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Evaluation of antimicrobial activity and bronchodialator effect of a polyherbal drug-Shrishadi

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ABSTRACT

Objective: To investigate antimicrobial and bronchodialator effect of hydroalcholic extract of polyherbal drug Shirishadi containing Shirisha (*Albezzia lebbeck*), Nagarmotha (*Cyprus rotandus*) & Kantakari (*Solanum xanthocarpum*). **Methods:** Antimicrobial activity was evaluated by disc diffusion method and MIC, MBC, MFC were calculated by micro dilution method. Hydroalcholic extract of this preparation was investigated for its phytochemical analysis, phenol and flavonoid were determined by spectrophotometric method and *in vivo* bronchodilator effect was analysed by convulsion time. **Results:** The phytochemical tests revealed presence of alkaloids, anthraquinones, carbohydrates, flavonoids, saponins and tannins. The antimicrobial result showed the MIC of 6.25 mg/mL against *Staphylococcus aureus* and 12.5 mg/mL for *Escherichia coli* and 12.5 mg/mL against remaining bacteria tested, with strong antifungal activity. The maximum inhibition zone is found against *Pseudomonas aeruginosa* with MIC 16 mg/mL. Drug showed significant bronchodilator effect with 27.86% & 36.13% increase in preconvulsion time of guinea pigs pretreated with 100 & 200 mg/kg body weight of extract. **Conclusions:** The study reveals that the extracts possess antibacterial activity and antifungal activity in a dose dependent manner. This antimicrobial property may be due to presence of several saponins, further studies are highly needed for the drug development.

1. Introduction

Albizzia lebbeck also known as tree of happiness is extensively used in various traditional medicines. In Chinese system of medicine it is used for relieving stress, anxiety & depression. Whereas in Ayurveda (Indian system of medicine) it is told to be Vishaghana *i.e.* destroying the toxins present in body. It is mainly used in allergic conditions such as allergic rhinitis, allergic asthma, urticaria *etc.* Research studies had shown that it possesses anti-histaminic & mast cell stabilizing property[1] by virtue of which it is supposed to work as anti-asthmatic drug[2]. It also has anti-inflammatory[3], antioxidant properties[4], antiallergic activity[5] and analgesic activity[6]. *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[7,8], hepatoprotective activity[9], antidiabetic[10], antioxidant potential[10,11], antihyperglycemic[11], larvicidal action[12]. *Cyprus rotundus* or Mustaka is thought to have originated in India and then spread from there during the past 2 000 years. Its uses in modern Ayurvedic medicine are primarily for treating fevers and digestive system disorders (diarrhea, vomiting, indigestion, *etc*). It has been reported to possess anti-inflammatory, anti-pyretic and analgesic activities[13].

Therefore, this study was planned to assess the hydroalcholic extract of Shirishadi-polyherbal drug for antiasthmatic effect and antimicrobial activity with preliminary phytochemical characterization to justify its use in treatment of infectious respiratory disorders.

2. Materials and methods

The plants *Albizzia lebbeck*, *Cyprus rotandus* and *Solanum*

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xanthocarpum 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. Shirishadi group contains Shirisha (*Albezzia lebbeck*), Nagarmotha (*Cyprus rotandus*) & Kantakari (*Solanum xanthocarpum*). Hydroalcoholic extraction (Distilled water: ethanol=2:1) of drug was 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.

2.1. Preliminary phytochemical screening

Chemical test was carried out on the aqueous and hydroalcoholic extract and on the powdered specimen using standard procedures to identify the constituents[14-16].

2.2. Screening of antibacterial activity

2.2.1. Media used

Muller-Hinton agar and broth (Hi-media, Mumbai, India), Sabouraud dextrose agar pH 7.3±0.2 (Hi-media), were used for antibacterial and antifungal activity respectively.

2.2.2. Test microorganism

A total of 7 bacterial strains *viz. Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27893, *Salmonella typhi* MTCC 3216, *Klebsialla pneumoniae*, *Salmonella enteriditis*, *Morganella morganii* (Gram-negative), and *Staphylococcus aureus* ATCC 25323 (Gram-positive) and four fungal strains namely *Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Candida tropicalis* ATCC 750, *Candida parapsilosis* ATCC 22019 were used in the investigation. All cultures were obtained from American Type Culture Collection (ATCC), MTCC, clinical strain preserved at Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India. The fresh bacterial broth cultures were prepared before the screening procedure.

