Original Article

Effect of time on extraction of Ashwagandha in various Hydroalcoholic compositions and their anti-inflammatory activity

Anupama Singh, Vikas Anand Saharan[1], Rajesh Garg[2], V.B. Gupta[2]
Department of Pharmacognosy, Jodhpur National University, Boranada, Jodhpur, Rajasthan, [1]Seth G. L. Bihani S. D. College of Technical Education, Sri Ganganagar, Rajasthan, [2]Department of Pharmacognosy, B. R. Nahata College of Pharmacy, Mhow-Neemuch Road, Mandsaur, Madhya Pradesh, India

Ashwagandha, is a plant of Solanaceae or nightshade family which have botanical name *Withania somnifera.* It is mainly cultivated in Madhya Pradesh, Rajasthan, Uttar Pradesh and many other states of India. Among all states Madhya Pradesh (Neemuch-Mandsaur region) is the major producer of Ashwagandha. Major phytoconstituents are withanolides and alkaloids. These phytoconstituents have many pharmacological activities such as anti-cancer, diuretic, immunomodulatory, anti-inflammatory, anti-stress. The yield of active constituents may vary with time, temperature, number of extractions, drug and solvent ratio. Extracts were prepared in different water and alcohol compositions at different time intervals. These prepared extracts were chromatographed and number of phytoconstituents was present. Some of the extracts were used for performing anti-inflammatory activity. The activity was performed by carrageenan-induced paw oedema method in rats. A few extracts were found effective reducing the oedema. Extract obtained at 15 h was found superior in anti-inflammatory activity which is proposed due to some additional phytoconstituents extracted at that point of time.

Key words: Anti-inflammatory activity, Ashwagandha, Time

INTRODUCTION

Withania somnifera, also known as Ashwagandha, is a plant in Solanaceae or nightshade family. Indian medicinal plant *Withania somnifera* have been used in the Ayurvedic system of medicine and have anti-arthritic, anti-bacterial, anti-oxidant, anti-diabetic, anti-tumour,[1-6] anti-inflammatory activity,[7] immunomodulatory activity[8] and analgesic activity,[9] anti-sertogenic activity, anabolic activity and anti-stress activity.[10] It also possesses adaptogenic, cardiotropic, cardioprotective and anticoagulant properties.[11] Many withanolides have been isolated from *Withania somnifera* which have important role in treatment of various disorders. Withaferin A and 3-b-hydroxy-2, 3- dihydrowithanolide F have shown promising antibacterial, anti-tumour, immunomodulating and anti-inflammatory properties.[12] The aerial parts contain 5-dehydroxywithanolide-R and withasomniferin-A.[13] Withacoagin, coagulin, and withasomidienone together with other withanolides and Withaferin-A showed significant anti-inflammatory activity.[14]

Various factors affects yield of active constituents such as time, temperature, number of extractions, drug and solvent ratio. But till date no such study on effect of factors has been reported with extracts of Ashwagandha. The present study aimed to prepare extracts of Ashwagandha at different time durations with diferent water and alcohol compositions, collected from Neemuch, Mandsaur region, Madhya Pradesh, to perform anti-inflammatory activity.

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MATERIALS AND METHODS

Drug and its Authentification

Ashwagandha roots were collected from Manasa Madhya Pradesh in the month of March. The crude drug was authenticated at KNK College of horticulture, Mandsaur Madhya Pradesh and assigned the voucher number BRNCP/W/003/2007/Ashwagandha.

Extraction

Drug was extracted using water and alcohol in different compositions and at different time intervals using soxhletion. The powdered drug was weighed and filled in the soxhlet apparatus. The soxhlet was fitted to the round bottom flask, and the assembly was attached to the condenser. The paraffin wax was used to seal the

Address for correspondence: Mrs. Anupama Singh, Department of Pharmacognosy, Jodhpur National University, Boranada, Jodhpur, Rajasthan, India. E-mail: anupama.cognosy@gmail.com
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assembly joints. The solvent (100% water, 75% water, 50% water, 25% water, 100% alcohol) for extraction was filled in round bottom flask and extraction was carried out at different time intervals and 40 extracts were prepared. After completion of the extraction procedure the extract was taken out and its amount in percentage was determined. On the basis of extracted amount and solvent used for extraction eight extracts were further selected from these 40 extracts for anti-inflammatory activity.

