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Phytochemical composition and In Vitro Antimicrobial and Antioxidant activities of Anti-Asthmatic Polyherbal Compounds

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ABSTRACT

The present study provides the health application at affordable cost. This study such as ethnomedicine keenly represents one of the best avenues in searching new economic plants for medicine.The present investigation was carried out to examine the qualitative phytochemical analysis, antibacterial and anti-oxidant effect of polyherbal drugs namely *Shirishadi* and *Bharangyadi.* The hydroethanolic extracts of drugs showed the presence of alkaloid, tannins, flavanoids, phlobatannins, saponins, terpenoids, cardiac glycosides, anthraquinones, proteins, amino acids and carbohydrates particularly reducing sugars. The estimations of total phenolics content in the selected groups was also done spectrophotometrically following the Folin – ciocalteau method and was found to contained 112mg/g & 50.98± 0.62 of phenolics respectively. Flavonoids were also estimated following the aluminium chloride method and 13.66 ± 1.67mg/g of flavonoids was found to present in *Bharangyadi* compound whereas *Shirishadi* contains 23.89 ± 4.62mg/g. $ABTS^{+}$ assay shows maximum inhibition of 82.27 ± 2.69 with EC_{50} 462.72 ± 4.56 for *Shirishadi* and *Bharangyadi* shows 64.2± 0.86 percent inhibition with EC_{50} 675.31 ± 4.24. Assessment of antimicrobial activity shows that *Bharangyadi* has more potent antibacterial and anti-fungal effect than *Shirishadi.* Antibacterial activity was best revealed by *Bharangyadi* compound against *Plesiomonas shigelloides* followed by *Pseudomonas aeruginosa* it showed strong antifungal property with maximum affinity against *Candida parapsilosis. Shirishadi* compound also showed effective antibacterial and antifungal activities with maximum inhibition zone against *Plesiomonas shigelloides* and *Candida tropicalis*.Both the drugs were tested against seven bacterial strains and four fungal strains.

Introduction

Ayurveda is the Ancient Indian medical science based on herbal remedies. Due to its intrinsic qualities, unique and holistic approaches as well as its accessibility and affordability, Ayurvedic system of medicine continues to be the best alternative care available for the majority of the global population. Plants constitute the centre-piece of therapy in this system of medicine for restoring or maintaining the well-being of the people. *Shirishadi* and *Bharangyadi* are self experienced polyherbal drugs used for the management of bronchial asthma in Ayurvedic system of medicine.Aim to search the active phytoconsistuent of both these drugs and to assess their anti-microbial activity and anti-oxidant activity and justify their use in infectious pulmonary diseases present study was carried out.

Shirishadi compound was hydroethanolic extract of *Shirisha* (*Albizzia lebbeck*), *Nagarmotha* (*Cyprus rotundus*) & *Kantakari* (*Solanum xanthocarpum*) in equal quantity and prepared by hot percolation method through soxhlet apparatus and by classical decoction method. *Bharangyadi* compound was hydroethanolic extract of *Bharangi* (*Clerodendrum serratum*), *Sati* (*Hedychium spicatum*) & *Pushkarmoola* (*Inula racemosa*) and prepared by same method as mentioned above. The drugs were in liquid form and administered as aerosol by Nebulization machine.

All these drugs were chosen on the basis of their action on respiratory tract diseases evidenced by literary review and well supported by previous research works[1-14]. The drugs were administered through Nebulizer machine which convert liquid form of the drug into mist/vapours from having particle size less than 0.5 µm.

Trial Drug's

1. Content of *Shirishyadi* Ayurvedic Nebulizer

Name of the Drug	Botonical name	Part Uesd	Approx. quantity in 100ml of extract
Shirisha	Albezzia lebbeck	*Twaka* (Bark)	20mg
Nagarmotha	Cyprus rotundus	*Kanda* (Rhizome)	20mg
Kantkari	Solanum xanthocarpum	*Panchanga* (whole plant)	20mg

2. Content of *Bharangyadi* Ayurvedic Nebulizer

Name of the Drug	Botonical name	Part Uesd	Approx. quantity in 100ml of extract
Bharangi	Clerodendrum serratum	Moola (Root)	20mg
Sati	Hedychium spicatum	Moola (Root)	20mg
Pushkarmoola	Inula racemosa	Moola (Root)	20mg

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Preperation of drug:

Methods employed for preparation of drugs are:

- Though classical Ayurvedic decoction preparation method.
- Through Soxhlet by hot percolation method.