2.2.3.Preparation of sample extract for microbiological assay

About 1 g of each extract was dissolved in 10 mL (100 mg/mL) of peptone water to obtain a stock solution and the working solution was prepared. The extract was diluted as 1:10 equivalent to 100 mg/mL and 1:5 dilution equivalent to 50 mg/mL, from which 5 μL was dispensed on a sterile disc of whatman's filter paper No.1 of 6mm diameter for susceptibility testing.

2.2.4.Antimicrobial susceptibility test

The disc diffusion method was used to screen the antibacterial activity and antifungal activity[17]. Muller Hinton agar (MHA) plates were prepared by pouring 15 mL of molten media into sterile petriplates. The fresh grown bacteria was suspended in sterile saline to achive concentration of 10^7 cfu/mL. This suspension was spread on the surface of MHA agar plates. The plates were allowed to dry for 5 min. The different concentrations of extract (20, 30, 50, 80 mg/mL) was put on 6 mm sterile disc of Whatman filter paper No.1. The disc was then placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37 ℃ for 24 h for bacteria and 48 h at 25 ℃ for fungal agents. At the end of incubation, inhibition zones were examined around the disc which if present were measured with transparent ruler in millimeters. This study was performed in triplicate.

2.3. Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

MIC was determined by micro-dilution method[18,19] using serially diluted (2 folds) plant extracts according to the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards, 2000)[20]. MIC of the extracts was determined by dilution of polyherbal drug of various concentrations. Equal volume of each extract and nutrient broth were mixed in wells of microtiter plate. Specifically 0.1 mL of standardized inoculums ($1-2\times10^7$ cfu/mL) was added in each tube. The plates were incubated aerobically at 37 ℃ for 18-24 h for bacteria and 48 h at 25 ℃ for fungal growth. Two control wells were maintained for each test batch. These included antibiotic control (containing extract and growth media without inoculum) and organism control (tube containing the growth medium, saline and the inoculum). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control were regarded as MIC. However, the MBC and MFC were determined by sub-culturing the test dilution on to a fresh drug free solid medium and incubated further. The highest dilution that yielded no bacterial or fungal colony was taken as MBC and MFC.

2.4. Preliminary phytochemical analysis

The preliminary phytochemical studies were carried out by the methods with some modifications[15,16]. 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.

2.4.1.Determination of total phenols (TP) by spectrophotometric method

TP concentration in different fractions of alcoholic extract was measured by Follin ciocalteau assay. Briefly, 5 mL of distilled water, 0.5-1.0 mL of sample, and 1.0 mL of Folin caiocalteau reagent was added to a 25 mL flask. The content was mixed and allowed to stand for 5-8 min at room temperature. Next 10 mL of 7% sodium carbonate solution was added followed by distilled water. Solution were mixed and allowed to stand at room temperature for 15 min, 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 0.5-5.0 mg/L GAE (R^2=0.999), giving an absorbance range of 0.050-0.555 absorbance units[21,22].

2.4.2. Determination of total flavonoid content

Total flavonoid content was measured by using aluminium chloride (2%) in which it was mixed with solution of test samples. Absorbance reading at 415 nm (Elico SL 177) was taken after 10 min against a blank sample consisting of 5 mL of sample solution and 5 mL of methanol without aluminium chloride. The total flavonoid content was determined using a standard curve of quercetin at 0-50 μg/mL. The average of three readings were used and then expressed in μg quercetin equivalent flavones per mg extract [21,22].

2.5. Studies on Histamine induced bronchospasm in guinea pigs

Guinea pigs of either sex weighing 350-500 g were selected and randomly divided into four groups each containing four animals. The drugs were administered orally in 0.5% sodium carboxymethyl cellulose (CMC). The group 1 was used as the control and given 1.0 mL of saline. The standard group was treated with ketotifen (1 mg/kg) as standard drug. The single dose treatments were given one and half an hour before the study. The following schedule of treatment was administered:

Group I: 1 mL CMC (Normal control);

Group II: Ketotifen (Positive control);

Group III: Hydroalcoholic extract of Shirishadi compound(100 mg/kg);

Group IV: Hydroalcoholic extract of Shirishadi compound (200 mg/kg).