Different extracts taken are as follows:

- A:100% Water::0% Alcohol (15 hours)
- B:100% Water::0% Alcohol (24 hours)
- C:75% Water::25% Alcohol (15 hours)
- D:75% Water::25% Alcohol (24 hours)
- E:50% Water::50% Alcohol (15 hours)
- F:50% Water::50% Alcohol (24 hours)

Table 1: Extraction yield at different time intervals

Time (in hours)	Amount extracted (%, w/w)				
	A (%)	B (%)	C (%)	D (%)	E (%)
03	18.38	11.04	19.55	17.44	5.48
06	20.46	18.36	25.02	23.55	14.76
09	21.31	28.43	32.96	23.70	14.84
12	22.57	30.84	26.78	27.61	16.00
15	27.08	42.00	32.00	37.32	17.12
18	14.22	16.60	21.79	23.64	20.84
21	11.32	25.85	18.17	22.20	20.68
24	29.47	28.91	23.07	25.88	13.52

Amount extracted shown in the table represents the mean values of three different extracts A: 100% water; B: 75% water and 25% alcohol; C: 50% water and 50% alcohol; D: 25% water and 75% alcohol; E: 100% alcohol

Table 2: Comparative R_f values of extracts in water at different time intervals

Solvent system	Extraction time (in hours)	No. of spots	R_f values			
			I	II	III	IV
Acetonitrile:water (75:25)	03	4	0.18	0.28	0.44	0.70
	06	3	0.30	0.46	0.75	–
	09	4	0.36	0.48	0.63	0.81
	12	3	0.18	0.41	0.69	–
	15	2	0.67	0.76	–	–
	18	2	0.70	0.80	–	–
	21	1	0.83	–	–	–
	24	3	0.53	0.71	0.80	–
Toluene:ethyl-acetate:acetic acid (65:33:2)	03	0	–	–	–	–
	06	0	–	–	–	–
	09	1	0.35	-	-	-
	12	0	–	–	–	–
	15	0	–	–	–	–
	18	0	–	–	–	–
	21	0	–	–	–	–
	24	0	–	–	–	–

Table 3: Comparative R_f values of extracts in hydroalcoholic compositions (75% water:25% alcohol) at different time intervals

Solvent system	Extraction time (in hours)	No. of spots	R_f values				
			I	II	III	IV	V
Acetonitrile:water (75:25)	03	3	0.37	0.71	0.81	–	–
	06	3	0.37	0.68	0.81	–	–
	09	3	0.47	0.74	0.84	–	–
	12	3	0.45	0.75	0.84	–	–
	15	4	0.31	0.42	0.66	0.82	–
	18	3	0.33	0.65	0.80	–	–
	21	2	0.57	0.88	–	–	–
	24	3	0.55	0.72	0.86	–	–
Toluene:ethyl-acetate:acetic acid (65:33:2)	03	4	0.26	0.36	0.63	0.88	–
	06	4	0.25	0.37	0.68	0.86	–
	09	4	0.28	0.39	0.73	0.87	–
	12	4	0.28	0.48	0.75	0.89	–
	15	4	0.29	0.47	0.72	0.86	–
	18	4	0.30	0.48	0.74	0.85	–
	21	5	0.27	0.39	0.53	0.68	0.86
	24	5	0.27	0.40	0.55	0.73	0.90

Table 4: Comparative R_f values of extracts in hydroalcoholic compositions (50% water:50% alcohol) at different time intervals

