

Study of Antiasthmatic Properties and Chemical Characterization of Indigenous Ayurvedic Compounds (Polyherbal Formulations)

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ABSTRACT

The treatment of diseases with pure pharmaceutical agents is a relatively modern phenomenon. Drugs derived from natural products are usually secondary metabolites and their derivatives. The purpose of these compounds in the organisms and their formation was little understood or investigated, primarily due to the lack of appropriate techniques and structural theory. Therefore, it should be of interest to ascertain just how important medicinal plants are throughout the world when used in the form of crude extracts. In the present study Phytochemical analysis of two self experienced polyherbal indigenous Ayurvedic compounds namely *Shirishadi* and *Bharangyadi* is carried out to evaluate the bio-active constituents responsible for anti-asthmatic properties of the drugs.

Keywords: *Shirishadi* polyherbal compound, *Bharangyadi* polyherbal compound, Phytochemical analysis, Bio-active constituents, Asthma.

INTRODUCTION

Asthma is a chronic inflammatory disease characterized by acute exacerbation of coughing, dyspnea, and wheezing and chest tightness. Worldwide Asthma cases are increasing at the rate of 50% every decade and according to WHO by the year 2020, Asthma along with COPD will become third leading cause of death. All the contemporary medicine use for the management of Asthma proves to be beneficial in acute condition and act as life saving remedies but long term use of all

these medicine can cause serious toxic side effects mainly that cause by using corticosteroid. It is said that asthma cannot be cured it can only prevented. Pathology of the disease once started cannot be arrested at any stage. Hence search has been started once again to explore traditional herbal medicine that are effective as well as safe in curing and preventing Asthma. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some

chemical substances that produce a definite physiological action on human system. In Ayurvedic system of medicine mainly polyherbal compounds are used for the treatment of Asthma. An interest to find out that whether mixing of more than one drug can change the phytochemical constituent of compound or it remain same and in same quantity and with the aim to explore the chemical constituent that are responsible for producing the antiasthmatic activities of all these drug, phytochemical screening of these drug was planned to carried out. *Clerodendrum serratum*, *Hedychium spicatum*, *Inula racemosa*, *Albizia lebbek*, *Cyprus rotandus* and *Solanum xanthocarpum* are extensively used in Ayurvedic system of medicine for the treatment of Bronchial Asthma. Their various medicinal uses in traditional medicine are reviewed with the estimation of phytochemical constituent present in these plants.

PLANT MATERIAL AND EXTRACTION

The plants *Clerodendrum serratum*, *Hedychium spicatum*, *Inula racemosa*, *Albizia lebbek*, *Cyprus rotandus* and *Solanum xanthocarpum* were collected from local market of Varanasi(India). The identification of the drugs was done by Prof. A.K. Singh, Department of dravyaguna, S.S.U., Varanasi. All the six drugs were divided into two groups of three each. Group *Shirishadi* contains *Shirisha* (*Albezzia lebbek*), *Nagarmotha* (*Cyprus rotandus*), & *Kantakari* (*Solanum xanthocarpum*). Group *Bharangadi* consists of *Bharangi* (*Clerodendrum serratum*), *Sati* (*Hedychium spicatum*) & *Pushkarmoola* (*Inula racemosa*). Hydroalcoholic Extraction (Distilled water: Ethanol = 2:1) of drug of both groups were done 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.

Phytochemical Screening

Chemical test were carried out on the aqueous & hydroalcoholic extract and on the powdered specimen using standard procedures to identify the constituent as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Carbohydrates

Molisch's Test

In a test tube containing 2 ml of extract, 2 drops of freshly prepared 10 percent alcoholic solution of α - naphthol was added. Then it was shaken and 2 ml of conc. sulphuric acid was added from sides of the test tube. So the violet ring was formed at the junction of two liquids, indicated the presence of carbohydrates.

Proteins

Biuret's Test

To 1 ml of test extract, 4% of sodium hydroxide solution and few drops of 1% copper sulphate solution were added. Formation of a violet red colour indicated the presence of proteins.

Amino Acid

Ninhydrin Test

3 ml of test solution and 3 drops of 5% ninhydrin solution in a test tube were heated in boiling water bath for 10 minutes. Formation of purple or bluish colour indicated the presence of amino acid.

Glycosides

Borntrager Test

3 ml of extract was treated with dilute Sulphuric acid then boil and filtered. Cold filtrate was treated with chloroform (equal volume) and shaken for some time. The organic layer is separated and treated with

dilute ammonia. Pinkish colour of ammonical layer indicated anthraquinone glycoside.

Test for Tannins

1 ml of hydroethanolic extract of Shirishadi and Bharangadi extract were taken separately in two test tubes and 10% of alcoholic ferric chloride solution was added. Dark blue or greenish colour indicates the presence of tannins.