***Bharangyadi* Compound**

- Color of the drug : Brown colored suspension
- pH : 4.83
- Specific gravity : 0.9779
- TLC : Stationary Phase : TLC silicate gel 60 F254
- Applying : Directly spotting
- Visualization : Examine under U.V. light, 254 nm

***Shirishadi* Compound**

- Color of the drug : Light violet colored suspension
- pH : 5.5
- Specific gravity : 0.8567
- TLC : Stationary Phase : TLC silicate gel 60 F254
- Applying : Directly spotting
- Visualization : Examine under U.V. light, 254 nm

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:

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

Extraction Yield, Total Phenol and Flavonoid contents of *Shirishadi* & *Bharangadi* Compound:

Extraction Yield (%)	12%
Total Phenolic Content (mg/g)	
Bharangadi Compound	50.98 ± 0.62 mg/g
Shirishadi Compound	112 ± 4.62mg/g
Total Flavonoid Content (mg/g)	
Bharangadi Compound	13.66 ± 1.67 mg/g
Shirishadi Compound	23.89 ± 4.62mg/g

Antioxidant Activity of Drugs:

Discussion:

The present study aim to evaluate the antioxidant effect of two polyherbal compounds namely *Shirishadi* & *Bharangyadi*. Both of these drugs are extensively use for the management of respiratory tract diseases since decades. The efficacy of these drugs are time tested and clinically proved but validation on scientific parameters is still lacking.Hydroethanolic extract of both these compounds was prepared and phytochemical analysis had been done. The preliminary phytochemical screening of ethanolic extract of compounds showed 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 (Tables- 1 & 6). 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 (MM Hassan, et.al),(H.Usman,et.al.2007). Phytochemical screening of the drugs showed that *Shirishadi* compound has highest Phenolic & Flavonoid content. Both these classes of compounds have good antioxidant potential and their effects on human nutrition and health are considerable (Havsteen, 2002; Gumul *et al.*, 2007). Natural phenolics exert their beneficial health effects mainly through their antioxidant activity by decreasing oxygen concentration, intercepting singlet oxygen, preventing 1st-chain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to non radical species, and breaking chains to prevent continued hydrogen abstraction from substances (Xu and Chang 2007).

The percentage of inhibition in ABTS in different concentration of *Shirishadi* extract like 50, 100, 200, 400,600,800 & 1000 µg/ml were observed as 15.61 ± 1.23, 28.93 ± 1.43, 37.46 ±1.46,49.05 ± 0.84, 59.08± 0.87, 71.67±2.67 and 82.27±2.69 respectively. *Bharangyadi* extract showed percentage of inhibition in different concentration as 12.49± 1.14, 21.96±1.14,28.31±1.6, 37.66±1.27, 47.29±0.84,56±0.85 and 64.2±0.86 with EC_{50} 675.31±4.24. *Shirishadi* showed highest ABTS radical scavenging activity as its EC_{50} found to be less than *Bharangyadi* (462. 72± 4.56). Ethanolic extracts were tested against the organisms namely *Plesiomonas shigelloides* (10, 11, 12,and 18mm for *Shirishadi* compound & 10,11.14 and 18mm for *Bharangyadi* compound), followed by *Pseudomonas aeruginosa* (9, 11, 13,and 15mm for *Shirishadi* compound and 10,14,19 and 21mm for *Bharangyadi* compound), *Salmonella typhi* (8,10, 12 and 13mm for *Shirishadi* & 10,12,13 and 16mm for *Bharangyadi* compound), *Shigella flexneri ATCC 21022* (9, 10, 11and 13mm for *Shirishadi* and 9,10,11 and 14mm for *Bharangyadi* compound), *Salmonella typhi MTCC 3216* (8,10,12 and 13mm for *Shirishadi* and 10,12,13 and 16mm for *Bharangyadi* compound), *Escherichia coli* ATCC 25922 (7,9,10 and 12 mm for *Shirishadi* and 11,13,15 and 18mm for *Bharangyadi* compound)respectively at concentrations 5, 10, 15, 20, 25 mg/ml. Antifungal screening shows that *Bharangyadi* extract has maximum affinity against *Candida parapsilosis* (17±0.41mm) followed by *Candida tropicalis* (16±0.36mm). *Shirishadi* extract shows highest zone of inhibition for *Candida tropicalis* (14±0.46).

