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Phytochemical, Preliminary Pharmacognostical and Antimicrobial Evaluation of Combined Crude Aqueous Extract

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Abstract: The aim of the present work was to evaluate the anti-microbial activity of Combined Crude ethanolic extract of *Datura stramonium, Terminalia Arjuna* and *Withania somnifera* in cup plate diffusion method for antibacterial and antifungal activity. The extracts were subjected to screening to detect potential antimicrobial activity against *Staphylococcus aureus, Bacillus subtilus, Escherichia Coli, K. Pneumonia, M. luteus and C. Albicans* with compare Ciprofloxacin standard drug. Minimum inhibitory concentrations (MIC) of the extracts were also determined against the four selected microorganisms showing zones of inhibition =10mm.Anti-inflammatory activity, Analgesic activity both aqueous and alcoholic extracts revealed significant effects ($p<0.01$) against thermal, chemical and forced induced stimuli in hot plate, tail immersion and tail clip method respectively as well as significant anti-inflammatory effects.

Key words: *Datura stramonium* · *Terminalia Arjuna* · *Withania somnifera* · Antibacterial activity · Analgesic activity · Anti-inflammatory activity

INTRODUCTION

Datura stramonium (Family: Solanaceae) is consist of dried leaves contain 0.25 % of alkaloids of stramonium. It is indigenous to Caspian region and in United States, South America, France, Germany and Hungary. The main active constituents of plant are atropine, hyoscyamine and scopolamine. It is used as a aphrodisiac, medicinal, psychotropic, sacred and antispasmodic[1-4]. *Terminalia arjuna* (Family: Combretaceae) is a dried stem bark. The tree is common in Indian peninsula it is grown by the side of stems and very common in Chotta-nagpur region. The pieces of various-sizes, about 15x10x1cm. According to ayurevedic text it is used as cardiotonic, styptic, febrifugal and antidysentric and diuretic. The main active constituents of the plant are 15% tannins, arjuonic acid, arjunogenin, arjunetine and arjunolone [1-4]. *Withania somnifera* (Family: Solanaceae) is consists of dried roots. The main active constituents are steroidal lactones, somniferine, somnine, somniferinine, tropane and anahydrine. It is used as sedative and hypnotic, hypotensive, as respiratory stimulant along with bradycardia.

MATERIALS AND METHODS

Collection of Plant Material and Authentication: *Datura stramonium (leaves), Terminalia Arjuna (bark), Withania somnifera (root)* were collected from SAGAR, M.P. The plant authenticated by comparing with the herbarium voucher specimen. The material was air dried under shade, powdered mechanically and stored in airtight containers. About 30 g of the powdered material was subjected individually for cold maceration with 95% ethanol for 7 days. This ethanolic extract was concentrated to dryness under reduced pressure and controlled temperature (50-60°C) to yield solid masses.

Drying and Size Reduction of Plant: The whole plant material of Datura stramonium (leaves), Terminalia Arjuna (bark). Was subjected to shade drying for about 10 weeks. The dried plant material was further crushed to powder and the powder was passed through the mesh 60 and stored in air tight container for further analysis.

Determination of Fluorescence Character: Fluorescence characters of powdered plant material with different chemical reagents were determined under ordinary and ultraviolet light [5] (Table 6).

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Determination of Physicochemical Parameters: The dried plant material was subjected for determination of physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value, moisture content, foreign organic matter, crude fibre [6], alcohol soluble extractive and water soluble extractive [7].

Extraction of Powdered Plant Material: The shade dried powdered plant material was subjected to sequential soxhlet extraction using the solvents of different polarity such as petroleum ether (40-60°), chloroform, ethanol and finally macerated with water so as to get respective extracts. Cold maceration was also done using ethanol and water. The extracts were filtered individually, evaporated to dryness and the percent yields of all the extracts were determined. All the extracts were then stored in a refrigerator till further analysis.

Preliminary Photochemical Analysis: Preliminary qualitative photochemical analysis of all the extracts was carried out by employing standard conventional protocols [8].

Thin Layer Chromatography: Successive extractions with n-hexane, chloroform and ethanol were carried out on the pulverized sample by maceration for 24 hrs. The extracts were filtered and concentrated on a rotary evaporator. Each concentrated extract was spotted on a normal phase plate previously activated at 110°C for 2 hrs, using a capillary tube. The plate was developed using mobile phase of hexane-ethyl acetate (12:3) for hexane extract and n-hexane- chloroform (2:1) for ethanol extracts.

RESULT AND DISCUSSION

Antibacterial and Antifungal Studies: The various extracts were tested for their effect on Gram +ve bacteria such as *Staphylococcus aureus, Bacillus subtilis* and Gram - ve bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. Fungi used for the present study were *Aspergillus niger* and *Candida albicans*. minimum inhibitory concentration of the extracts was evaluated by cup plate diffusion method for antibacterial and antifungal activity [9-11].

