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Phytochemical and Biochemical Constituents Evaluation of Polyherbal and Herbomineral Formulation

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Abstract:

Qualitative analysis of plant extracts involves identifying the presence or absence of various phytochemical constituents without quantifying their amounts. For herbal medicines and dietary supplements, qualitative analysis ensures the presence of bioactive compounds that contribute to their purported health benefits. It helps verify the authenticity and quality of herbal products by confirming the presence of specific phytochemical markers. This research work was aimed to determine the presence of various phytochemical in herbal formulation.

Keywords: Herbs, Phytochemical, biochemical test, Qualitative analysis.

INTRODUCTION

Phytochemicals are naturally occurring, biologically active chemical compounds in plants. Phytochemicals are protective and disease-preventing particularly for some forms of infectious diseases such as HIV and degenerative diseases like anti-inflammatory diseases etc. The most important action of these chemicals with respect to human beings is somewhat similar in day today function as antioxidants. Plants are natural reservoir of medicinal agents almost free from side effects caused by synthetic chemicals (Rizvi et al., 2009).

Phytochemistry is rapidly expanding area with the new technique for the analysis of organic plant components (Harbrone, 1973). In order to discover new bioactive compound extracts of simultaneously evaluated by chemical screening (Wink, 1999). Modern methods of extractions are based on chemical polarity and solubility because an aqueous extract/organic extract might be prepared and further analysis are required for cleanup and purifications followed by bio-separation of components within the fraction by chromatography methods but final analysis of Mass Spectroscopy or NMR of the selected sample is necessary in order to unambiqusly identified the compound of interest (Kaufmann, 1999).

This research work was aimed to evaluate qualitative and quantitative determination of phytochemical and biochemical constituents of polyherbal and herbomineral formulation.

Materials and method

The bark of Pterocarpus marsupium (Asana), Cinnamomum zeylanicum (Tvak), rhizome of Curcuma longa (Haridra), fruit pericarp of Terminalia chebula (Haritaki) fruit pericarp & Root of Plumbago zeylanica (Chitraka) were collected for the polyherbal formulation.

The samples were shade dried at room temperature and then ground to a fine powder in a mechanic grinder. The powdered material was then mixed and extracted using various solvent extraction (petroleum ether, chloroform, methanol, ethanol and aqueous) in the ratio 1:10 using Soxhlet apparatus. After extracting all colouring material of the solvent was removed by evaporating on water bath which give rise to a solid mass of the extract.

Qualitative analysis

Plants are found to be the sources of many chemical compounds, most of which account for their various uses by man. The medicinal values of the plants rely in the presence of certain chemical substances that produce a definite physiological effect on the human body. Phytochemical screening was done according to the published standard method.

Quantitative analysis

The ethanolic extract of Polyherbal formulation and herbomineral formulation was analysed for some of the biochemical constituents by standard procedures.

Estimation of alkaloids: Dragendorff's reagent: This is a chemical reagent used as a general test for alkaloids. It forms precipitates with many alkaloids, producing an orange-red to brown precipitate.

Estimation of flavanoids: Flavonoids react with magnesium acetate in the presence of concentrated sulfuric acid to produce a pink, red, or purple color. A sample extract is mixed with magnesium acetate solution and then sulfuric acid is added. The color development indicates the presence of flavonoids.

Estimation of phenols: Phenols react with ferric chloride (FeCl3) to form colored complexes. A sample extract or solution is mixed with ferric chloride solution. The color change observed (often violet, blue, or green depending on the phenol concentration and structure) indicates the presence of phenols.

Estimation of Tannin: Hedge and Hofreiter method: Tannins react with ferric ammonium sulfate (FAS) in the presence of sulfuric acid (H2SO4) to form a blue-black or green-black complex. The intensity of the color formed is directly proportional to the amount of tannins present in the sample.

Estimation of saponin: Saponins form stable foams when shaken with water and can be quantified based on the height and stability of the foam formed. A sample solution is shaken vigorously with water in a graduated cylinder or similar container. The height of the foam is measured after a specified time, which correlates with the saponin concentration.