Later the animals were exposed to an aerosol of 0.2% histamine and time for pre convulsion state was noted for each animal.

3. Results

Ancient Indian system of medicine (Ayurveda) is mainly based on herbal treatment. Here Shirisha (*Albezzia lebbeck*), Nagarmotha (*Cyprus rotandus*) & Kantakari (*Solanum xanthocarpum*) alone & in combination, were extensively used in the management of various infections & allergic diseases. The extractive yield (12 g/100 g) of dry plant material of polyherbal drug was obtained by hydroalcoholic solvent. The preliminary phytochemical screening of hydroalcholic extract of *Shirishadi* compound is presented in Table 1, showing the presence of alkaloids, phenolic groups, flavonids, saponins, steroids, reducing sugars, tannins and anthraquinones, cardiac glycosides, phlobatanins along with carbohydrate, amino acid & protein. In addition to the phytochemical screening, on the basis of number of secondary metabolites antibacterial efficacy was determined (Table 2). Ethanolic extract was tested against the organisms namely *Escherichia coli* ATCC 25922 (11, 13, 15 and 18 mm) followed by *Pseudomonas aeruginosa* ATCC 27893 (10, 14, 19, and 21 mm), *Plesiomonas shigelloides* ATCC 14029 (10, 11, 14, and 18mm), *Salmonella typhi* MTCC 3216 (10, 12, 13, and 16 mm), *Shigella flexneri* ATCC 21022 (9, 10, 11, and 14 mm) *Candida parapsilosis* ATCC 22019 (10, 12, 14, and 17 mm), respectively at concentrations 20, 30, 50, 80 mg/mL. The MIC

Table 1.
Preliminary phytochemical screening of shrishadi polyherbal drug.

Constituents	*Albezzia lebback*	*Cyprus rotandus*	*Solanum xanthocarpum*
Alkaloids	+	+	+
Aminoacids	+	+	+
Cardiac glycosides	+	+	+
Flavones	+	+	+
Quinones	+	+	+
Saponins	−	−	+
Steroids	−	+	+
Sugars	−	−	+
Tannins	+	+	+
Triterpenes	+	+	+
Carbohydrates	+	+	+
Protein	+	−	+

+ = Present, − = Absent.

Table 2.
Determination of MIC, MBC, MFC values.

Microorganism	MIC (mg/mL)	MBC (mg/mL)	MFC (mg/mL)
Pseudomonas aeruginosa ATCC 27893	16.00	18.00	−
Klebsialla pneumonia	12.50	14.50	−
Salmonella typhi MTCC 3216	12.50	13.00	−
Escherichia coli ATCC 25922	12.50	14.00	−
Staphylococcus aureus ATCC 25323	6.25	8.00	−
Salmonella enteriditis	12.50	13.00	−
Morganella morganii	12.50	14.00	−
Candida albicans ATCC 90028	12.50	−	14.00
Candida krusei ATCC 6258	12.50	−	14.00
Candida tropicalis ATCC 750	12.50	−	14.50
Candida parapsilosis ATCC 22019	12.50	−	15.00

Table 3.
Antimicrobial activity measured by zone of inhibition of Shrishadi polyherbal drug (mm).

Microorganism	20 mg/mL	30 mg/mL	50 mg/mL	80 mg/mL	Standard drugs
Pseudomonas aeruginosa ATCC 27893	9.00±0.22	11.00±0.34	13.00±0.50	15.00±0.12	28 (Tobramycin)
Plesiomonas shigelloides ATCC 14029	10.00±0.11	11.00±0.09	12.00±0.31	18.00±0.22	26 (Tetracycline)
Salmonella typhi MTCC 3216	8.00±0.48	10.00±0.24	12.00±0.60	13.00±0.36	25 (Ciprofloxacin)
Escherichia coli ATCC 25922	7.00±0.18	9.00±0.26	10.00±0.31	12.00±0.35	26 (Norfloxacin)
Staphylococcus aureus ATCC 25323	10.00±0.49	11.00±0.51	12.00±0.37	14.00±0.35	24 (Ampicilin)
Shigella flexneri ATCC 12022	9.00±0.15	10.00±0.34	11.00±0.94	13.00±0.16	28 (Ciprofloxacin)
Candida albicans ATCC 90028	7.00±0.48	8.00±0.15	10.00±0.51	12.00±0.42	25 (Fluconazole)
Candida krusei ATCC 6258	8.00±0.90	9.00±0.23	11.00±0.46	13.00±0.34	16 (Amphotericin B)
Candida tropicalis ATCC 750	9.00±0.25	10.00±0.98	13.00±0.54	14.00±0.46	20 (Fluconazole)
Candida parapsilosis ATCC 22019	9.00±0.61	10.00±0.42	11.00±0.71	13.00±0.56	25 (Fluconazole)

