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Research Article

INVESTIGATION OF PHYTOCHEMICAL, MINERAL CONTENT, AND PHYSIOCHEMICAL PROPERTY OF A POLYHERBAL EXTRACT

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ABSTRACT

Objective: The objective of the present study was to investigate the phytochemical, mineral content, and physiochemical properties of a polyherbal extract (PE).

Methods: Fresh plants *Punica granatum* (rind), *Catharanthus roseus*, *Gymnema sylvestre*, *Cissus quadrangularis*, *Garcinia cambogia*, *Tinospora cordifolia*, *Terminalia Arjuna*, *Urginea indica*, *Ficus racemosa* were selected for the PE. The plants were collected from various areas in and around Coimbatore district. The plants were washed, air dried, and coarsely powdered. 10 g of each plant powder has undergone various extract analysis for its phytochemical screening. The coarse extract called PE is been tested for physiochemical properties and its mineral content.

Results: The presence of secondary metabolites such as flavonoids, glycosides, phenolic compounds, and tannins in all the extract but highest in the hydroethanolic extract. The physiochemical properties showed the appropriate pH and solubility of PE.

Conclusion: Our findings provide that PE contain medicinally important secondary metabolites for the treatment of various diseases like cancer, cardiovascular diseases, and diabetes mellitus in the traditional folk medicine.

Keywords: *Punica granatum* (rind), *Catharanthus roseus*, *Gymnema sylvestre*, *Cissus quadrangularis, Garcinia cambogia, Tinospora cordifolia, Terminalia Arjuna, Urginea indica, Ficus racemosa*, Cardiovascular diseases, Polyherbal extract.

INTRODUCTION

Plants are a rich source of a diverse type of medicines in different countries and produce a diverse array of bioactive molecules, the source of potential and powerful drugs [1]. Thus, natural products with pharmacological or biological activities still play a very important role in medicine [2]. Plant extract has a potential application as natural medicine and to treat diseases of the human health [3]. Herbal drugs are prescribed widely even when their biological active compounds are unknown, because of their effectiveness, lesser side effects, and relatively low cost [4]. Plants are known to be the source of many chemical compounds and also used medicinally in different countries and are a source of many potent and powerful drugs [5].

Medicinal plants have a global distribution although they are most abundant in the tropics [6]. Medicinal plants are a rich source of antioxidant property [7]. Medicinal plants are the plants whose parts as extracts, infusions, and powders are used in the treatment of different diseases of humans, plants, and animals [8]. Medicinal plants are sources of important therapeutic aids for alleviating human ailments [9]. Many medicinal plants traditionally used for thousands of years are present in a group of herbal preparation of the Indian traditional health care system [10]. Medicinal plants are the backbone of traditional means, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis [11].

Phytochemicals are natural and non-nutritive plant bioactive chemical compounds that have protective or disease preventive properties against external stress and pathogenic attack [7]. Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine [12]. The plant-derived phytochemicals with therapeutic properties could be used as a single therapeutic agent or as combined formulations in drug development [13,14]. The choice of technique depends largely on the solubility properties and volatilities of the compounds to be separated [10]. The phytochemical investigation of a plant may involve extraction of plant materials, phytochemical screening, separation and isolation of the constituents, characterization of the isolated compounds [7].

The natural herbal products either as pure compounds or as standardized plant extracts provided unlimited opportunities for new drug leads because of the uncompared availability of diversities of chemical [12]. Therefore, researchers are increasingly turning their keen attention toward folk medicine from plants which leads into developing better natural drugs against diseases. Nowadays the usage of herbal drug is gaining greater acceptance from the medical and public profession due to their positive contribution and influence on health and life [15].

Pharmaceutical and scientific communities have recently received the attention of the medicinal plants and various publications have documented the therapeutic worth of natural compounds to validate the claims of their biological activity [16]. World Health Organization (WHO) described the plant as a plant with one or more organs which contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1]. Biodiversity studies revealed that the plant kingdom has not been exhausted based on the species of medicinal plants which are yet to be discovered.

Thus, plants have been used in treating human diseases for thousands of years. The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines [17]. Hence, this medicinal plant was chosen for our present study with main objectives to screen the phytochemicals constituents for further analysis.