Conclusion:

Polyherbal compounds are source of potential antioxidant for radical scavenging. The highly positive correlation of antiradical scavenging activity and total polyphenolic content in Polyherbal compounds indicates that polyphenols are the important components which could be used for the free radical scavenging activity. This is the first scientific study on the antiradical efficiency of the herbal nebulizer drugs. This study shows that crude ethanolic extracts of *Bharangyadi & Shirishadi* compounds found to have significant antibacterial and antifungal activity and may be used for treatment of infectious respiratory diseases. It can also be concluded that antimicrobial activity of *Bharangyadi* compound is higher than that of *Shirishadi* compound.

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Table No. 1 : TLC Result of hydroalcoholic extracts of polyherbal drugs

Extracts	Solvent system	Number of components	Distance of spots (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

Table No. 2 : Analysis of Phytochemical constituent in different medicinal plants

Plants → Phhytochemical	Albezzia lebback	Cyprus rotundus	Solanum xanthocarpum	Clerodendrum serratum	Hedychium spicatum	Inula racemosa
Alkaloids	+	-ve in Aq. + in EtoH	+	+	-ve (by Dragondroff's test)	+
Flavonoids	+	+	+	+	-ve	+
Saponin	+ in Aq. - ve in EtoH	-ve	+	+	+	-ve
Tannin	+	+	+	+	-ve	+
Glycosoides	-ve	+	+	+	-ve	+
Steroids	-	+	+	+	-ve	+
Carbohydrates	+	+	+	+	+	+
Proteins	+	-ve	+	+	+	-ve
Amino acid	+	+	+	+	-ve	-ve
Cardiac glycoside	+	-ve in Aq + in EtoH	+	+	-ve	-ve
Phlobatannis	+	+	+	+	-ve	+
Reducing sugar	+	+	+	+	+	-ve
Anthraquinone	+	+	+	+	+	-ve

Table 3 : Showing the main constituent and probable mode of action of some Antiasthmatic Ayurvedic drugs:

Plants	Part used	Extract/ Active principle	Probable mechanism of action
Albizzia lebbeck	Stem bark	Aqueous extract M.C.-Catechin	Mast cell stabilizing effect[15], antiallergic & antioxidant activity[16,17]. 
Cyprus rotundus	Root	Aqueous extract/ alcholic extract M.C.-Sesquiterpenes	Anti-Inflammatory[18], Antimutagens[19] and Radical scavengers, Antioxidant activities.  
Solanum xanthocarpum	Whole herb	Aqueous/alcoholic extract M.C.-Salasodin, Apigenin,Stigmasterol, Carpesterol, Diosegenin	Bronchodilator[20], Antiallergic property[21], Anti-inflammatory 
Clerodendrum serratum	Root	Aqueous / alcholic extract M.C.- Apigenin-7-glucoside	Antihistamine[22] ,antiallergic & bronchodilator activities[23] 
Hedychium spicatum	Rhizome	Ethanolic extract M.C.- Hedychenone a terpene	Anti-inflammatory[24], analgesic effect, reduce total eosinophil count[25]. 
Inula racemosa	Root	Aqueous / alcholic extract. M.C.- Inulin	Antihistaminic[26], Anti- serotonergic. 

Table 4: $ABTS^+$ radical scavenging activity (%). $ABTS^+$ scavenging activity of different concentrations of hydroalcholic *Shirishadi* extract

Concentration	% of Inhibition
50 µg/ml	15.61±1.23
100	28.93±1.43
200	37.46±1.46*
400	49.05±0.84**
600	59.08±0.87**
800	71.67±2.67**
1000 µg/ml	82.27±2.69**
EC_{50}	462.72±4.56

Table 5 : $ABTS^+$ radical scavenging activity (%). $ABTS^+$ scavenging activity of different concentrations of hydroalcholic *Bharangyadi* extract.

Concentration	% of Inhibition
50 µg/ml	12.49±1.14
100	21.96±1.14
200	28.31±1.6
400	37.66±1.27*
600	47.29±0.84*
800	56±0.85**
1000 µg/ml	64.2±0.86**
EC_{50}	675.31±4.24

Table 6 : Determination of MIC, MBC, MFC values for *Shirishadi* compound

Microorganism	MIC(mg/ml)	MBC(mg/ml)	MFC(mg/ml)
Pseudomonas aeruginosa ATCC 27893	15	16	-
Klebsialla pneumonia	12.5	14.5	-
Salmonella typhi MTCC 3216	12.5	13	-
Escherichia coli ATCC 25922	12.5	12	-
Staphylococcus aureus ATCC 25323	6.25	14	-
Shigella flexneri	12.5	13	-
Plesimonas shigelloides	12.5	18	-
Candida albicans ATCC 90028	12.5	-	12
Candida krusei ATCC 6258	12.5	-	13
Candida tropicalis ATCC 750	12.5	-	14
Candida parapsilosis ATCC 22019	12.5	-	13

Table 7 : Antimicrobial activity measured by zone of inhibition (in mm) of *shirishadi* polyherbal drug.