Table 4.
Effect of polyherbal formulation on histamine-aerosol in guinea pigs (n=4).

Treatment	Dose	Onset of convulsion in sec.	Protection (%)	% Increase in preconvulsion time	% Increase in preconvulsion time
Control (saline)	1 mL/kg	91.45±0.09	0	0.00±0.00	0.00±0.00
Ketotifen fumarate	1 mg/kg	1 028.00±4.55	90***	33.93±3.12	33.93±3.12
Shirishadi compound	100 mg/kg	800.00±0.40	80**	27.85±3.96***	27.85±3.96***
Shirishadi compound	200 mg/kg	860.00±0.67	86***	36.13±3.68***	36.13±3.68***

**P < 0.01, ***P < 0.001 *vs.* control; (χ^2 test with Yate's correction).

and MBC value of 12.5 and 14 mg/mL against *Staphylococcus aureus* and 12.5 and 15 mg/mL for *Escherichia coli* and 10-20 mg/mL against remaining organism tested listed in Table 3. Estimation of total phenolic and total flavonoid content showed that the compound has (112±4.62) mg/g of total phenol & (23.89±3.24) mg/g of total flavonoid. Exposure of guinea pigs pretreated with ethanolic extract of Shirishadi polyherbal compound (100 & 200 mg/kg body weight) had shown that there was 80% and 86% protection against bronchoconstriction caused by histamine with 28% and 36% increment in preconvulsion time (Table 4).

4. Discussion

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[23,24]. *In vitro* antimicrobial test results shows the susceptibility test against Gram positive and negative bacteria with strong antifungal activity of ethanolic extract. The ethanolic extract exhibited considerable level of inhibition against the entire test organism compared to standard drug. This is suggestive of the presence of some compounds or groups in the extract with similar mechanism of action to that of standard drug used in bacterial and fungal activity.

Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative. These differences may be attributed to fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure. Alternatively, the passage of the active compound through the Gram negative cell wall may be inhibited. It is thought that observed differences may result from the doses used in this study. In addition, microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains[22].

The highest activity was exhibited by crude extract against *Pseudomonas aeruginosa* (16 mg/mL) and *Candida parapsilosis* (15 mg/mL) and lowest against *Staphylococcus aureus* (6.25 mg/mL) suggested its efficacy in pneumonia, bacteremia, candidiasis and urinary tract infections. However, it may be suggested that plant extracts exhibiting diameters of zones of inhibition > 10 mm is considered as active[24-28]. Thus it is believed that the extract is better antimicrobial agents for various pathogenic fungus and bacteria. The pharmacological activities of the drug may be contributed due to the presence of secondary metabolites. Pharmacological study of *Albizia lebbeck* showed that it possess anti-inflammatory, antihistaminic & mast cell stabilizing properties[29]. It is found that anti-allergic activity of *Albizzia lebbeck* is due to catechin, whereas anti-histaminic activity is supposed to be due to saponins[30]. *Solanum xanthocarpum* is found to have bronchodilator activity due to solasodine. The result shows that drug has brochodilator effect probably by acting as histamine antagonist and may inhibit the H_1 receptors present on bronchial smooth muscles[31].

Thus it could be suggested that Shirishadi polyherbal drug is effective in bronchial asthma through multitargated drug action. It shows both anti-histaminic and antimicrobial properties.

Conflict of interest statement

We declare that we have no conflict of interest.

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