Solvent system	Extraction time (in hours)	No. of spots	R_f values			
			I	II	III	IV
Acetonitrile:water (75:25)	03	4	0.25	0.58	0.77	0.85
	06	4	0.21	0.58	0.78	0.86
	09	3	0.40	0.66	0.82	–
	12	4	0.39	0.54	0.74	0.87
	15	4	0.41	0.54	0.75	0.89
	18	4	0.31	O.48	0.62	0.75
	21	4	0.31	0.45	0.68	0.81
	24	2	0.73	0.84	–	–
Toluene:ethyl-acetate:acetic acid (65:33:2)	03	3	0.26	0.47	0.86	–
	06	3	0.27	0.46	0.86	–
	09	3	0.20	0.51	0.94	–
	12	3	0.18	0.55	0.94	–
	15	3	0.61	0.80	0.95	–
	18	4	0.25	0.38	0.64	0.90
	21	4	0.24	0.37	0.63	0.89
	24	3	0.21	0.40	0.88	–

Table 5: Comparative R_f values of extracts in hydroalcoholic compositions (25% water:75% alcohol) at different time intervals

Solvent system	Extraction time (in hours)	No. of spots	R_f values					
			I	II	III	IV	V	VI
Acetonitrile:water (75:25)	03	3	0.24	0.52	0.83	–	–	–
	06	3	0.28	0.58	0.87	–	–	–
	09	3	0.27	0.47	0.83	–	–	–
	12	6	0.25	0.35	0.46	0.58	0.75	0.85
	15	5	0.24	0.34	0.58	0.75	0.85	–
	18	3	0.19	0.43	0.75	–	–	–
	21	3	0.19	0.40	0.74	–	–	–
	24	3	0.18	0.40	0.74	–	–	–
Toluene:Ethyl-acetate:Acetic acid (65:33:2)	03	0	–	–	–	–	–	––
	06	0	–	–	–	–	–	–
	09	0	–	–	–	–	–	–
	12	0	–	–	–	–	–	–
	15	0	–	–	–	–	–	
	18	6	0.26	0.34	0.45	0.52	0.78	0.87
	21	6	0.26	0.35	0.45	0.51	0.80	0.88
	24	6	0.26	0.35	0.45	0.51	0.80	0.88

- G:25% Water::75%Alcohol (15 hours)
- H:25% Water::75%Alcohol (24 hours)

Chromatographic Profile of Different Extracts

Thin layer chromatography (TLC) was performed to identify the different constituents present in different extracts. Different solvent systems such as acetonitrile: water (75:25) and toluene: ethyl-acetate: acetic acid (65:33:2). Iodine chamber was used as a detecting reagent for prepared TLCs.

Anti-inflammatory Activity

Both male and female albino rats weighing, 100-200 g, were divided into 11 different consisting of five rats in each group (one standard, two control groups (simple control and model control) and eight test groups). All the rats were kept for overnight fasting. Simple control received the vehicle (2%, w/w gum acacia dispersion in distilled water) and model control received the carrageenan (1%, w/w dispersion in distilled water). Test groups received carrageenan (1%, w/w dispersion in distilled water) and the extract (1 g/kg of body weight) and standard (10 mg/kg of body weight) group was administered indomethacin. The carrageenan (0.05 ml of 1%, w/v concentration) was administered by subcutaneous injection into the plantar side of the left hind paw. Carrageenan-induced inflammation in half an hour. Extracts were administered orally to test groups. The left hind paw was marked with ink at the level of the lateral malleolus and immersed

Table 6: Comparative R_f values of extracts in alcohol at different time intervals

Solvent system	Extraction time (in hours)	No. of spots	R_f values			
			I	II	III	IV
Acetonitrile:water (75:25)	03	3	0.21	0.28	0.51	–
	06	3	0.20	0.29	0.53	–
	09	4	0.16	0.42	0.62	0.75
	12	4	0.16	0.40	0.62	0.75
	15	3	0.22	0.56	0.85	–
	18	3	0.23	0.55	0.83	–
	21	4	0.16	0.40	0.61	0.74
	24	4	0.18	0.41	0.60	0.73
Toluene:ethyl acetate:acetic acid (65:33:2)	03	4	0.21	0.71	0.82	0.94
	06	4	0.21	0.71	0.82	0.94
	09	3	0.20	0.41	0.68	–
	12	3	0.21	0.66	0.71	–
	15	4	0.24	0.71	0.78	0.87
	18	4	0.23	0.69	0.76	0.85
	21	4	0.23	0.74	0.82	0.96
	24	4	0.23	0.73	0.83	0.95

in mercury up to this mark. The paw volume was measured with plethysmometer immediately after oral administration of extracts, 3 and 6 and 24 h from oral administration.[15]

