Extraction of Ashwagandha by conventional extraction methods and evaluation of its anti-stress activity

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The present study was conducted to compare the yield and the antistress activity of Ashwagandha (*Withania somnifera*) extracts, using two extraction methods: hot continuous percolation and maceration. Various parameters like temperature, extraction time (10 hours) solvents (water, alcohol, hydroalcohol) and drug-solvent ratios (1:6, 1:8, 1:10) were fixed. The highest yield was found to be 16.96% w/w by maceration process using water (1:8). The activity of different extracts was done by Plus-Maze model using alprazolam as the standard drug. Significant result was found in water extract and hydroalcoholic (1:8) extract prepared by maceration method and also in hydro-alcoholic extract (1:8) prepared by soxhlet process.

Key words: Alprazolam, anti-stress activity, elevated plus maze, extraction, *withania somnifera*

INTRODUCTION

Extraction may be defined as the process of removal of desirable soluble constituents from a substance, leaving out those which are not required with the aid of solvents.[1] There are various method of extraction *i.e.,* maceration, hot continuous extraction, percolation, decoction, ultrasound extraction, supercritical fluid extraction, microwave-assisted extraction, etc.[2] Stress is defined as a state of affair involving demand on physical or mental energy. Stress is a psychological and physiological response to events that upset our personal balance in some way.[3] *Withania somnifera* (Solanaceae, part used root), popularly known as Ashwagandha or Winter Cherry[4] is a well-known anti-stress drug. It is a green shrub found throughout the drier parts of India, Pakistan, Afghanistan, Sri Lanka, Congo, South Africa and Egypt. In India, it is widely grown in Madhya Pradesh, Uttar Pradesh, Punjab, Gujarat and Rajasthan.[5] It mainly contains withanolides, glycowithanolides and withaferine.[6] In this regard, our aim was to identify a suitable extraction method for ashwagandha and to correlate the extraction method with its bioactivity.

MATERIALS AND METHODS

Extraction by Maceration and Hot Continuous Percolation Process

100 g of *W. somnifera* dried roots were exhaustively extracted with various solvents (alcohol, water, hydro alcohol (50:50)), using different drugs – solvent ratios (1:6, 1:8, 1:10) by hot continuous percolation (10 hours) and maceration methods (10 hours). The extracts were dried and the percentage yields of extracts were determined. Preliminary phytochemical test was carried out to identify the nature of phytoconstituents present in the extracts.[7,8]

Pharmacological Screening

Acute toxicity studies

Acute toxicity study was done according to OECD (organisation for economic cooperation and development) guideline no. 420.[9] Female Albino mice (20–25 g) were used for this study. After the sighting study, starting dose of each extract was given to five animals, which were observed for 14 days for any behavioral change and death.

Anti-stress Activity

Animals

Mice (20–25 g) were selected and housed under standard laboratory condition for a period of seven days prior to the experiment. Experimental protocols were approved by our Institutional animal ethical committee, which follows guidelines of CPCSEA/ IAEC reg. no. 918/ac/05/ CPCSEA.

Anti-stress activity was assessed by elevated plus maze method. Mice (20–25g) were selected, weighed and divided into various groups of five animals each. Group 1 was kept as normal control, received only vehicle, Group 2 was standard control, treated with 1

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mg/kg of Alprazolam and remaining groups were treated orally with various extracts prepared using maceration and hot continuous percolation methods. After 30 minutes, the animals were placed in the centre of the maze, head facing towards open arm. Parameters like first preference of mouse towards open or closed arm were noted and number of entries in open and closed arm for 5 minutes was recorded.[10]

Statistical Analysis

Analysis was done using one-way analysis of variance (ANOVA). *P* value less than 0.05 was considered as statistically significant. Results are presented as mean ± standard errors (SE).

RESULTS AND DISCUSSION

Percentage Yield

Percentage yield of different extracts by soxhlet and maceration was given in Tables 1 and 2. Comparatively the percentage yield of extracts obtained by maceration method was higher than that of the extracts prepared by soxhlet method. The extract prepared using water as the solvent showed higher yield in both methods when compared to other two solvents, alcohol and hydro alcohol (50:50). Highest yield was found in the extract prepared by maceration method using 1:8 drug-solvent ratios.

Acute Toxicity Study

The acute toxicity study showed that all the extracts of *W. somnifera* were safe up to 2,000 mg/kg body weight. Therefore, 2,000 mg/kg dose was considered as a safe dose for the extracts of *W. somnifera***.**

Anti-stress Activity

The alcoholic extracts prepared using both the methods at 100 and 200 mg/kg did not produce any significant increase in the duration of stay in the open arm, when compared to the vehicle control group. The reference drug alprazolam (1.0 mg/kg po), water extract by maceration (1:8, 200 mg/kg p.o.), Hydro alcoholic extract by Soxhlet (1:8, 100 mg/kg po) and Hydro alcoholic extract by maceration (1:8, 200 mg/kg po) significantly increased duration of stay in the open arms, indicating anti-stress activity. The results are given in Tables 3-5.

Withanolides are the main active constituents reported to produce antistress activity. Comparatively the activity was found better in extracts prepared by maceration method. This indicates that the extracts prepared by maceration technique contain more amounts of withanolides, particularly when using water as the solvent.