METHODS

Preparation of polyherbal formulation and solvent extraction

Each 1 g of a poly herbal formulation contains equal amount of *Punica granatum* (rind), *Catharanthus roseus*, *Gymnema sylvestre*, *Cissus*

quadrangularis, *Garcinia cambogia*, *tinospora cordifolia*, *Terminalia Arjuna*, *Urginea indica*, *Ficus racemosa*. The plants were authentified in Botanical Survey of India, Coimbatore. 10 g of the dried powder of each plant was taken and cold macerated with pet ether, chloroform and (1:1) ratio of hydroethanolic solvent with occasional stirring for 3 days.

After 3 days, the suspensions was filtered through a fine muslin cloth and the filtrate was evaporated to dryness at low temperature (<40°C) under reduced pressure in a rotary evaporator. The yield of crude extract of each solvent extraction was found to be petroleum ether - 2.35%, chloroform extract - 3.57% and hydroethanolic extract - 9.64%, which were stored in an air-tight desiccator's and used for further analysis.

Organoleptic properties [18]

Organoleptic evaluation refers to the evaluation of formulation by color, odor, taste, texture, etc. The organoleptic characters of the sample were carried out based on the method described by Siddique *et al*.

Physiochemical properties [19]

Determination of total ash

Total ash determination constitutes detecting the physiological ash (ash derived from plant (tissue) and non-physiological ash (ash from extra generous matter, especially sand, and soil adhering to the surface of the drug). For its detection, 2 g of powdered material of the formulation was placed in a suitable tared crucible of silica previously ignited and weighed. The powdered drug was spread into an even layer and weighed accurately. The material was incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccator, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible and that of crucible with total ash.

Acid insoluble ash

The ash obtained as above was boiled for 5 minutes with 25 ml of dilute hydrochloric acid; the insoluble matter was washed with hot water and the percentage of acid-insoluble ash was calculated.

Water soluble ash

The ash was boiled for 5 minutes with 25 ml of water; collected insoluble matter in an ashless filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash with reference to the air-dried drug was calculated.

Alcohol soluble extractive value

A volume of 5 g of coarsely powdered air-dried drug was macerated with 100 ml of alcohol in a closed flask for 24 hrs, shaking frequently during 6 hrs and allowed to stand for 18 hrs. It was then filtered rapidly taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish at 105°C to constant weight and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried drug and is represented as a % value.

Water soluble extractive value

A volume of 5 g of coarsely powdered air-dried drug was macerated with 100 ml of chloroform water in a closed flask for 24 hrs, shaking frequently during 6 hrs, and allowed to stand for 18 hrs. Then the contents were filtered and the filtrate was evaporated to dryness. The water soluble extractive percentage was calculated.

Loss on drying

Loss on drying is the loss of mass expressed as percent w/w. About 10 g of drug sample of the formulation was accurately weighed in a dried and tared in flat weighing bottle and dried at 105°C for 5 hrs. Percentage was calculated with reference to initial weight.

Determination of pH

The pH of the formulation in 1% w/v and 10% w/v of water soluble portions was determined using standard glass electrode at 240 according to the prescribed standard method in Indian Pharmacopoeia.

Fluorescence analysis [20]

A volume of 1 mg of powdered drug of formulation were exposed to ultraviolet light at a wavelength of 366 nm and in daylight while wet after being treated with different reagents.

Minerals estimation

For analysis of K, Na, and Ca the Crude was taken in precleaned and constantly weighed silica crucible and heated in a muffle furnace at 400°C till there was no evolution of smoke. The crucible was cooled in desiccator at room temperature. The ash totally free from carbon moistened with Conc. H_2SO_4 and heated on hot plate till fumes of sulfuric acid get evolved the silica crucible with sulfated ash was again heated at 600°C in muffle furnace till the weight of sample conc. HCl to obtain solution for determination of K, Na, Ca, and Cr through flame photometry (FPM), standard solution of each mineral was prepared and calibration curve drawn for each element using FPM [21]. For determination of iron 1 g of sample in 125 ml deionized water was taken in conical flask pH adjusted to 2-3 by using Congo red paper. 5 drops of the variamine blue indicator was added then content was warmed at 400°C on a hot plate and titrated with standard 0.05 M ethylenediaminetetraacetic acid (EDTA) the initial blue color changes to gray just before the end point and final drop of reagent changes to yellow. Concentration of Iron was calculated by using the formula 1 mol.

EDTA≡1 mol Iron.