RESULTS AND DISCUSSION

Extraction

Ashwagandha was extracted using different solvent compositions at different time intervals. Extracts were dried and results in (%, w/w) are presented in Table 1. On the basis of extracted amount and solvent (cost factor) used for extraction, eight extracts were further selected from those 40 extracts. As presented in Table 1 extracted amount increased up to 15th h. Further it decreased after 15th h than again it increased at 24th h. The fluctuation was caused in extracted amount, the decrease in overall extractive was probably due to degradation and increase at 24 h was anticipated as a result of appearance of plant parts in the extract or appearance of any other constituent. Therefore, selected extracts were subjected to chemoprofiling and bioprofiling.

Chromatographic Profile of Different Extracts

Thin layer chromatography (TLC) was performed to identify the different constituents present in different extracts. Results are shown in Tables 2–6 and Figure 1. Phytoconstituents having R_f values 0.18, 0.24, 0.35, 0.40, 0.47, 0.55, 0.61, 0.72, 0.73, 0.80, 0.88, were present in the solvent system (toluene: ethyl-acetate: acetic acid:65:33:2).[16]

Anti-inflammatory Activity

The animal studies were conducted as per the approved protocol of institutional animal ethical committee of B. R. Nahata College of Pharmacy, Mandsaur, Madhya Pradesh, India.

Table 7: Anti-inflammatory activity

Groups	Time (in hours)		
	0	3	6
Control	0.42±0.04	0.45±0.03	0.43±0.02
Model control	0.75±0.07	0.77±0.01	0.67±0.03
A	0.76±0.02	0.71±0.00	0.69±0.01
B	0.77±0.02	0.70±0.01	0.66±0.01
C	0.77±0.01	0.68±0.01**	0.65±0.01*
D	0.79±0.00	0.67±0.09**	0.67±0.01
E	0.78±0.10	0.72±0.03	0.68±0.01*
F	0.75±0.02	0.70±0.01	0.64±0.04
G	0.78±0.01	0.67±0.07**	0.46±0.08***
H	0.76±0.02	0.66±0.01***	0.67±0.02
Standard	0.76±0.01	0.68±0.00*	0.41±0.00***

*P<0.05 Significant w.r.t model control of same group; **P<0.01 Significant w.r.t model control of same group; ***P<0.001 Significant w.r.t model control of same group

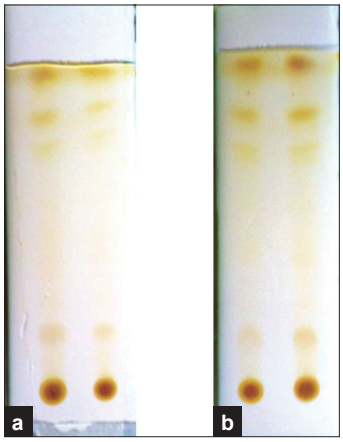

Figure 1: Chromatographic profile in (a) acetonitrile: water (75:25); (b) toluene: ethylacetate: acetic acid (65:33:2) of 50% alcoholic extract

It was performed by carragenean induced paw oedema method results are shown in Figures 2-4 and Table 7. When the selected extracts were administered to test groups

