Comparative Evaluation of Phenol And Flavonoid Content of Polyherbal Drugs

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Summary

Background: In Ayurvedic system of medicine mainly polyherbal compounds are used for treatment of various infections. As several reports are on various individual phytochemical, phenolic and flavonoid content but none is having comparative study of polyherbal drugs. **Objective**: Comparative qualitative phytochemical, phenolic, and flavonoid content, TLC analysis was studied of two polyherbal drug named Bharangyadi and Shrishadi. **Methods**: Hydroalcoholic Extraction (Distilled water: Ethanol = 2:1) was done by hot percolation method through soxhlet apparatus. Qualitative phytochemical study was performed using various standard methods. Phenolic content was estimated using Follin ciocalteau reagent, flavonoid using aluminium chloride (2%) reagent as as quercetin equivalent and TLC analysis using hexane, chloroform and methanol solvent. **Result**: The phytochemical analysis showed the presence of alkaloid, saponin, tanins, terpenoids, steroids, glycosides, phenols, flavonoids. Shrishadi contain higher phenolic (112±4.62 mg/g) and flavonoid contents (23.89±3.24 mg/g) compare to bharangyadi polyherbal drug. Ayurveda is traditional system of Indian medicine using mainly herbal products for curing diseases. TLC analysis shows six spots using chloroform and methanol solvent in both the polyherbal drug, while three and four spots using other solvent.

Keywords: Bharangyadi Polyherbal, Shirishadi Polyherbal, Phenol, Flavonoid, TLC, Folin-Ciocalteu, Quercetin.

Introduction

Plants have been associated with the human health from time immemorial and they are the important source of medicines since the down of human civilization. In spite of tremendous developments in the field of allopathic medicines during the 20th century, plants still remain one of the major sources of drugs in modern as well as in traditional systems of medicine. The medicinal plants are rich source of secondary metabolites like alkaloids, glycosides, steroids, and flavonoids, which are potential source of drugs. Approximately, one third of all pharmaceuticals are plant origin. In Ayurvedic system of medicine mainly polyherbal compounds are used for treatment of various infections. *Clerodendrum serratum, Hedychium spicatum, Inula racemosa*, *Albizzia lebbeck, Solanum xanthocarpum* and *Cyprus rotundus* are extensively used in Ayurvedic system of medicine in treating various ailments. Bharangyadi polyherbal is a mixture of *Clerodendrum serratum, Hedychium spicatum* and *Inula racemosa.* Shirishadi polyherbal constitute *Shirisha, Nagarmotha, & Kantakari* is one such preparation.

As all these Plants are able to synthesize a multitude of organic molecules/ phytochemicals, referred to as “secondary metabolites”[1,2] . These molecules play variety of role in the life span of plants, ranging from structural ones to protection. Phenolic compounds are regarded as one such group that is synthesized by plants during development[1,3] and in response to conditions such as infection, wounding ,UV radiation[4,5] etc. Approximately 8000 naturally occurring compounds belong to the category of “phenolics”. Phenols are associated with diverse functions, including nutrient uptake, protein synthesis, enzyme activity, photosynthesis; structural components and allelopathy[6-8] . Phenolics show an array of health promoting benefits in

human health. They are of current interest due to their important biological and pharmacological properties, especially the anti-inflammatory[9] , antioxidant[10] , antimutagenic and anticarcinogenic activities[11, 12] .They are widespread in plant based foods and human consume it. The estimated range of consumption is 25mg to 1g per day, depending on diet[13].

Flavonoids are universal within the plant kingdom. More than 5000 flavonoids have been identified in nature[14]. They functions as stress protectants in plant calls by scavenging reactive oxygen species produced by the photosynthetic electron transport system[15]. Due to UV-absorbing properties, flavonoids protect plants from the UV radiation of the sun and scavenge UV-generated reactive oxygen species[16]. Flavonoids are considered as important components in the human diet, although they are generally considered as non-nutrients. Flavonoid intake can range between 50 and 800 mg per day, depending on the diet consumption.
Albizzia lebbeck also known as tree of happiness is extensively used herb in various traditional medicines. Research studies had shown that it possesses anti-histaminic & mast cell stabilizing property[17] by virtue of which it is supposed to work as anti-asthmatic drug[18]. It also has anti-inflammatory[19], antioxidant properties[20], antiallergic activity[21] and analgesic activity[22]. *Solanum xanthocarpum* known as kantakari in Ayurveda is very effective in respiratory tract disorders. It is found to have strong bronchodilator effect along with anti-inflammatory property [23, 24], hepatoprotective activity[25], antidiabetic[26], antioxidant potential[26, 27], antihyperglycemic[27], larvicidal action[28]. *Cyprus rotundus* or Mustaka reported to possess anti-inflammatory, anti-pyretic and analgesic activities[29].

