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PHYTOCHEMICAL EVALUATION OF POLYHERBAL FORMULATION

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ABSTRACT

Background: Evaluation of herbal formulation is essential in order to assess the quality of the drugs, based on the concentration of their active principles. This article reports on the Phyto-chemical analysis of polyherbal formulation, an Ayurvedic Polyherbal formulation used in treating painful disorders. **Objective:** The experiment was carried out with the aim of evaluating various chemical constituents of polyherbal formulation. **Methods:** polyherbal formulation, *vedanasthapana gana vati* was prepared and same used for the analysis. Evaluation of the poly herbal formulation is possible by following modern scientific quality control procedures for the finished product. The prepared "polyherbal formulation" is evaluated phyto-chemically by subjecting it to various tests like qualitative analysis and TLC. The present formulation has been used for clinical study, showed efficacy in relieving pain. **Results:** The Qualitative tests on polyherbal formulation revealed the presence of sugars, proteins, alkaloids, flavanoids and tannins. The richness of various chemical constituents in the formulation is confirmed by the TLC study. **Conclusion:** The present phytochemical evaluation of polyherbal formulation showed the presence of various chemical constituents.

Key words: Polyhedral formulation, *Vedanasthapana, ganavati*

INTRODUCTION

The increasing interested in the use of plant based formulations is leading to a fast growing market for Ayurvedic, neutracetical and poly herbal formulations. The development of stable poly herbal formulations is a challenging task because of the large number of varied chemical compound present in the different medicinal plants. Hence, the entire herbal drug or herbal drug preparation is regarded as active drug substance, regardless of whether constituents with defined therapeutic activity are known. This difficulty has been acknowledged in the draft of the Strategic Plan for Regional Traditional Medicine of the World Health Organization.

The chemical incompatibility leads to changes in the chemical nature, solubility, absorption and therapeutic response of these drugs. Therefore, during the formulation of new drugs or the reformulation of existing products, the interaction study between active markers of various plant extracts and commonly used excipients should be carried out thoroughly. However, no universally accepted protocol is available for evaluating

the compatibility of drugs with different excipients. Assessment of possible compatibility between active component and different excipients along with the evaluation of stability are crucial part of a normal study prior to the final formulation.

In compatibility studies, temperature variation is one of the most important parameter to induce rapid chemical and physical alternation in formulations, which is determined by quantification of the active constituents over the time. Unlike a single chemical entity that forms the basis of conventional medicine, traditional Ayurvedic medicine views the polyherbal preparations as they induce combined therapeutic activity this creates a challenge in the development of a stable polyherbal formulation[1].

OBJECTIVE: To evaluate phytochemicals of poly herbal formulation

MATERIALS AND METHODS

Poly herbal formulation: Polyherbal formulation of *Vedanasthapana gana* contains following dravyas, namely, *Shala, Katphala, Kadamba, Padmaka*, and *Mocha rasa, Tumba, Shirisha, Ashoka, Vanjula* and *Elavaluka* mentioned in charaka samhita fourth chapter of sutra sthana[2].

Table 1: Showing contents of formulations

No	Drug (Sanskrit name)	Botanical name	Family
1	*Shala*	*Shorea robusta*	Dipterocarpeaceaa
2	*Katphala*	*Myrica nagi*	Myricaceae
3	*Kadamba*	*Anthocephalus indicus*	Rubiaceae
4	*Padmaka*	*Prunus cerasoides*	Rosaceae
5	*Mocha rasa*	*Salmalia malabarica*	Bombacaceae
6	*Tumba*	*Zanthoxylum aramatum*	Rutaceae
7	*Shirisha*	*Albizzia lebback*	Leguminoseae
8	*Ashoka*	*Sarraca ashoka*	Leguminoseae
9	*Vanjula*	*Salix tetrasperma*	Salicaceae
10	*Ela valuka*	*Prunus avium*	Rosaceae

TABLE 2: Components of the Formulation

No	Drug (Sanskrit name)	Botanical name	Family	Chemical constituents
1	*Shala*	*Shorea robusta*	Dipterocarpeaceaa	Sterols, Methyl sterols
2	*Katphala*	*Myrica nagi*	Myricaceae	Myricitrin, Myrisetin,Tanin
3	*Kadamba*	*anthocephalus indicus*	Rubiaceae	cinchotannic acid, Beta sitosterol
4	*Padmaka*	*Prunus cerasoides*	Rosaceae	Taxifolin Amygdaline
5	*Tumba*	*Zanthoxylum aramatum*	Rutaceae	Berberine Dictamnine
6	*Mocha rasa*	*Salmalia malabarica*	Bombacaceae	Tannin Saponin