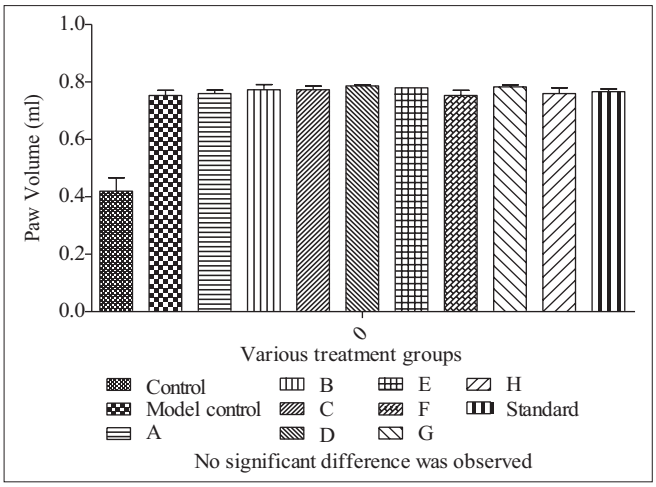

Figure 2: Paw volume of control, model control and treated rats at 0 h after carragenan administration

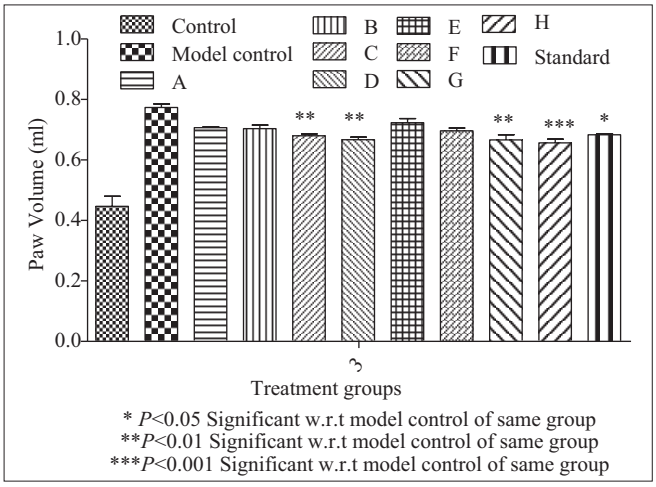

Figure 3: Paw volume of control, model control and treated rats at 3 h after carragenan administration

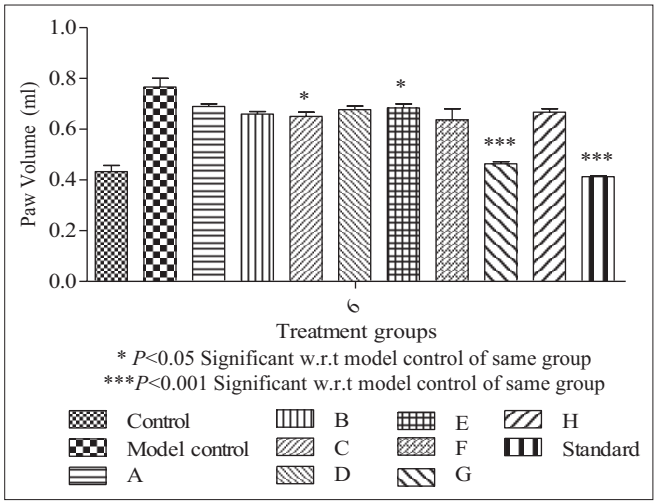

Figure 4: Paw volume of control, model control and treated rats at 6 h after carragenan administration

significance difference in inflamed paw volume was observed in all the test groups but the level of significance varied among different groups. At 0 h, no significant difference in the paw volume was observed of the inflamed rats, yet the groups C, D and G showed significant difference (P<0.01) with respect to (w.r.t) model control and standard while H showed significant difference (P<0.001) w.r.t model control after 3 h of treatment. When the treatment was continued to 6 h, showed significant decrease of inflammation in groups C and E rats (P<0.05) was observed and in group G rats significance difference (P<0.001) w.r.t model control.[17]

CONCLUSION

The present study provides an insight that how phytoconstituents in plants may vary with geographical regions, solvents, methods of extraction and time duration of extraction. TLCs performed showed presence of 4-5 different phytoconstituents in different extracts.

Further, animal studies revealed that sample C, D, E, G and H were effective in controlling inflammation. However, extracts C, D, G and H showed anti-inflammatory activity after 3 h and extract C, E and G showed anti-inflammatory activity after 6 hr. Considering the economic point of view (because less alcohol is being consumed in the process), extractive amount (42%, w/w) and animal activity extract C is more recommended.

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