Bharangi (*Clerodendrum serratum*) is found to have anti-inflammatory[30,31], antihistaminic, antiallergic, antioxidant and hepatoprotective properties[32]. In Ayurvedic system of medicine, it is mainly used in respiratory tract diseases. Sati (*Hedicium spicatum*) is found to possess hypotensive, hypoglycaemic, anti-inflammatory, vasodilator, antispasmodic, tranquillizer, anti-bacterial, anti-fungal, CNS-depressant, hypothermic, spasmolytic & analgesic effects[33,34]. Pushkarmoola (*Inula racemosa*) has been found prove beneficial for cardiovascular system, angina and dyspnoea[35,36].

Therefore this study was planned to evaluate comparative phenolic, flavonoid content and TLC analysis of hydroalcholic extract of Shirishadi, Bharanyadi polyherbal drug with preliminary phytochemical characterization.

Material And Methods

Plant material and preparation of Extract

The plants of polyherbal drugs were collected from local market of Varanasi (India). The identification of the plants was done by Prof. A. K. Singh, Department of Dravyaguna, S.S.U.,Varanasi. Air shade dried and pulverized plants parts were extracted with Hydroalcoholic solvent (Distilled water: Ethanol = 2:1) separately by hot percolation method through soxhlet apparatus. Thereafter extract was dried using rotary evaporator and dried extract was put to the process of standardization. This extract was used to investigate the total content of phenols and flavonoids.

Preliminary Phytochemical Screening

Chemical test were carried out on the aqueous and hydroalcoholic extract and on the powdered specimen using standard procedures to identify the constituents [37, 38]. The plant extract was assayed for the presence of alkaloids, proteins, free amino acids, anthraquinone, glycosides, flavonoids, tannins, phenolic compounds, carbohydrates, saponins, phytosterol and triterpenes.

Estimation of total phenolic content

Total Phenolic concentration in different fractions of alcoholic extract was measured by Folin ciocalteau assay[39, 40]. Briefly, 5ml of distilled water, 0.5-1.0ml of sample, and 1.0ml of Folin ciocalteau reagent was added to a 25ml flask. The content was mixed and allowed to stand for 5-8min at room temperature. Next 10ml of 7% sodium carbonate solution was added followed by distilled water. Solution were mixed and allowed to stand at room temperature for 15min, and then absorbance was recorded at 750 nm. TP content was standardized against gallic acid and expressed as milligram per liter of gallic acid equivalents (GAE). The linearity range for this assay was determined as 0.5-5.0mg/l GAE (R^2=0.999), giving an absorbance range of 0.050-0.555 absorbance units. Experiments were performed in triplicates and results were recorded as mean ± SEM (Standard Error Mean).

Estimation of total flavonoid content

Total flavonoid content was measured by using aluminium chloride (2%)[41, 42] in which it is mixed with solution of test samples. Absorbance reading at 415nm (Elico SL 177) were taken after 10 min against a blank sample consisting of 5ml of sample solution and 5ml of methanol without aluminium chloride. The total Flavonoid content was determined using a standard curve of quercetin at 0-50µg/ml[43]. The average of three readings were used and then expressed in µg quercetin equivalent flavones per mg extract. Experiments were performed in triplicates and results were recorded as mean ± SEM (Standard Error Mean).

Thin layer chromatography-(TLC): Thin layer chromatography (TLC) was used to separate the shrishadi and Bharangyadi extract into different spots on the chromatplate. The chromatograms developed on the microscope slide, were dried and observed visually for the various different part of polyherbal extract components. The developing solvent used in different extract are hexane: ethyl acetate(9:1) and chloroform:methanol(9.5:5).

