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"Antimicrobial and Anti-inflammatory effect of an Indigenous Ayurvedic Drug- *Bharangyadi*"

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ABSTRACT

The main objective of the present investigation is to evaluate the antiinflammatory & antimicrobial activity of ethanolic extract of Bharangyadi polyherbal compound on albino rats. Bharangyadi compound consist of three herbal drugs namely- Bharangi (Clerodendrum serratum), Sati (Hedychium spicatum) & Kantakari (Solanum xanthocarpum).In Ayurveda (ancient Indian system of medicine) all these herbs alone or in combination with other herbs are commonly used in the managmant of bronchial asthma.

In the carrageenan-induced rat paw edema test for acute inflammation, the extract of *Bharangyadi* compound in doses of 50mg, 200 mg and 500 mg/kg body weight showed 78.75% and 79% and 81.75% inhibition of edema, respectively, at the end of 4h which is comparable to that of standard (endomethacin) i.e. 92%. The antimicrobial activity of the plant extract was assayed against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa ATCC* 27893, *Salmonella typhi* MTCC 3216, *Aeromonas hydrophila* ATCC 7966, *Escherichia coli* ATCC 35218, *Shigella flexineri* ATCC 12022, *Bacillus subtilis, Plesiomonas shigelloides* ATCC 14029 (Gram-negative), *Staphylococcus aureus ATCC 25323* (Gram-positive) and *Candida albicans* ATCC 90028,*Candida krusei* ATCC 6258, *Candida tropicalis* ATCC 750, *Candida parapsilosis* ATCC 22019 using the disc diffusion and micro dilution techniques. The result showed the MIC of 12.5 mg/ml against *Staphylococcus aureus*, *Escherichia coli* and *Candida* species and 10-20 mg/ml against remaining bacteria tested. The study revealed that the extracts possessed antibacterial and antifungal activity in a dose dependent manner. Thus use of this herbal preparation in the treatment of respiratory tract infections may be justified.

KEY WORDS: Antibacterial activity, *Bharangyadi* Polyherbal drug, *in-vitro*, phytochemical, ethanolic extract, antifungal, anti-inflammatory.

INTRODUCTION

Asthma is a common disease that is rising in prevalence worldwide with the highest prevalence in industrialized countries. Asthma affect about 300 million people worldwide and it has been estimated that a further 100 million will be affected by 2025. Asthma is defined as disorder characterized by chronic airway inflammation and increased airway responsiveness resulting in symptoms of wheezing, cough, chest tightness, and dyspnea. It is characterized functionally by the presence of airflow obstruction which is variable over short periods of time or is reversible with treatment. It is not a uniform disease but rather a dynamic clinical syndrome which has a number of clinical patterns. Current asthma therapy lack satisfactory success due to adverse effect, hence patients are seeking complementary and alternative medicine to treat their asthma. Medicinal plant used for the treatment of asthma should have anti-inflammatory, immunomodulatory,antihistaminic, smooth-muscle relaxants and allergic activity. The basic pathology of Asthma starts with the process of inflammation so to show the antiasthmatic activity of drug first step involve to demonstrate the anti- inflammatory activity of drug. In Ayurvedic system of medicine mainly polyherbal compounds are used for the treatment of Asthma. Clerodendrum serratum, Hedychium spicatum and Inula racemosa, are extensively used in Ayurvedic system of medicine for the treatment of Bronchial Asthma. Bharangi (Clerodendrum serratum) is found to have anti-inflammatory, antihistaminic, antiallergic, antioxidant and hepatoprotective properties. 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. Pushkarmoola (Inula racemosa) has been found prove beneficial for cardiovascular system, angina and dyspnoea. Bharangyadi is a mixture of Clerodendrum serratum, Hedychium spicatum and Inula racemosa.

Therefore this study was planned to assess the ethanolic extract of polyherbal compound (Bharangyadi) for antimicrobial, preliminary phytochemical characterization and anti-inflammatory activity to justify its use in treatment of infectious respiratory disorders.

MATERIALS AND METHODS:

PLANT MATERIAL AND EXTRACTION

The plants Clerodendrum serratum, Hedychium spicatum and Inula racemosa 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. Bharangyadi group contains Bharangi (Clerodendrum serratum), Sati (Hedychium spicatum) and Pushkarmoola (Inula racemosa) Hydroalcoholic Extraction (Distilled water: Ethanol = 2:1) of drug 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.

Drugs and Chemicals

Aminopyrine, Carrageenan, Pentazocin, Endomethcin & Acetic acid were purchused from Sigma-Aldrich, US.

