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PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF POLYHERBAL FORMULATION

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ABSTRACT

In Ayurveda, single or multiple herbs (polyherbal) are used for the treatment. The Ayurvedic literature Sarangdhar Samhita' highlighted the concept of polyherbalism to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity. In this study a combination of Tinospora cordifolia, Terminalia chebula and Zingiber officinale are used for evaluation. This combination have a number of pharmacological activity like immunomodulatory digestive, antioxidative, hepatoprotective activities etc.So it is necessary to find out the pharmacognostic and phytochemical evaluation of the formulation. For the first time the above mentioned herbal compound in the powder form was subjected to pharmacognostical evaluation, phytochemical screening and physico-chemical analysis. The results of the phytochemical analysis indicated the presence of alkaloids, tannins, phenolic compounds, carbohydrates, glycosides and saponin glycosides which is necessary for the pharmacological activity. Physico chemical evaluation shows it has low moisture content, high water soluble extractive value and low ash content indicating better stability, active constituents are more soluble in water. Microscopical evaluation of the powder showed the presence of tannin content from haritaki collapsed epicarp cells of haritaki, pitted stone cells of haritaki, annular scalariform vessels and simple starch of ginger,

border pitted vessels and simple and compound starch grains of guduchi, disturbed collenchyma cells of guduchi, disturbed cork cells of guduchi.

KEYWORDS: Polyherbal Tinospora cordifolia, Terminalia chebula, Zingiber officinale, phytochemical, pharmacognostic evaluation.

INTRODUCTION

When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity.In this study a combination of Tinospora cordifolia, Terminalia chebula and Zingiber officinale are used for evaluation. This combination have a number of pharmacological activity like immunomodulatory digestive, antioxidative ,hepatoprotective activities etc.So it is necessary to find out the pharmacognostic and phytochemical evaluation of the formulation.

METHODOLOGY

1. Organoleptic properties

Colour: Light grey

Odour: Pungent

Taste: Bitter

Texture: Soft

Touch: Smooth

2. Phytochemical screening[1,2]

Phytochemical screening for the identification of various phytoconstituents such as alkaloids, carbohydrates, steroids, cardiac glycosides, flavonoids, carbohydrates, amino acids, phenolics, naphthoquinones and tannins according to standard methods were performed.

Test for carbohydrate

- Molisch test: A small quantity of the extracts was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's reagent and formation of brick red colour confirmed the presenceof reducing sugar.
- Fehling's test: Equal volume of Fehling A (coppersulphate in distilled water) and Fehling B (potassium tartrate and sodium hydroxide in distilled water) reagents were mixed with few drops of crude extract is added and boiled, a brick red precipitate of cuprous oxide forms, if reducing sugar are present.

Test for glycosides

- Borntrager's test: 200 mg crude extract was mixed with 2 ml of dilute sulphuric acid and 2 ml of 5 % aqueous ferric chloride solution, boiled for 5 minutes which lead to oxidation to anthraquinones, indicating the presence of glycosides.

Test for Alkaloids

- Mayer's test: Crude extract was mixed with Mayer's reagent (potassium mercuric iodide solution). Cream colour precipitate was formed, indicating the presence of alkaloids.
- Dragendroff's test: Crude extract was mixed with Dragendroff's reagent (potassium bismuth iodide solution). Reddish brown precipitate was formed which suggested the presence of alkaloids.
- Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Test for Flavanoids

- Alkaline reagent test: Crude extract was mixed with few drops of sodium hydroxide solution. An intense yellow colour was formed. Yellow colour turned to colorless on addition of few drops of diluted acid, marked the presence of flavanoids.
- Lead acetate test: To a solution of 0.5 g extract in water, about 1ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.

Test for Proteins

- Millions test: 3ml extracts were dissolved in 10ml of millions reagent. White precipitate is formed which on warming the white precipitate dissolves giving red coloured solution.
- Biurette test : 3ml of the extract was mixed with 4% sodium hydroxide and few drops of 1% copper sulphate solution were added, violet or pink colour not appeared. To 3ml of extract few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple colour. On addition of alkali, it becomes dark violet.

Test for Tannins

- Ferric chloride test: Crude extract was mixed with ferric chloride. Blue green colour appeared, suggested the presence of tannins.

3. Physico-chemical studies[2]

Determination of ash value

Determination of total ash

2-4g of the sample was weighed in a crucible and was spread evenly and ignited slightly increasing the temperature to 500-600 degree Celsius until it turns white, it was cooled in a dessicator and weighed.