7	*Shirisha*	*Albizzia lebback*	Leguminoseae	Teflitinin, Sayanitin,Saponin
8	*Vetasa*	*Salix tetrasperma*	Salicaceae	Hydrocyanic acid, Volatile oil,Salicylic acid
9	*Ela valuka*	*Prunus avium*	Rosaceae	Prunasin (D-mandelonitrile-β-glucoside), Quercetin-3-0-rutinosyl-7,
10	*Ashoka*	*Sarraca ashoka*	Lleguminoseae	Tannic acid,Gallic acid, Tannin and Catechin

CHEMICALS: DPPH, Folin ciocalteu reagent, ferrous sulphate, ascorbic acid, gallic acid, ferric chloride, trichloroacetic acid, sodium carbonate, sodium nitroprusside, NED, sulphonillic acid, quercitin, dextrose, sodium citrate, citric acid, sodium chloride, thiobarbutaric acid, potassium ferricyanide, ammonium molybdate, sulphuric acid, potassium acetate, TBA, deoxy ribose, EDTA, PMS, NBT, NADH were of analytical grade and are obtained from SRL, Rankem and Merck.

PREPARATION OF THE EXTRACTS

Polyherbal formulation: The polyherbal formulation was extracted with different solvents viz water, dietylether, methanol, acetone, chloroform, and hexane. Approximately 1.5g of polyherbal formulation was dissolved in each solvent in different tubes and kept overnight at 4^0C, after that the solvents with extract were filtered and the filtrates are stored at 4^0C for further uses.

PHYTOCHEMICAL ANALYSIS ESTIMATION OF TOTAL POLYPHENOLIC CONTENT by singlet V L., *et.al.*, Method[3]

PRINCIPLE:

This is based on the principle that polyphenol reacts with folin ciocalteau's reagent gives blue colored chromogen in alkali medium, which can be measured at 760nm and the concentration of polyphenol in extract were calculated by using standard curve prepare with gallic acid as per the procedure in Heinsonen et al 1998.

REAGENTS REQUIERD

- Folin ciocalteau's (FC) reagent (1:1 v/v)
- Sodium corbonate 10%
- Gallic acid solution in water 1mg/ml

PROCEDURE: The total polyphenolic content was determined colorimetrically using folin ciocalteau's method for extracts. Aliquot (0-5µl) of gallic acid was taken in the tubes. Volume of all the tubes was made up to 1ml with distilled water. The extracted also diluted accordingly; 1ml of FC reagent and 2ml of 10% sodium corbonate was added to each of the tubes. After 30 mins absorbance was read at 760nm against a blank. Concentration of polyphenol in *polyherbal formulation* extracts was calculated using standard curve and expressed as % concentration.

DETERMINATION OF PHENOLICS AND SAPONINS BY TLC Rivas et., al., [4]

PRINCIPLE

The test sample is applied as a spot on the precoated silica G plate and then placed in a reservoir of mobile phase that allowed passing over the plate. By simple capillary action the mobile phase moved rapidly across the layer.

REAGENTS REQUIERD

- TLC plate, sprayer,
- capillary tubes,
- Solvent system and sample.

PROCEDURE: Phenolics were separated with acetic acid: chloroform (45:5) and the saponin was developed with chloroform: methanol: water (13:7:2) and detected with 10% H_2SO_4. The *Rf* values of the spots were calculated as the ratio of the distance travelled by the solute to that by the solvent front.

$$Rf = \frac{distance\ travelled\ by\ compound\ (sample)}{distance\ travelled\ by\ solvent}$$

ESTIMATION OF TOTAL PROTEINS

The protein content was determined by Lowry's method, taking BSA as the standard (0.075g) 50µl of *polyherbal formulation* extract were taken and the volume is made up to 1ml using distilled water, this is followed by addition of 5ml of Lowry's reagent. The reaction mixture was allowed to stand for 10minuts at room temperature, 0.5ml of 1:1 dilution of folin ciocalteau's reagent added and allowed to stand 30 minutes at room temperature. And absorbance was measured at 670 nm. The total protein content was calculated using standard BSA calibration curve.[5]