Experimental animal:

Adult Charles Foster Albino rats (150± 30g) of either sex were obtained from the Animal Research Branch of the Institute of Medical Sciences, Banaras Hindu University, Varanasi . The animals were housed in polyvinyl cages and. were fed on commercial pellet diet (Amrut, Pranav Agro Industries Ltd, India). They were group housed under standard conditions of temperature (22 ± 20C), relative humidity (60 ± 5%) and 12:12 light/dark cycle, where lights on at 0700 and off at 1900 h). The saline fed group served as control and one group was treated with a standard drug in each protocol. Before experimentation, the animals were kept on fast for 24 h but water was given ad libitum except during experimental test period. During experiments, animals were also observed for any alteration in their general behavior.

Anti-inflammatory study

Carrageenin,a sulphated polysaccharide,extracted from sea weed, has been extensivelyused to induce inflammatory reaction in a number of animal species. Winter et al. (1962) introduce the carrageenin edema of rat hind paw for assay of anti-inflammatory drugs. The reproducibility of the fact that inflammation entirely depends upon local inflammatory reaction devoid of anigenic properties, has made carrageenin the most widely employed phlogistic agent. For the present experiment, carrageenin suspension was prepared as a homogenous suspension of powder in 0.9% sodium chloride solution (sterile normal saline) with the help of mortar & pestel. A volume of 0.1ml of suspension was injected through a 26 gauge needle into the plantar surface of the right hind paw below the plantar aponeurosis 1h after the oral administration of test materials. The volume of hind paw of the rats upto the ankle joint was measured plethysmographically, by the mercury displacement method. The volume was measured 1h, 2h ,3h, 4h & 24 after the administeration of drug. The extract was administered at 50, 200 and 500 mg/kg body weight. Endomethacin 25 mg/kg body weight was used as standard anti-inflammatory agent.

SCREENING OF ANTIBACTERIAL ACTIVITY

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.

Preparation of sample extract for microbiological assay

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

Antimicrobial Susceptibility Test

The disc diffusion method was used to screen the antibacterial activity and antifungal activity. Muller Hinton agar (MHA) plates were prepared by pouring 15ml 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°C for 24 hr for bacteria and 48 hr at 25^0C 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.

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

MIC was determined by micro-dilution method using serially diluted (2 folds) plant extracts according to the National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards, 2000) . 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 a wells of microtiter plate. Specifically 0.1 ml of standardized inoculums (1-2 ×10^7cfu/ml) was added in each tube. The plates were incubated aerobically at 37°C for 18-24 h for bacteria and 48h at 25^0C 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 was 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.

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.

Microorganism	MIC(mg/ml)	MBC(mg/ml)	MFC(mg/ml)
1. Pseudomonas aeruginosa ATCC 27893	18	20	-
1 Klebsialla pneumonia	12.5	14.5	-
2 Salmonella typhi MTCC 3216	10	12.5	-
3 Escherichia coli ATCC 25922	12.5	15	-
4 Staphylococcus aureus ATCC 25323	12.5	14	-
5 Salmonella enteriditis	20	25	-
6 Morganella morganii	12.5	14	-
7 Candida albicans ATCC 90028	12.5	-	13
8 Candida krusei ATCC 6258	12.5	-	14
9 Candida tropicalis ATCC 750	12.5	-	14.5
10Candida parapsilosis ATCC 22019	12.5	-	15

Table 1: Determination of MIC, MBC, MFC values for Bharangyadi compound:

Microorganism	Zone of inhibition (in mm)				
	Extract Concentration (mg/ml)				Standard drugs
	20	30	50	80	(10µg/disc)
Pseudomonas aeruginosa ATCC	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	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 2	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 (Amphotericin B)
Candida tropicalis ATCC 750	9±0.60	11±0.13	13±0.78	16±0.36	20 (Fluconazole)
Candida parapsilosis ATCC 220	10±0.34	12±0.12	14±0.67	17±0.41	25 (Fluconazole)

Table 2: Antimicrobial activity measured by zone of inhibition (in mm) of Bharangyadi polyherbal drug

Constituents	Clerodendrum serratum	Hedychium spicatum	Inula racemosa
Alkaloids	+	-	+
Amino acids	+	+	-
Cardiac Glycosides	+	-	-
Flavones	+	-	+
Quinones	+	+	+
Saponins	+	+	-
Steroids	+	-	+
Sugars	+	-	+
Tannins	+	-	+
Triterpenes	+	+	-
Carbohydrates	+	+	+
Protein	+	-	-

Table 3. Preliminary phytochemical screening of Bharangyadi polyherbal drug

GROUP	% Increase in Paw Volumes (ml × 1000) ± SEM (percent inhibition)				
	1h	2h	3h	4h	24h
Control	1.78 ± 0.77	3.0 ±0.15	3.61 ± 0.20	4.0± 1.2	1.93 ± 0.11
Standard (Indomethacin 25mg/kg)	0.72 ± 3.7 59%	0.69 ± 0.60 77%	0.67 ± 0.66 81%	0.65 ± 0.37 92%	0.66 ± 0.52 66%
Bharangyadi 50mg/ Kg bwt	0.96 ± 0.12 48.87%	0.92 ± 0.03 69.3%	0.82 ± 0.01 77.28%	0.87± 0.05 78.75%	0.78± 0.03 46.62%
Bharangyadi 200mg/Kg bwt	1.05 ± 0.11 41.01%	0.90 ± 0.02 70%	0.73 ± 0.02 80%	0.84± 0.03 79%	0.85 ± 0.03 53.3%
Bharangyadi 500mg/Kg bwt	0.76 ± 0.003 57.3%	0.76± 0.003 57%	0.69± 0.004 80.8%	0.73 ± 0.002 81.75%	0.81 ± 0.02 50.36%