Inoculum: The microorganisms were inoculated into SBCB and incubated at 35±2° C for 6hrs. The turbidity of the resulting suspension was diluted with SBCB to match with 1 McFarland turbidity standard. This level of turbidity is equivalent to approximately 5.0×10^6 CFU/ml.

Agar Well Diffusion Method: Muller-Hinton agar plates were inoculated by streaking the swab over the entire sterile over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure even distribution of the inoculum. As a final step the rim of the agar was also swabbed. After allowing the inoculum to dry at room temperature, 9-mm-diameter wells were bored in the agar. Each extract was check for antimicrobial activity by introducing 100iL of 400ìg/ml concentration into triplicate wells. Simultaneously, ciprofloxacin and Amoxicillin were used as positive control at a concentration of 1ig/ml. and the dilution medium for the positive control was respective solvents. The plates were allowed to stand at room temperature for 1 hour for extract to diffuse into the agar and then they were incubated at 35±2°C for 24 hr.0.1 ml of overnight grown nutrient broth culture of the bacteria was transferred aseptically to sterile glass Petri dish. Sterile molten nutrient agar (45°C) was then poured, mixed uniformly rotating the plate and allowed to solidify. Cups were made out in the centre of the seeded nutrient agar plate using a sterile cork borer (6mm). The various extracts of the leaf of different concentrations viz. 10,20,50, 100, 200 and 400 mg/ml were made using dimethyl sulphoxide (DMSO) as a diluting solvent. The samples were added with a sterile micropipette to each of the cups. The plates were then incubated at 37°C for 24 hrs. Plates with cups containing only DMSO served as a control (Table 2, 3).

Determination of Minimum Inhibitory Concentration (MIC): The MIC was determined only for the three organic solvent extracts which showed positive antimicrobial activity against four tested microorganisms by modified agar well diffusion method. In this technique, a twofold serial dilution of the extracts was prepared by first reconstituting in dimethylsulphoxide (DMSO). They were diluted in sterile distilled water to achieve a decreasing concentration range of 30 mg/ml to 0.09mg/ml. A 100 µl volume of each dilution was introduced in

Table 1: Phytochemical group test for the *Datura stramonium, Terminalia Arjuna, Withania somnifera*

S. No.	Phytoconstituents	Extract of leaves
1	Saponins	+
2	Flavonoids	+
4	Glycosides	-
5	Tannins	-
6	Alkaloid	+

+Present, - Absent

Table 2: Antimicrobial activity of *Datura stramonium, Terminalia Arjuna, Withania somnifera* aqueous extract of different micro organisms

	Zone of Inhibition in (mm)					
Sample Conc. in %	*S. Aureus*	*B. Subtillis*	*E. Coli*	*P.aeruginosa.*	*A. niger*	*C. albicans*
400	27	24	20	22	24	19
200	23	21	18	19	22	18
100	24	19	21	18	16	21
50	21	17	20	22	18	19
20	25	16	24	20	15	22
10	18	19	17	17	17	20
5	14	16	16	21	14	19

Table 3: Antimicrobial activity of antibiotics on different micro organisms *Datura stramonium, Terminalia Arjuna, Withania somnifera*

	Microorganisms (inhibition zone in mm)					
	Gram-positive			Gram-negative		
Standard Drug	*S. aureus*	*B.subtilus*	*P.aeruginosa*	*E.coli*	*A. niger*	*C. albicans*
Ciprofloxacin	28	26	23	25	26	24
Amoxicillin	25	23	21	24	19	-

Table 4: Anti-inflammatory activity

Compounds	Average Change in paw volume after 2hours (Mean± SEM)	% Inhibition of paw edema after 3 hours (Mean)	Average Change in paw volume after 5 hours (Mean ± SEM)	% Inhibition of paw edema after 5hours (Mean)
Control	0.75 ± 0.02	--	0.82 ± 0.02	--
01	0.71 ± 0.04**	12.11	0.76 ± 0.02**	13.43
02	0.56 ± 0.02*	33.17	0.80 ± 0.02*	41.53
03	0.69 ± 0.03*	13.19	0.72 ± 0.03*	22.40
04	0.63 ± 0.02*	48.55	0.76 ± 0.02*	29.81
-05	0.73 ± 0.02*	55.74	0.62 ± 0.02*	21.39
Indomethacin	.20 ± 0.03*	81.22	0.52 ± 0.03*	61.91
One way F	43.12		56.32	-----
ANOVA df P	11,81		34,15	
	<0.001		<0.001	

n =6 in each group.*P<0.001,**P<0.01 compared to control

The results were analyzed for statistical significance using one -way ANOVA followed by Dunnet's test