Determination of acid insoluble ash

25 ml of dilute hydrochloric acid was added into the total ash, covered with a watch glass and ignited for 5 minutes. It was filtered, washed with hot water until neutral and filter paper was dried and the ash was transferred back to the crucible, dried on a hot plate and was weighed.

Determination of water soluble ash

25 ml of water was added into the total ash, covered with a watch glass and ignited for 5 minutes. It was filtered, washed with hot water until neutral and filter paper was dried and the ash was transferred back to the crucible, dried on a hot plate and was weighed.

$$\text{Percentage ash value} = \frac{\text{Initial weight taken}}{\text{Weight of ash}} \times 100$$

Determination of extractive value

Water soluble extractive value

5 grams of the coarse drug was macerated with 100ml of water for 24 hours with occasional shaking for the first 6 hours and then left aside for 18 hours. It is filtered taking precautions to avoid loss of solvent. it is evaporated in a flat container at 105 degrees until constant weight. The percentage of water soluble extractive value was calculated with reference to the air dried drug.

Alcohol soluble extractive value

5 grams of the coarse drug was macerated with 100ml of alcohol for 24 hours with occasional shaking for the first 6 hours and then left aside for 18 hours. It is filtered taking precautions to avoid loss of solvent. It is evaporated in a flat container at 105 degrees until constant weight .the percentage of water soluble extractive value was calculated with reference to the air dried drug.

5. Powder analysis[3]

Sieve size/powder fineness: 75 g of the sample was accurately weighed and passed through various sieves numbered 16,22 ,44,100 and shaken for 20 minutes successively and the powder remaining on each sieve was weighed and average particle size was determined .

6. Thin layer chromatography[2]

100 gram of silica gel G was dissolved in sufficient amount of water and was coated on the glass plate. Solvent system chosen was toluene : ethyl acetate :acetic acid (7:2:1). Ethanolic extract was dissolved in sufficient ethanol to make up a concentration of 1mg/ml .The spots were made 1 cm from the bottom of the glass slide. The glass plate was kept in to the chamber after chamber saturation and allowed to run 2-3rd of the glassplate. $R_f$value was calculated.

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute.}}{\text{Distance travelled by the solvent front}}$$

RESULTS

- **Preliminary phytochemical screening**

Table 1

Constituents	Tests	Presence/absence
Carbohydrates	Molischs Fehlings	+ +
Alkaloids	Dragendorffs test Wagners test Mayers test	+ + +
Phenols	Ferric chloride test	+
Proteins	Millions test Biurette test	- -
Tannins	Ferric chloride test	+
Flavanoids	Alkaline test	+

- **PARTICLE SIZE**

Table 2

Sieve size	Pore size	Weight of powder(n)	n/gx100	Cumulative frequency	Mean pore diameter (d)	nd	
16	1000	13	26	26	855	11115	**Total =19473.24**
22	710	5.7	11.4	37.4	532.5	3032.4	
44	355	18	36	73.4	252.5	4545	
100	150	10.41	20.82	94.22	75	780.75	
Fine powder		2.05	4.1	98.32			
		Total=49.16					

Average particle size=$\frac{\sum nd}{\sum n}$ =389.46

RESULT OF THIN LAYER CHROMATOGRAPHY

	Ethanol extract
Solvent system	toluene : ethyl acetate :acetic acid(7:2:1)
Distance traveled by the spot1 Distance travelled by spot 2	5.5 cm 3.1cm
Distance traveled by the solvent front	5.5 cm
R_f value of spot 1 Rf value of spot 2	0.56 0.27

Photography of ethanolic extract of the polyherbal formulation

DISCUSSION

- The aqueous extract showed positive tests for carbohydrates, tannins, phenols, alkaloids, glycosides indicating their presence.
- The water soluble extractive value was found to be 39.68 %
- The alcohol soluble extractive value was found to be 10.75%
- Average particle size= 239.3423g
- Water soluble ash value= 7.87
- Acid insoluble ash value= 8.97
- Rf value of spot 1 =0.56
- Rf value of spot 2 = 0.27

CONCLUSIONS

Preliminary phytochemical screening showed the presence of active constituents necessary for the pharmacological activity. Pharmacognostic study revealed the Ash values, extractive value and Particle size and helped to determine the average particle size, level of contamination and adulterants. Less extractive value indicates presence of exhausted materials, adulteration. High ash is indicative of contamination, substitution or carelessness in preparing the formulation. Rf value of spot 1 & 2 stand within the range of standard Rf values of flavonoids and phenolic compounds.

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