Test for Phlobatannins

Deposition of a red precipitate when an extract of each group was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for Saponin

About 2g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Flavonoids

Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harbrone, 1973; Sinoda test).

5 ml of dilute ammonia solution were added to a portion of aqueous filtrate of plant extract followed by addition of concentrated H_2SO_4 . A yellow colouration observed in extract indicates the presence of flavonoids. The yellow colour disappeared on standing.

Few drops of 1% aluminium solution were added to a portion of filtrate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was heated with 10ml of ethyl acetate over a steam bath for 3min. The mixture was filtered and 4 ml of the filtrate was shaken with 1ml of dilute

ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

A piece of mg ribbon was added to 1ml ethanolic extract of Shirishadi Group and then 1ml of conc.HCL was added. Pink or red colour indicates the presence of flavonoids.

Test for Steroids

Two ml of acetic anhydride was added to 1 ml (0.05g) of ethanolic extract of Shirishadi & Bharangadi compound separately and 2ml of H_2SO_4 was then added to it. The colour changed from violet to blue or green in sample indicating the presence of steroids.

Test for Terpenoids (Salkowski test)

Five ml of each extract mixed with 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Cardiac Glycosides (Keller- Killani test)

Five ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of conc. H_2SO_4 . A brown ring of the interface indicates a doxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for Alkaloids

A drop of ethanolic extract of each compound was spotted on pre-coated TLC plate and sprayed by modified Dragendroff's reagent. Orange colour of the spot indicates the presence of alkaloids.

Test of Anthraquinone

One ml of ethanolic solution of both extract were mixed with 10% FeCl₃ solution, 1ml of HCL solution, heated and filtered. Filtrate was shaken with diethyl ether and it is further extracted by strong ammonia. Pink or red deep colour indicates the presence of Anthraquinone.

Test of Reducing Sugar

The extract solution was mixed with Fehling solution and heated for 5 minutes. Appearance of red colour indicates the presence of reducing sugar.

Qualitative analysis of phytochemical constituent of *Shirishadi* Compound

Principle Phytochemicals in *Clerodendrum serratum*

Serratogenic acid, queretaroic acid, some phytosterol, saponins, glycosides, arabinose etc. The bark of the root contains phenolic glycoside and saponin as active ingredient. Saponin is very helpful as an antihistaminic agent. A new compound, serratin, was isolated from the essential oil of *Clerodendron serratum* along with lupeol. Apigenin-7-glucoside, C₂₁H₂₀O₁₀ (7-(β-D-glucopyranosyloxy)-5-hydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one).

Pharmacodynamic properties

Antihistamine and ant allergic activity

Saponin and D-mannitol are found to have the Antihistaminic and antiallergic effect¹.

Anti inflammatory and Antipyretic effect²

Pharmacognostical studies on the root of *Clerodendrum serratum* shows anti-inflammatory, anti-nociceptive, and antipyretic effects.

Apigenin-7-glucoside (flavonoid) was obtained as yellowish brown amorphous solid has demonstrated anti-inflammatory³, antimicrobial⁴, hepatoprotective⁵ and antidiarrheal⁶ properties. The compound also showed significant protection against Alzheimer's disease in mice⁷.

Principal Phytochemical Constituents of *Hedichium spicatum* (Sati)

The dried rhizome of the plant contains essential oil, starch, resins, organic acids, and glycoside, albumen and saccharine are also present. The essential oil has ether ester of p-methoxy cinnomic acid, d-sabirene cineole, sesquiterpenes and pentadecane, methyl paracumarine acetate. It contains B sitosterol and its β-D glycosides.

Pharmacodynamic properties

Anti-inflammatory and analgesic activity

The crude ethanolic extract of rhizomes possesses anti-inflammatory and analgesic activity. The anti-inflammatory activity was mainly localized in the hexane fraction, from which 1% of pure active constituent hedychenone a terpene has been isolated. The analgesic activity was more prominent in the benzene fraction. The root stalk is useful in local inflammation, nausea, asthma, bronchitis and in pain. The rhizome of the plant is carminative, stimulant and tonic⁸.

Antiasthmatic effect

Clinical study proved that rhizome of *Hedychium spicatum* has antiasthmatic effect⁹.

Antimicrobial effect

Essential oil extracts from the rhizome of *H. spicatum* showed antimicrobial activity. Petroleum ether and chloroform extracts showed inhibitory activity against gram (+), gram (-) bacterial cultures, including a strain

of methicillin and vancomycin resistant *Staphylococcus aureus* and fungal cultures. Terpenoid compositions of the rhizome of *H. spicatum* also showed significant antimicrobial activity against *Staphylococcus aureus*, *Shigella flexneri*, *Pasteurella multocida* and *Escherichia coli*. Ethanol extract of fruits of *H. spicatum* was reported to possess antibacterial and antifungal properties against *Salmonella sps.* *Escherichia coli* and filamentous fungi¹⁰.