For determination, magnesium 0.1 g of sample was taken in 25 ml of 5 M hydrochloric acid in 100 ml graduated flask and the volume was made up to mark adding distilled water, standard solution is prepared by using 1 g magnesium metal in 50 ml of 5 M HCl, and solution made to 100 ml with distilled water then few drop of solochrome black T indicator was added and adjusted to pH to 10.1 the color developed is read at 520 nm and concentration of Mg was calculated spectrophotometrically by using standard graph [22]. For determination of phosphorous, 2 g sample of each plant material taken in 100 ml conical flask, two spoons of Darco-G-60 is added followed by 50 ml of 0.5 M NaHCo3 solution, next flask was corked, and allowed for shaking for 30 minutes on shaker. the content was filtered and filtrate was collected in flask from which 5 ml filtrate was taken in 25 ml volumetric flask to this 2 drops of 2, 4-paranitrophenol and 5 N H2SO4 drop by drop was added with intermittent shaking till yellow color disappear, content was diluted about 20 ml with distilled water and then 4 ml ascorbic acid was added then the mixture was shaken well and the intensity of blue color at 660 nm on colorimeter was measured. The absorbances were compared and concentrations of phosphorous using standard value were calculated [23].

Phytochemical investigation [24]

For the preliminary phytochemical analysis ethanol, water, acetone, chloroform, and hydroethanolic extract were investigated by the presence or absence of different phytoconstituents such as alkaloids, phenols, terpenoids, steroids, sugar, tannin, glycosides, and flavonoids, etc. were detected by usual prescribed methods.

RESULTS

Organoleptic properties

The result obtained for the formulation was a coarsely powdered material with fragrant odor, dark brown color.

Physiochemical properties

The physiochemical properties of the PE were tabulated in the Table 1.

Solubility properties
The solubility properties of the formulation were tabulated in the Table 2.

The fluorescent analysis
The fluorescent analysis was examined under the ultraviolet and daylight were tabulated in the Table 3.

Determination of minerals
The minerals of the formulation were tabulated in the Table 4.

Phytochemical investigation
The preliminary phytochemical investigation shows the presence or absence of various.

Primary and secondary metabolites in different solvent extraction which are helpful in predicting their therapeutic properties which tabulated in Table 5, it was found that most of the extracts were shown less conformity to active constituents like flavonoids, triterpenoids, etc. which are highly required for availing antioxidant property. However, hydroethanolic and ethanolic extract was found to show positivity to maximum number of phytochemical constituents. Based on ICH guidelines, the bulk extraction was carried out with 50% hydroethanol. Around 2 kg of grounded coarse powder of poly herbs were subjected for bulk extraction in 1:2 ratio and the yield was found is 300 g.

DISCUSSION

In recent years, there has been a great demand for plant derived products in developing countries. These products are increasingly being sought out as medicinal products, nutraceuticals, and cosmetics [14]. Due to lack of infrastructures, skilled manpower reliable methods, and stringent regulatory laws most of these manufacturers produce their product on very tentative basis [18]. The organoleptic evaluation provides the simplest and quickest means to establish the identity and thereby ensure quality of a particular sample and these features are useful in judging the material in its entirety and in powder form [19]. In this study, it was revealed that the crude drug used for the preparation of formulation lie within the significant limit with good quality.

Table 1: Physiochemical properties of the formulation

Parameters %	Yield (w/w)
Total ash	10.6
Moisture content	16.11
Crude fat	1.9
Crude protein	2.3
Crude fiber	13.6
Crude carbohydrate	43.12
pH	6.03

Table 2: Physical properties of the formulation

Parameters	Solubility
Acetone	Insoluble
Chloroform	Insoluble
Ethanol	Soluble
Water	soluble

Table 3: Fluorescent analysis of the formulation

Reagents	Daylight	UV light
Powder+Conc. H_2SO_4	Reddish brown	Reddish brown
Powder+aluminum ferric chloride	Blackish green	Blackish green
Powder+picric acid	Yellowish blue	Yellowish blue
Powder+aqueous mercuric chloride	Light brown	Dark brown

UV: Ultraviolet

Table 4: Minerals of the formulation

Parameters	Yield (mg/g)
Calcium	2.0
Magnesium	1.5
Iron	4.6
Phosphorus	3.4
Sodium	1.84
Potassium	1.15

The amount of minerals and earthy materials in the plant material was indicated by total ash content. The water-soluble extractive value indicated the presence of sugar, acids, and inorganic compounds. Less or more extractive value indicates the addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating [20,21]. Loss on drying at 105°C was determined since the presence of excess moisture is conclusive to the promotion of mold and bacterial growth, and subsequently to deterioration and spoilage of the drug [4]. If the rate is too slow, the drying rate also changes, here the rate of formulation dried was proper, so the drying limit was also proper. The pH of 1% w/v and 10% w/v solution revealed that the formulation was suitable for human use. The fluorescent analysis of powder indicates the nature of phytoconstituents present or adulterant. In this study, there was no such fluorescent material found in the formulation.