Estimation ofglycoside: Glycosides are hydrolyzed by acids or enzymes to release the aglycone and sugar. Legal's test specifically detects the presence of the aglycone portion (often an alcohol or phenol) after hydrolysis. The sample is treated with hydrochloric acid (HCl) or other suitable acid to hydrolyze glycosides. The liberated aglycone is then detected using specific reagents or by observing characteristic color changes.

Estimation of steroids: Zak"s method Sample Preparation: The sample containing steroids is dissolved or extracted using a suitable solvent. A portion of the sample solution is mixed with nitric acid (HNO3) and then sulfuric acid (H2SO4). The mixture undergoes a reaction that produces a colored complex, typically pink to red in color. The intensity of the color is measured spectrophotometrically at a specific wavelength, often around 540 nm

Estimation of protein: Proteins in alkaline solution react with copper ions (Cu^{2+}) in the presence of sodium potassium tartrate (Biuret reagent) to form a colored complex.The sample is mixed with Biuret reagent and incubated. The development of a purple color indicates the presence of proteins.

Estimation of carbohydrate: Benedict's reagent (copper sulfate in alkaline solution) reacts with reducing sugars (monosaccharides and some disaccharides) to form a colored precipitate (red, orange, or yellow). The sample is mixed with Benedict's reagent and heated in a water bath. The color change indicates the presence and concentration of reducing sugars (Shaikh Jr et al., 2020).

Results and Discussion

Plants are the best-known sources of secondary metabolites which are classified by their chemical structure and/or of physical properties in one or more of the following groups: alkaloids, terpenoids, steroids, saponins, resins and peptides (Drager, 2002; Breemen et al., 2001). Secondary metabolites are chemicals produced by means of secondary reactions resulting from primary metabolism carbohydrates, amino acids and lipids (Ting, 1982). Plants have an almost limitless ability to synthesise aromatic substances mainly secondary metabolites (Mallikharjuna et al., 2007). These secondary metabolites are known to exhibit diverse biochemical and pharmacological effects. The preliminary phytochemical studies of Polyherbal formulation and herbomineral formulation with petroleum ether, chloroform, methanol, ethanol and aqueous polyherbal extract were performed to detect the presence of following phytochemical namely alkaloids, flavonoids, phenols, tannins, saponins, steroids, glycosides, carbohydrates and proteins are given in table 1.

Table 1: Phytochemical screening of Polyherbal formulation and herbomineral formulation

Phytochemical Constituents	**Different Solvents**				
	Petroleumether	**Chloroform**	**Methanol**	**Ethanol**	**Aqueous**
Alkaloids	-	-	+	+	-
Flavonoids	-	-	-	+	+
Phenols	-	-	+	+	+
Tannins	+	+	-	+	-
Saponins	-	-	-	+	+
Glycosides	-	-	+	-	-
Steroids	+	+	+	+	+
Proteins	+	+	+	+	+
carbohydrates	+	+	+	+	+

+: presence; -: Absence;

from the above results, it was inferred that more secondary metabolites were present in ethanolic and aqueous extract of Polyherbal formulation and herbomineral formulation; therefore it is utilized for further experimental studies.

The qualitative data presented in the table1 of Polyherbal formulation and herbomineral formulation reveals the presence of wide range of secondary components alkaloids, flavonoids, phenols, tannins, saponins, steroids, carbohydrates and proteins. The petroleum ether and chloroform extracts showed the presence of tannins, steroids, proteins and carbohydrates. The methanolic extract of Polyherbal formulation and herbomineral formulation exhibited the presents of alkaloids, phenols, glycosides, steroids, carbohydrates and proteins, while the ethanolic extract showed the presence of alkaloids, flavonoids, phenols, tannins, saponins, steroids, carbohydrates and proteins. Flavonoids, phenols, saponins, steroids, carbohydrates and proteins where present in the aqueous extract of Polyherbal formulation. From the above table 1 results it was inferred that broad range of secondary metabolites were present in the ethanolic extract of Polyherbal formulation and herbomineral formulation. Hence, it is utilized for experimental analysis.

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