Anti-oxidant effect

Terpenoid compositions of rhizome of *H. spicatum* were found to possess antioxidant activity¹¹.

Phytochemical Composition of *Inula racemosa* (Pushkarmoola)

Roots contains inulin (10%) and an essential oil (1.3%) containing alantolactone. At least four sesquiterpene lactones have been isolated from *Inula*. Isoalantolactone, Dihydroalantolactone, Dihydroisoalantolactone, Beta sitosterol, Daucosterol, Innuolide are some other active compound found in *Inula racemosa*.

Anti Allergic effects

Preliminary studies with the ethanolic extract of roots of *Inula racemosa* exhibited antiallergic and antiasthmatic properties, the later being more pronounced. Specific studies for bronchodilator properties on isolated trachea were performed and found it a potent bronchodilator. The extract also protected guinea-pigs against various experimental asthma, plant pollen etc. It possessed antihistaminic as well as anti-5-HT activity, suggesting its use in bronchial asthma^{12,13}.

Anti-inflammatory effects

"Ethanol 70 % extracts of sun-dried roots of *Inula racemosa*, in doses of 100, 150 and 200 mg/ kg i.p., produced dose-dependent anti-inflammatory effect experimentally¹⁴.

Antimicrobial effect

The essential oil of *Inula racemosa* was tested for antibacterial and antifungal activity, and was found moderately effective against *S. aureus*, *Ps. aeruginosa*, *B. anthracis*. The essential oil of the root exhibited anthelmintic activity against earthworms and tapeworms, but it was less potent than piperazine citrate. Alantolactone and isoalantolactone exhibited antidermatophytic activity¹⁵.

Phytochemicals in *Albizia lebbek*

Chemical Composition

A. Lebbek contains 3',5 Dihydroxy 4', 7 dimethoxy flavone and N Benzoyl L phenyl alaninol. The beans of the plant contain albigenic acid-a new triterpenoid saponin¹⁶. The plant also contains saponins, macrocyclic alkaloids, phenolic glycoside and flavonols.

Pharmacological properties:

Analgesic and Anti-inflammatory Properties¹⁷

Anti-allergic activity of *Albizzia lebbek*

Results support the conclusion that *Albizzia lebbek* at different concentrations has got potent mast cell stabilizing property and the IC (50) value of *Albizzia lebbek* was found to be 85microgm/ml. This inhibitory potential of catechin from *Albizzia lebbek* is perhaps due to modulation of two important effectors' functions, histamine release and cytokine expression of antigen -IgE activated mast cell¹⁸.

Antioxidant activity of *Albizzia lebbek*

Study in alloxan diabetic rats showed *Albizzia lebbek* is a promising plant in respect to its antioxidant potential to alleviate diabetes¹⁹.

Anti-asthmatic activity of *Albizzia lebeck*

Saponin fraction & seed extract of plant significantly reduces the number of ruptured mast cells, in both mesenteric buds and peritoneal fluid obtained from sensitized rats and this effect was identical in both types of systemic Anaphylaxis (Active & Passive). *Albizzia lebeck*- Inhibits degranulation of mast cells, synthesizes reaginic type antibodies and has a pharmacological action like Disodium Cromoglycate. Oral administration of *Albizzia* bark (ethanol extract) provided adrenal protection against histamine-induced bronchospasm²⁰.

Immunomodulatory

The immunomodulatory effect of the bark of *Albizzia lebeck* was evaluated studying humoral and cell mediated immune responses. The *lebeck* treated mice developed higher serum antibody titers compared to the control group. Delayed type hypersensitivity response was suppressed in mice treated with *Albizzia lebeck* extract²¹.

Phytochemicals in *Cyperus rotundus*

Chemical Composition

The tubers contain fat, sugar, gum, carbohydrates, essential oil, albuminoid matter, starch fibre and ash. There are traces of an alkaloid. The essential oil is reported to contain about 27 compounds. In recent years, with the help of ultrasound technology, the extraction of plant alkaloids, glycosides, biologically active substances is make possible. With the help of ultrasonic extraction process flavonoids are also found in root.

Pharmacological properties

Antihypertensive Effect

Aqueous extract of *C. rotundus* caused a decrease in mean arterial blood pressure in rats in a dose dependent manner.

At the dose of 3mg/kg the mean arterial blood pressure was found to reduce by 42.6% from its control²².

Anti-Inflammatory, Anti-Pyretic and Analgesic activities

Research studies showed that the oil of *Cyperus rotundus* possesses anti-inflammatory, anti-pyretic and analgesic activity^{23,24}.