Table 1: Percentage yield of different extracts by soxhlet (% w/w)

Ratios	A	B	C
1:6	5.06	3.48	9.33
1:8	11.88	7.52	10.44
1:10	9.83	5.16	12.30

A - Water, B - Alcohol and C - Hydroalcohol

Table 2: Percentage yield of different extracts by maceration (% w/w)

Ratios	A	B	C
1:6	14.08	4.80	12.08
1:8	16.96	3.48	12.60
1:10	16.18	6.62	15.08

A - Water, B - Alcohol and C - Hydroalcohol

Table 3: Anti-stress activity of water extracts

Groups	Dose (Mg/kg)	Stay in open arm (Mean±SEM)	Stay in closed arm (Mean±SEM)
Normal control	–	58.33±1.66	241.67±1.66
Standard control	1 mg/kg	133.33±16.66*	59.00±30.23**
Water S (1:6)	100 mg/kg	176.00±1.11	126.00±1.15
Water S (1:8)	100 mg/kg	160.33±51.46	139.67±51.46
Water S (1:10)	100 mg/kg	179.00±76.64	121.00±76.64
Water S (1:6)	200 mg/kg	171.67±8.81	123.33±8.81
Water S (1:8)	200 mg/kg	98.33±37.12	139.67±51.46
Water S (1:10)	200 mg/kg	176.00±50.34	162.33±36.68
Water M (1:6)	100 mg/kg	17.66±5.00	282.33±5.04
Water M (1:8)	100 mg/kg	251.67±28.91	201.67±37.12
Water M (1:10)	100 mg/kg	30.66±14.07	269.33±14.07
Water M (1:6)	200 mg/kg	59.33±8.24	24.67±4.63
Water M (1:8)	200 mg/kg	201.00±55.10*	65.00±32.53**
Water M (1:10)	200 mg/kg	42.00 ±16.16	258.00 ±16.16

ANNOVA followed by Dunnet test, Value are Mean + SE (n = 5); significance *vs.* control group: ***P* < 0.01, **P* < 0.05, S - Soxhlet and M – Maceration.

Table 4: Anti-stress activity of alcoholic extracts

Groups	Dose (Mg/kg)	Stay in open arm (Mean±SEM)	Stay in closed arm (Mean±SEM)
Normal control	–	58.33±1.66	241.67 ±1.66
Standard control	1 mg/kg	133.33±16.66*	59.00±30.23**
Alcohol S (1:6)	100 mg/kg	85.000±7.683	141.67±68.57
Alcohol S (1:8)	100 mg/kg	32.66±2.33	267.00±2.33
Alcohol S (1:10)	100 mg/kg	91.66±7.83	101.00±56.29
Alcohol S (1:6)	200 mg/kg	32.00±5.68	266.00±3.78
Alcohol S (1:8)	200 mg/kg	23.66±6.55	267.33±2.33
Alcohol S (1:10)	200 mg/kg	57.33±23.05	242.67±23.03
Alcohol M (1:6)	100 mg/kg	80.00±15.50	213.00±11.17
Alcohol M (1:8)	100 mg/kg	81.00±18.90	219.00±18.90
Alcohol M (1:10)	100 mg/kg	87.66±27.16	212.3±27.16
Alcohol M (1:6)	200 mg/kg	87.00±13.65	213.00±31.19
Alcohol M (1:8)	200 mg/kg	117.67±14.25	182.33±14.25
Alcohol M (1:10)	200 mg/kg	175.00±31.19	125.00±31.19

ANNOVA followed by Dunnet test, Value are mean ± SE (n = 5); significance *vs.* control group: ***P* < 0.01, **P* < 0.05, S - Soxhlet and M – Maceration.

Table 5: Antistress activity of hydro-alcoholic extract

Group	Dose (mg/kg)	Stay in open arm (Mean±SEM)	Stay in closed arm (Mean±SEM)
(Normal)	-	58.33±1.66	241.67±1.66
(Standard)	1 mg/kg	133.33± 16.66*	59.00±30.23**
Hyd alc S. (1:6)	100 mg/kg	20.00±2.88	280.00±2.88
Hyd alc S. (1:8)	100 mg/kg	200.00±14.43*	150.33±76.85**
Hyd alc S. (1:10)	100 mg/kg	134.67±45.16	165.33±45.16
Hyd alc S. (1:6)	200 mg/kg	62.667±5.36	237.33±5.63
Hyd alc S. (1:8)	200 mg/kg	165.67±76.85	133.33±22.04
Hyd alc S. (1:10)	200 mg/kg	157.00±4.04	133.67±5.85
Hyd alc M. (1:6)	100 mg/kg	67.000±31.43	213 .00±11.17
Hyd alc M. (1:8)	100 mg/kg	152.67±16.54	147.33±76.54
Hyd alc M. (1:10)	100 mg/kg	89.33±30.86	210.67±30.86
Hyd alc M (1:6)	200 mg/kg	74.667±29.23	225.33±29.23
Hyd alc M (1:8)	200 mg/kg	259.00±14.22*	41.00±14.22**
Hyd alc M (1:10)	200 mg/kg	107.67±49.34	196.33±47.36

ANNOVA followed by Dunnet test, Value are Mean + SE (n = 5); significance vs. control group: $^{**}P < 0.01$, $^{*}P < 0.05$, S - Soxhlet and M - Maceration

From this study, it was concluded that the maceration method, using water as the solvent, for Ashwagandha *(Withania Somnifera)* was found to be the better technology than hot continuous percolation process. The percentage yields and the antistress activity was found better in hydro alcoholic and aqueous extracts in maceration technology than the hot continuous percolation process.

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