Table 4: Anti-inflammatory activity of crude extract of Bharangyadi Compound by Carrageenan induced rat paw edema

*Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test): **P<0.001. All values are means of individual data obtained from six rats (n = 5).

Groups	Change in Paw Volume expessed as Mean ± SE (per hour)				
	1h	2h	3h	4h	24h
Bharangyadi 50mg/ 100g bwt	1.06 ± 0.068**	0.99± 0.006**	0.93 ± 0.03**	0.89± 0.003**	0.78 ± 0.04**
Bharangyadi 200mg/100g bwt	1.10 ± 0.05**	0.97 ± 0.01**	0.93 ± 0.02**	0.89± 0.05**	0.77 ± 0.02**
Bharangyadi 500mg/100g bwt	0.95 ± 0.03**	0.95 ± 0.03**	0.83 ± 0.06**	0.76± 0.06**	0.66 ± 0.03**
Endomethacin (25mg	0.72 ± 0.03**	0.69 ± 0.06**	0.67 ± 0.06**	0.65 ± 0.03**	0.66 ± 0.03**

* Values are mean ± SEM (n =3); One-way ANOVA; df = 4, 10; 14,**P<0.001, *P<0.05 compared to control.

Table: 5, Intergroup comparison of Bharangyadi Compound treated and Endomethacin treated group with control by using One Way ANNOVA followed by Post- Hoc test.

RESULTS AND DISCUSSION:

In the carrageenan-induced rat paw edema test (table 4)for acute inflammation, the extract of Bharangyadi compound in doses of 50mg, 200 mg and 500 mg/kg body weight showed 78.75% and 79% and 81.75% inhibition of edema, respectively, at the end of 4h which is comparable to that of standard (endomethacin) i.e. 92%.

Carrageenan-induced inflammation in the rat paw represents a classical model of edema formation and hyperalgesia, which has been extensively used in the development of nonsteroidal anti-inflammatory drugs and selective COX-1-2 inhibitors (Winter CA,et.al 1962). Several lines of evidences indicate that the COX-2-mediated increase in prostaglandin (PG) E_2 production in the central nervous system (CNS) contributes to the severity of the inflammatory and pain responses in this model. In the paw, the early phase was associated with increases in PGE_2 and thromboxane (TX)B_2 levels and

with a peak of COX-2 (Vinegar R,et.al.1969). Therefore, the inhibition of carrageenan induced inflammation by the extract of Bharangyadi compound could be due to the inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. The present study on extract of Bharangyadi compound has demonstrated that this compound has significant analgesic and anti-inflammatory properties, and thus can be use in Bronchial asthma and other inflammatory conditions.

The ethanolic extract exhibited considerable level of inhibition against all the 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.

The highest activity was exhibited by crude extract against Pseudomonas aeruginosa (21±0.42 mm) and Candida parapsilosis (17±0.41) and lowest against S. aureus (15±0.35) suggesting its efficacy in pneumonia, bacteremia, candidiasis and urinary tract infections. The lowest activity was exhibited by residual portion against Shigella flexneri (14±0.36 mm). However, it may be suggested that plant extracts exhibiting diameters of zones of inhibition > 10mm considered active. In this line, it may be suggested that the extract is better antimicrobial agents for various pathogenic fungus and bacteria. The pharmacological activities of the drug contributed by the presence of secondary metabolites. The anti-histaminic and anti allergic properties of Clerodendrum serratum is attributed to the presence of saponin. Apigenin-7-glucoside (flavonide) present in Clerodendrum serratum has demonstrated anti-inflammatory, antimicrobial, hepatoprotective and antidiarrheal properties. Hedychenone a terpene present in Hedychium spicatum shows antiinflammatory and analgesic activities. Ethanolic extract was tested against the organisms E.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 17mm) respectively at concentrations 20, 30, 50, 80 mg/ml. The MIC and MBC value of 12.5 and14 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-1.

This study shows that crude ethanolic extracts of Bharangyadi compound found to have significant antibacterial and antifungal activity and may also be used for infectious respiratory diseases. This study support the traditional use of Bharangi (Clerodendrum serratum), Sati (Hedychium spicatum) & Pushkarmoola (Inula racemosa) in the treatment of respiratory tract diseases mainly bronchial asthma, bronchitis, pneuomonia.

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