Microorganism	Zone of inhibition (in mm)				
	Extract Concentration (mg/ml)				Standard drugs
	20	30	50	80	(10µg/disc)
Pseudomonas aeruginosa ATCC 27893	9±0.22	11±0.34	13±0.50	15±0.12	28 (Tobramycin)
Plesiomonas shigelloides ATCC 14029	10±0.11	11±0.09	12±0.31	18±0.22	26 (Tetracycline)
Salmonella Typhi MTCC 3216	8±0.48	10±0.24	12±0.60	13±0.36	25(Ciprofloxacin)
Escherichia coli ATCC *25922*	7±0.18	9±0.26	10±0.31	12±0.35	26 (Norfloxacin)
Staphylococcus aureus ATCC 25323	10±0.49	11±0.51	12±0.37	14±0.35	24 (Ampicilin)
Shigella flexneri ATCC 12022	9±0.15	10±0.34	11±0.94	13±0.16	28(Ciprofloxacin)
Klebsialla pneumonia	10± 0.16	12± 0.32	13±0.13	14± 0.52	28(Ciprofloxacin)
Candida albicans ATCC 90028	7±0.48	8±0.15	10±0.51	12±0.42	25 (Fluconazole)
Candida krusei ATCC 6258	8±0.90	9±0.23	11±0.46	13±0.34	16 (Amphotericin B)
Candida tropicalis ATCC 750	9±0.25	10±0.98	13±0.54	14±0.46	20 (Fluconazole)
Candida parapsilosis ATCC 22019	9±0.61	10±0.42	11±0.71	13±0.56	25(Fluconazole)

Table 8 : Determination of MIC, MBC, MFC values for *Bharangyadi* compound

Microorganism	MIC (mg/ml)	MBC (mg/ml)	MFC (mg/ml)
Pseudomonas aeruginosa ATCC 27893	18	21	-
Klebsialla pneumonia	12.5	21	-
Salmonella typhi MTCC 3216	10	16	-
Escherichia coli ATCC 25922	12.5	18	-
Staphylococcus aureus ATCC 25323	12.5	15	-
Shigella flexneri	12.5	14	-
Plesimonas shigelloides	12.5	18	-
Candida albicans ATCC 90028	12.5	-	15
Candida krusei ATCC 6258	12.5	-	15.5
Candida tropicalis ATCC 750	12.5	-	16
Candida parapsilosis ATCC 22019	12.5	-	17

Table 9 : Antimicrobial activity measured by zone of inhibition (in mm) of *Bharangyadi* polyherbal drug.

Microorganism	Zone of inhibition (in mm)				
	Extract Concentration (mg/ml)				Standard drugs
	20	30	50	80	(10µg/disc)
Pseudomonas aeruginosa ATCC 27893	10±0.57	14±0.63	19±0.90	21±0.42	30 (Tobramycin)
Klebsialla pneumoniae	10±0.57	14±0.63	19±0.90	21±0.42	28 (Ciprofloxacin)
Plesiomonas shigelloides ATCC 14029	10±0.41	11±0.09	14±0.31	18±0.22	25 (Tetracycline)
Salmonella Typhi MTCC 3216	10±0.48	12±0.24	13±0.30	16±0.36	28 (Ciprofloxacin)
Escherichia coli ATCC *25922*	11±0.68	13±0.50	15±0.61	18±0.32	26 (Norfloxacin)
Staphylococcus aureus ATCC 25323	10±0.49	10±0.51	11±0.37	15±0.35	24 (Ampicilin)
Shigella flexneri ATCC 12022	9±0.60	10±0.23	11±0.24	14±0.36	32 (Ciprofloxacin)
Candida albicans ATCC 90028	10±0.48	12±0.10	13±0.71	15±0.72	25 (Fluconazole)
Candida krusei ATCC 6258	10±0.34	11±0.29	13±0.45	15±0.65	16 (AmphotericinB)
Candida tropicalis ATCC 750	9±0.60	11±0.13	13±0.78	16±0.36	20 (Fluconazole)
Candida parapsilosis ATCC 22019	10±0.34	12±0.12	14±0.67	17±0.41	25 (Fluconazole)

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