisch's Test: Few drops of Molisch's reagent were added to an aqueous extract followed by vigorous shaking. Thereafter, 1.0 ml of conc. H_2SO_4 was added carefully by sliding down the walls of the tube gently to form two layers. Appearance of a brown ring separating the solution into two layers indicates the presence of carbohydrate.

b. Fehling's Test: Equal volumes of Fehling A and Fehling B were mixed, 2ml of this solution was added to extract and boiled. Formation of red brick precipitate at the bottom of the test tube indicates the presence of carbohydrates.

c. Benedict's Test: 2ml of Benedict`s solution was added to extract and boiled. Formation of reddish brown precipitate indicates the presence of carbohydrates.

d. Iodine test: 2ml of iodine solution was added to the extract. Dark blue or purple coloration indicates the presence of complex carbohydrates (Starch).

2. Test for glycosides: Tests for glycosides were performed as follows:
a. Legal' test: To the few ml of extract, 1ml of pyridine and a few drops of sodium nitroprussside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearence of pink to red colour showed the presence of glycosides.

b. Borntrager's test: Few ml of extract treated with chloroform and then the chloroform layer was separated. To this equal quantity of diluted ammonia solution was added. Ammonia layer acquired pink colour, showing the presence of glycosides.

3. Test for Proteins
a. Biurette test: To the sample 5 drops of biurette reagent was added and heated for few minutes, Appearance of blue colour indicates the presence of protein.

b. Ninhydrin test: To the sample 3 drops of Ninhydrin reagent was added and heated for few minutes. Purple colour appearance indicates presence of proteins or amino acids.

4. Test for alkaloids
a. Dragendroff's test: To the extract Dragendroff's reagent (potassium bismuth iodine solution) was added. Production of reddish brown precipitate indicates the presence of alkaloids.

b. Mayer's test: To the extract, Mayer's reagent (potassium mercuric

iodine solution) was added. A reddish brown precipitate production indicates the presence of alkaloids.

c. Wagner's test: To the extract, Wagner's reagent (iodine- potassium iodide solution) was added. A reddish brown precipitate indicates the presence of alkaloids.

5. Test for phenols: 2ml of 2% ferric chloride solution was added to the extract. Blue green or purple coloration indicates the presence of phenols.

6. Test for tannins
a. Ferric chloride test: A 5%solution of ferric chloride in 90% of alcohol was prepared. Few drops of this solution was added in to extract. Dark green or deep blue colour indicates the presence of tannins.

b. Lead acetate test: A 10% w/v solution of basic lead acetate is added to the extract. Precipitate obtained indicates the presence of tannins.

7. Test for saponins
a. Foam test: The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15minutes. There is formation of one layer of foam, it indicates the presence of saponins.

8. Test for flavanoids
a. Shinoda's test: Small quantity of the extract is dissolved in ethanol. To this pieces of magnesium are added followed by conc. hydrochloric acid drop wise and heated. Appearance of magenta colour showes the presence of flavonoids.

9. Test for anthroquinones: Approximately 0.1 g of the extract was mixed with 5.0 ml of chloroform and agitated for 5.0 min. The solution was filtered and equal volume of ammonia was added to the filtrate and agitated again. A brick red colour in the upper aqueous layer indicates the presence of free anthroquinones.

10. Test for phytosterol and terpenes
a. Liebermann-Burchard Test: The first portion is mixed with 1 ml of acetic anhydride followed by the addition of 1.0 ml of concentrated Sulfuric acid gently down the side of the test tube to form a layer underneath. The formation of a reddish violet colour at the junction of the two liquids and a green colour in the chloroform layer would indicate the presence of terpenes.

b. Salowski's Test: To the second portion of the solution is added 2.0 ml of concentrated Sulfuric acid carefully down the side of the tube so that the sulfuric acid forms a layer. No reddish brown colour at the interface would indicate the absence of sterols.

Quantitative estimation of the Primary and Secondary metabolites
Primary and secondary metabolites were estimated by using standard procedures [4-10].

RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis
The hydroalcoholic extract of Urizone (HAEU) was evaluated for phytochemical screening and the results are given in Table 1 given below

The hydro-alcoholic extract of Urizone was subjected to various phytochemical tests, which showed the presence of alkaloids. Tannins, flavones, glycosides, phenolic compounds, proteins, phytosterol and saponins.

Table: 1. Qualitative analysis of phytoconstituents in HAEU

S. No.	Phytochemical constituents	HAEU
1	Alkaloids	+
2	Tannins	+
3	Flavones	+
4	Glycosides	+
5	Phenolic compounds	+
6	Proteins	+
7	Quinones	-
8	Phytosterols	+
9	Reducing sugars	-
10	Saponins	+
11	Anthroquinones	-

+ Present, - Absent

Quantitative Estimation of Primary metabolites content in HAEU

The estimation of primary metabolites content in HAEU is given in Table 2.

Table: 2. Quantitative estimation of primary metabolites in HAEU.

S. No.	Name of the metabolite	% w/w
1	Total Carbohydrates	11.10
2	Total proteins	2.05
3	Total lipids	0.17

Quantitative Estimation of Secondary metabolites content in HAEU
The estimation of secondary metabolites content in HAEU is given in below Table 3.

Table: 3. Quantitative estimation of secondary metabolites in HAEU

S. No	Name of the secondary metabolite	Concentration
1	Alkaloid content	79.68 mg/gm of plant material
2	Phenolic content	282 mg/gm of Gallic acid equivalents
3	Tannins content	125.5 mg/gm of Catechin equivalents
4	Flavonoid content	0.784 mg/gm of Quercetin equivalents
5	Phytosterols	0.11 mg/g of plant material
6	Saponins	0.245 mg/g of plant material

In the preliminary screening analysis found Alkaloids, Tannins, polyphenolics, glycosides and Saponins. The plant Secondary metabolites are produced in plants to maintain the normal physiological functions. Recent years most of the researchers are showing interest in secondary metabolites isolation and analysis which are great therapeutic value[11,12]. In the present study secondary metabolites are estimated in the nephroprotective proprietary polyherbal formulation using standard methods. The total alkaloid content present in the sample was found to be 79.68 mg/gm of plant material. The total phenolics content was 282 mg/gm of Gallic acid equivalents, Tannins 125.5 mg/gm of Catechin equivalents, Flavonoid content 0.784 mg/gm of Quercetin equivalents, Phytosterols 0.11 mg/g of plant material and Saponins 0.245 mg/g of plant material.

CONCLUSION

The present study is to conclude that the hydroalcoholic extract of Urizone has potential to have a medicinal value due to presence of phytoconstituents like Alkaloids, Polyphenolics, Tannins, Flavonoids, Phytosterols and Saponins which are responsible for many activities. Further, *in vitro* and *in vivo* studies are in progress to prove safety and efficacy.

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CONFLICTS OF INTEREST

There are no conflicts of interests.

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