The retention factor was calculated using:

$$Rf = \frac{\text{Distance move by the substance(cm)}}{\text{Distance move by the solvent(cm)}}$$

Results And Discussion

Bharangyadi polyherbal extractive value was found to be higher than shrishadi polyherbal drug. Plant extracts with high phenolic content also enclosed high flavonoid content. Estimation of total phenolic and total flavonoid content showed that shrishadi extract was having maximum phenolic content (112±4.62 mg/g) in µg Gallic acid equivalents (GAE) followed by Bharangyadi

extract (50.98±1.81 mg/g) with a very less flavonoid content in Bharangyadi extract (13.66 ±0.54 mg/g) and Shrishadi (23.89±4.62 mg/g) extracts in µg of quercetin equivalents (QE). The amount of total phenolic and flavonoids for the test samples are summarized (Table 1 and 2). The preliminary phytochemical screening of hydroalcholic extract of polyherbal drugs are presented in Table 3, showing the presence of alkaloids, phenolic groups, flavonids, saponins, steroids, reducing sugars, tannins and anthraquinones, cardiac glycosides, phlobatanins along with carbohydrate, amino acid & protein. The result of TLC analysis using ethyl acetate: chloroform: methanol solvent mixture as shown in table 4 which revealed three to six spots in different solvent.

Flavonoids has been considered to effect on human nutrition and health as it show antioxidant activity, and their mechanism of action are through scavenging or chelating process[44, 45]. It has been recognized that phenolic compounds are a class of antioxidant compounds which act as free radical terminators[46].These compounds such as flavonoids, which contain hydroxyl functional groups, are responsible for antioxidant effect in the plants [47, 48]. The pharmacological activities of the drug contributed by the presence of secondary metabolites. In these classes (such as alkaloids, saponins, tannins, anthraquinones and flavonoids) of compounds are known to have activity against several pathogens and therefore could suggest their traditional use for the treatment of various illness [49].

Table 1. Extraction Yield, Total Phenol and Flavonoid (mg/g) contents of Bharangadi Polyherbal drug:

Extractive value	12%
Total Phenolic Content	50.98 ±1.81 mg/g
Total Flavonoid Content	13.66 ±0.54 mg/g

Table 2. Extraction Yield, Total Phenol and Flavonoid (mg/g) contents of shrishadi Polyherbal drug:

Extractive value	12%
Total Phenolic Content	112 ±4.62 mg/g
Total Flavonoid Content	23.89±4.62 mg/g

Table 3. Preliminary phytochemical screening of polyherbal drugs

Constituents	*Clerodendrum serratum*	*Hedychium spicatum*	*Inula racemosa*	*Albezzia lebback*	*Cyprus rotandus*	*Solanum xanthocarpum*
Alkaloids	+	-	+	+	+	+
Amino acids	+	+	-	+	+	+
Cardiac Glycosides	+	-	-	+	+	+
Flavones	+	-	+	+	+	+
Quinones	+	+	+	+	+	+
Saponins	+	+	-	-	-	+
Steroids	+	-	+	-	+	+
Sugars	+	-	+	-	-	+
Tannins	+	-	+	+	+	+
Triterpenes	+	+	-	+	+	+
Carbohydrates	+	+	+	+	+	+
Protein	+	-	-	+	-	+

Table 4: TLC Result of hydroalcoholic extract of polyherbal drug

Extracts	Solvent system	Number of components	Distance of spot (cm)	Solvent front (cm)	Rf value
shrishadi	Hexane:ethyl acetate (9:1)	3	11.5, 9.4, 7.3	13	0.88, 0.77, 0.56
	chloroform: methanol (9.5:5)	6	11.2, 9.2, 5.7, 3.9, 2.9, 2.4	13	0.86, 0.70, 0.43, 0.30, 0.22, 0.18
Bharangyadi	Hexane:ethyl acetate (9:1)	4	10, 5.6, 4.9, 3.3	13	0.76, 0.43, 0.37, 0.25
	chloroform: methanol (9.5:5)	6	11.5, 8.5, 6.3, 5.2, 4.3, 3.4	13	0.88, 0.65, 0.48, 0.40, 0.33, 0.26

Conclusion

This study indicates that both polyherbal hydroalcoholic extracts showed presence of high amount of phenolic and flavonoid compounds along with secondary metabolites from various medicinally important plants suggesting the use of polyherbal compounds for treatment of various infections.

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