Antimutagenic and Radical Scavenger Activity

Investigation of extracts from *Cyperus rotundus* showed that it has antimutagenic and radical scavenger activities²⁵⁻²⁷.

Phytochemicals in *Solanum xanthocarpum*

Chemical Composition

Alkaloids: Solasodine is the main constituent isolated from the berries of the plant, together with solanine in the unripe fruits and solacarpidin **Sterols:** Carpesterol, β -Sitosterol and norcarpesterol, **Polyphenols:** Chlorogenic, isochlorogenic, neochlorogenic and caffeic acids and the flavonoids quercitrin and apigenin glycosides are present in the fruits.

Pharmacological properties

Antifertility activity

This has been demonstrated in various species. The crude alcoholic extract of the seeds showed spermicidal activity on rat epididymal spermatozoa which was 100% effective after 60 days of treatment at a dose of 100mg/kg²⁸.

Antiasthmatic and Respiratory activity

A whole plant extract of *S. xanthocarpum* produced a significant improvement in certain parameters of pulmonary functions in asthmatic subjects.

Clinically the herb has also proved to be useful in treating bronchitis²⁹.

Anti-inflammatory Effect

Studies showed that *Solanum xanthocarpum* has potent anti-inflammatory activity, maximum at the dose concentration of 500mg/kg³⁰.

Hypoglycaemic activity

Studies on the fruit extract of *Solanum xanthocarpum* showed that it possess hypoglycaemic activity³¹.

Antibacterial and Antifungal activities of *Solanum xanthocarpum* Leaf

Antimicrobial and antifungal properties of water and alcoholic extracts of leaves of *Solanum xanthocarpum* Leaf were tested in gram positive and gram negative bacterial strains using agar gel diffusion method. It was observed that alcoholic extract was most effective as it showed bactericidal activity in the gram negative and gram positive strains and against fungi also. The test compound exhibited moderately to good anti bacterial and antifungal activity³².

RESULTS

The present study carried out on the polyherbal compound revealed the presence of medicinally active constituents. The phytochemical character of both the compound are investigated and summarized in Table 1. Carbohydrate, Protein, Amino acid, Glycosides, Tannins, Flavonoids, Cardiac glycosides, Saponins, Phlobatannins, Terpenoids, Reducing sugar, Anthraquinone. Steroid was absent in both *Shirishadi* and *Bharangadi* compound. Alkaloid was not present in *Shirishadi* compound. As Alkaloid is also absent in aqueous extract of *Cyperus rotandus* may be the cause for absence in compound. As it was found that Alkaloids, Flavonoids, Tannin, Glycosides, Steroid & Cardiac glycosides is absent in *Hedychium*

spicatum but were found to be present in compound *Bharangadi*.

Quantitative estimation of percentage crude chemical constituents in compound is summarized in Table 3. Phenolic (112%) & Flavonoid (23%) contents was found to be highest in *Shirishadi* compound.

DISCUSSION

The Phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the plants studies showed that presence of steroid in the *Shirishadi* compound two of the ingredients namely *Solanum xanthocarpum* and *Cyperus rotandus* have steroid according to Puneet kumar *et.al.*, 2010 & M.R. Heble *et.al.*, 1968., whereas steroid is not found in *Albezzia lebbeck* according to Rahula Chulet *et.al.*, 2010. Similarly steroid is present in *Bharangadi* compound having *Clerodendrum serratum*, *Hedychium spicatum* & *Inula racemosa* as main ingredient. Among these three, steroids is only found in *Clerodendrum serratum* according to Banerjee *et al.*1969, Anonymous 2005, whereas it is absent in *Hedychium spicatum*.

CONCLUSION

Phytochemical screening of both polyherbal drugs showed that both the drugs are having anti-asthmatic properties by virtue of specific phytoconstituents in addition to other pharmacodynamic properties.

ACKNOWLEDGEMENT

Authors were grateful to Prof. Y.B. Tripathi, Professor and Head, Department of Medicinal Chemistry, Faculty of Ayurveda, IMS, BHU, for providing required technical support and guidance in executing phytochemical studies.

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Table 1. Qualitative analysis of the phytochemical of polyherbal compound

Groups Phytochemical ↓	Shirishadi Compound	Bharangadi Compound
Carbohydrate	+	+
Protein	+	+
Amino acid	+	+
Glycosides	+	+
Alkaloids	-ve	+
Tannins	+	+
Steroids	+	+
Saponins	+	+
Phlobatannins	+	+
Terpenoid	+	+
Flavonoid	+	+
Cardiac glycoside	+	+
Reducing sugar	+	+
Anthraquinone	+	+