Minerals are the inorganic substances present in all body tissues and fluids and their presence is necessary for the maintenance of bone, blood coagulation, acid base, equilibrium, enzyme activity, osmotic regulation, etc. [25]. The preliminary phytochemical analysis indicates the nature of phytoconstituents. Hydro-ethanolic extract showed the maximum presence of phytoconstituents than the acetone and chloroform extract. Phenolics are one of the major largest and most ubiquitous groups of plant metabolites that can be found ubiquitously in certain plants [26], which are considered as bioactive and non-nutritional compounds, due to their antioxidant properties, against free radicals effects that exhibit various significant biological activities [27]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [21].

Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against a wide array of microorganism *in-vitro* [21]. They are also an effective antioxidant and exhibit stronger anticancer, cardiovascular activities. Flavonoids are capable of treating certain physiological disorder and diseases [22]. Saponins have been implicated as bioactive antibacterial agents of plants, which are a glycoside, have the property of precipitating and coagulating red blood cells, which are occurring widely in plants [21].

Plant steroids are known to be important for their cardiotonic activities, possess insecticidal, and antimicrobial properties. They are also used in nutritional preparation, herbal medicine, and cosmetics. The efficiency of plant derived antimicrobials is needed to be determined completely [26]. Steroids have been reported to have antibacterial properties and they are very important compounds especially due to their relationship with compounds like sex hormones [4]. Steroids and saponins were responsible for central nervous system activities [23].

CONCLUSION

The present investigation suggest that the polyherbal formulation of medicinal plants have the appropriate pH was evaluated by various standardization parameters such as physicochemical standards, organoleptic parameters, and safety evaluation showed that the contents of formulation presents within the permissible limits as per WHO. The therapeutic potential of the formulation may be due to the presence of various phytochemicals present in the hydro-ethanolic extract. Further studies are too carried out with different analytical and biochemical and also by *in-vivo* methods.

Table 5: Phytochemical investigation of the formulation

Qualitative analysis	Ethanol extract	Water extract	Chloroform extract	Hydroethanolic extract	Acetone extract
Alkaloids					
Dragendroff's test	+++	+	-	+++	-
Wagner's test	+++++	++	+	+++	-
Mayers test	+++	+	-	++	-
Hager's test	+	+++	-	++	-
Flavonoids					
Alkaline reagent	+++	+++	-	++	-
shinodas test	+++	+++	-	+++	-
Saponins	-	-	-	-	-
Starch					
T1	+	+	+	++	-
Soluble starch	+	+	-	++	-
Carbohydrates					
Fehlings test	+	+	-	++	-
Benedicts test	++	+++	-	+	-
Molischs test	+	-	-	++	-
Alurone grains	-	-	-	+	-
Proteins					
Millons test	+++	+	-	+++	-
Biurets test	++	+	-	-	-
Ninhydrin	+	++	-	-	-
Phenols					
Ferric chloride test	+++	+++	-	+++	-
Lead acetate test	++	+++	-	+++	-
Libbermanns test	+++	++	-	+++	-
Gelatin test	+	+	+	++	-
Steroids					
Libbermanns burchards test	++	+	-	+++	-
Salkowsi test	+	+	-	+++	-
Tannins					
Ferric chloride test	+++	++	-	++	-
Lead acetate test	++	+++	-	+	+
Phlobatannins	++	++	-	-	-
Glycosides					
Killani synthesis	+++	+++	-	+++	-
Bonntragers test	+++	+++	+	+++	+
Coumarian test	+++	++	+	+++	+
Terpenoids	++	+++	-	-	-
Lignins					
T1	-	++	-	-	-
T2	-	+	+	-	-
Acedic Compounds	+	-	-	+	-
Napthoquinones	-	-	-	-	-
Alurone grains	-	-	-	-	-
Cellulose	-	-	-	-	-
Thiols	-	+	-	-	-
Terpenoids	+	-	-	-	-
Lignin	+	+	+	-	-
Gum+mucilage	-	-	-	-	-
Protein+amino acid					
Millons test	-	-	-	+++	+
Biurets test	-	-	+	-	+
Ninhydrin test	+	-	-	+	+
Inulin	-	-	-	-	+
Suberin	-	-	-	-	-
Thiols	-	-	+	-	

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