ESTIMATION OF TOTAL CARBOHYDRATES

The total carbohydrate content was determined by phenol-Sulphuric acid method, taking glucose as standard (100 mg).100µl of *polyherbal formulation* extract were taken and the volume is made up to 1ml using distilled water, this was followed by addition of 1ml of 5% phenol and 5ml of 96% H_2SO_4. The reaction mixture was allowed to stand for 20 minutes at 25-30^{0}C and the absorbance is read at 490 nm. The total carbohydrate content was calculated by using standard glucose calibration curve.[6]

ESTIMATION OF TOTAL FLAVONOID

The aluminum chloride colorimetric method was modified from the procedure reported by Woisky and Salatino. Quercetin (0-100µg) was used to make the caliberation curve. 0.1 ml of *polyherbal formulation* extract was taken and the volume is made up to 1ml using methanol, this was followed by additional of 1ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The reaction mixture was allowed to stand for 30 minutes at room temperature and then the absorption was measured at 415nm. The total flavonoid content was calculated using standard curve.[7]

QUALITATIVE TEST

HAGER'S TEST: Alkaloids give yellow colour precipitate with Hager's reagent (saturated solution of picric acid).

TERPENOIDS: 5ml of each *polyherbal formulation* extract was mixed in 2ml of chloroform; 3ml of concentrated H_2SO_4 was then added to form a layer. A reddish-brown precipitate colouration at the interface formed indicated the presence of terpenoids.

STEROIDS: 1ml of the poly herbal formulation extracts was dissolved in a few drops of acetic acid. It was gently warmed and cooled under the tap water and a drop of concentrated H_2SO_4 was added along the sides of the test tube. Appearance of green color indicates the presents of steroids.[8]

DISCUSSION

In living system free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules leading to various disease conditions, especially lysis. Many synthetic drugs protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidant from food supplements and traditional medicine. The antioxidant and poly phenol content of polyherbal formulation was determined by using different extracts. The carbohydrates and protein content of polyherbal formulation was determined by using different extracts. It contains 155.405mg/1g of carbohydrates in water extract of polyherbal formulation and 41.55mg/1g of proteins in water extracts of polyherbal formulation (Table.3). The polyherbal formulation also contains saponins and phenolics were determined by thin layer chromatography (Fig. 1 and Fig.2).The total phenolic and Flavonoids contents of polyherbal formulation was determined in different solvent extracts (Table 4). By qualitative test the Alkaloids, Terpenoids and Steroids are determined (Table 5).

Table 3: Total Proteins and carbohydrates present in polyherbal formulation

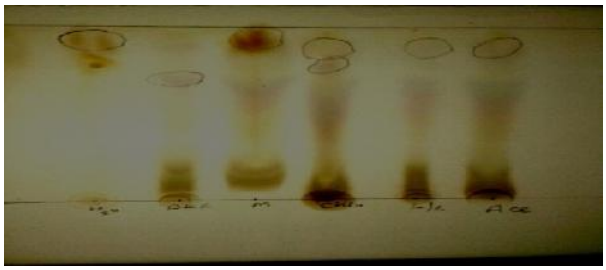

Fig 1: TLC separation of saponins

Fig2: TLC separation of phenolic

Polyherbal formulation	PROTEINS	CARBOHYDRATES
Water extracts	41.55mg	155.405mg

Polyherbal formulation extracts	TPC	FLAVONOIDS
Water extract	7.4324mg	0.8108mg
Hexane extract	0.4888mg	5.689mg
Acetone extract	4.3706mg	43.636mg
Chloroform extract	2.244mg	0.01066mg
Diethyl ether extract	0.00722mg	35.556mg
Methanol extract	4.554mg	31.1735mg

Table 4: Total Polyphenolic contents and Flavonoids of Polyherbal formulation

QUALITATIVE TEST

Polyherbal formulation extracts	ALKALOIDS	TERPENOIDS	STEROIDS
Water extract	Ab	Ab	Ab
Hexane extract	Ab	Pr	Ab
Acetone extract	Pr	Ab	Ab
Chloroform extract	Pr	Ab	Ab

Diethylether extract	Ab	Pr	Ab
Methanol extract	Pr	Pr	Ab

Table 5: Alkaloids, Terpenoids and Steroids in Polyherbal formulation (Ab- Absent, Pr- Present)

CONCLUSION

The results, indicates that Polyherbal formulation contains polyphenols, saponins, phenolics, proteins, carbohydrates, flavonoids, alkaloids, terpenoids and steroids.

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