A P value < 0.05 was considered significant

Table 5: Analgesic Activity

		Post Drug reaction time in sec. (Mean ± SEM)			
Compound	Pre drug Reaction time in sec (Mean ± SEM)	30 Min. (Mean±SEM)	60 Min. (Mean±SEM)	90 Min. (Mean±SEM)	180 Min. (Mean±SEM)
Control	0.45 ± 0.02	--	0.71 ± 0.02	--	0.45 ± 0.02
01	0.38 ± 0.04**	17.11	0.66 ± 0.02**	12.43	0.38 ± 0.04**
02	0.36 ± 0.02*	23.17	0.70 ± 0.02*	31.53	0.36 ± 0.02*
03	0.41 ± 0.03*	33.19	0.63 ± 0.03*	12.40	0.41 ± 0.03*
04	0.43 ± 0.02*	38.55	0.56 ± 0.02*	34.81	0.43 ± 0.02*
05	0.40 ± 0.02*	45.74	0.62 ± 0.02*	21.39	0.40 ± 0.02*
Tramadol HCl	.40 ± 0.03*	65.22	0.60 ± 0.03*	61.91	.40 ± 0.03*
One -way F	33.19		46.26	-----	33.19
ANOVA df	16,31		41,52		16,31
P	<0.001		<0.001		<0.001

n= 6 in each group.*P<0.001,**P<0.05 compared to control

The results were analyzed for statistical significance using one -way ANOVA followed by Dunnet's test. A P value < 0.05 was considered significant

Table 6: Fluorescence analysis of powder

Drug	Short ultra violet (256 nm)	Long ultra violet (365 nm)	Visible ultraviolet (400 -800nm)
Powder	pink	Black	red
Powder + Water	Emerald Green	red	Green
Powder + Conc. HCl	Dark Green	red	Dark Green
Powder + Conc. H_2SO_4	Green	Black	Black
Powder + Conc. HNO_3	pink	Black	Orange
Powder + NaOH	red	Black	red
Powder +Methanol	pink	Black	Green
Powder + Acetone	yellow	Black	Green
Powder + NaOH (0.1 N)	pink	Black	pink
Powder + (0.1M) HCl	yellow	Black	red

wells in the respective agar plates already seeded with the standardized inoculum (5×10^6 cfu/ml) of the test microbial strain. All the test plates were incubated at 37°C for 24 hrs.

Anti-Inflammatory Activity [6]: Healthy inbred Wister albino rats of either sex, (150-180 g) were selected and housed in polypropylene cages at a well-ventilated, temperature-controlled (30±1°C) animal room with food and water *ad libitum*. Animals were periodically weighed before and after experiments. Animals were divided in four groups of six animals each. The control group receives vehicle orally, while other groups received either test drug or standard drug. The animals were treated with drugs by oral route and subsequently one hour after treatment, 0.1ml of 1% suspension of carageenan in normal saline was injected to the sub planter region of left hind paw to induce edema. The paw volume was measured initially at 1, 3 and 5 hours after carageenan injection using plathismometer. The difference between the initial and subsequent reading gave the actual edema volume which was compared with control. The difference of average values between treated animals and control group is calculated for each time interval and statistically evaluated. The percent inhibition is calculated using the formula as follows- % edema inhibition = $[1-(Vt/Vc)^*] 100$. Vt and Vc are edema volume in the drug treated and control groups respectively (Table 4).

Analgesic Activity [6]: Analgesic activity was measured by tail flick method using the radiant type analgesiometer. Basal reaction times to radiant heat were taken by placing the tip of the tail on the radiant heat source. Swiss albino mice (25-30 g) of either sex were divided into different groups (control, test and standard) containing six animals each. For each animal, the tail flick reaction time was obtained thrice before drug administration and mean was used as pre drug reaction time. After the administration of drug, the tail flick reaction times were measured at 30, 60, 90 and 180 minutes. The test and standard drug were intraperitoneally given, while the control group received only vehicle. The animals were administered a 30 mg/kg (body weight) dose of the test drugs and 22.8 mg/kg (body weight) dose of standard drug (tramadol HCl) (Table 5).

Statistical Analysis: The data were analyzed by one-way ANOVA. According to this test, there was a significant difference between the drug treated groups and control at the level of $P<0.05$. Results, expressed as Mean ± SEM were evaluated using the t-test. Values of $P < 0.05$ were considered statistically significant.

CONCLUSION

From our investigation, for screening combined crude ethanolic extract of *Datura stramonium (leaves), Terminalia Arjuna (bark), Withania somnifera (root)* the results obtained confirmed therapeutic potency of some plants used in traditional medicine. The plants could be potential source of new antimicrobial agent. Polyherbal formulation possesses potent anti-inflammatory activity as it inhibits maximum edema at 5 hrs, which was comparable to that of standard Indomethacin. Since, serotonin, histamine and prostaglandins are the major mediators of inflammation, anti inflammatory effect of polyherbal formulation could be due to inhibition of either their synthesis or release possibly due to inhibition of the enzyme cycloxygenase leading to inhibition of prostaglandin synthesis at third stage of inflammation. Based on the results of the present study, it can be concluded that polyherbal formulation showed signification anti inflammatory activity and analgesic activity.

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