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Development of Extraction Protocol for Ashwagandha and Evaluation of its Anti - Stress Activity

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ABSTRACT: The aim of my present study is to compare the percentage yield and antistress activity of different extracts of ashwagandha which are prepared by two methods like microwave assisted extraction and ultrasonication. Some parameters were fixed like solvents (alcohol, water, hydro alcohol) and Drug solvent Ratio (1:6, 1:8, 1:10). The antistress activity of different extracts of ashwagandha was carried out by using elevated plus maze model using alprazolam as a standard drug. The highest percentage yield was found in solvent water in the ratio of (1:10) by microwave method and antistress activity was found significantly in Water in ratio (1:8). However, the microwave assisted extraction technique is better than the ultrasonication.

Keywords: Microwave assisted Extraction, Ultrasonication, Ashwagandha, Antistress activity, Elevated Plus Maze model.

I. INTRODUCTION:

Plants have always been an exemplary source of medicine and far of the currently available drugs are derived directly or indirectly from them. Thanks to economic constraints, providing modern medical healthcare in developing countries like India remains a far - reaching goal.[1,2] Screening the crude plant extracts for the required activity is among the foremost important operations in medicinal plant research, and extraction is that the primary crucial step of the tactic.[3] For the identification of bioactivity of crude extracts, it becomes necessary to optimize the extraction methodology, so on achieve maximum possible extraction efficiency. Obtaining better quality and high efficiency of extraction from herbs being significant, one possesses to optimize the extraction methods for better extraction efficiency. [4,5] With the increasing demand for herbal medicinal products, herbal manufacturers aim at using the foremost appropriate extraction technologies to supply extracts of defined quality.[6] Quality of an extract is influenced by several factors like, plant parts used as starting material, solvent used for extraction, extraction procedure, and plant material: solvent ratio, etc.[7]Withania somnifera are alsocalled Ashwagandha or Winter Cherry which is having anti-stress Property. It is cultivated throughout the drier parts of India, Pakistan, Afghanistan, Sri Lanka, Congo, South Africa and Egypt. In India, it is mainly grown in Madhya Pradesh, Uttar Pradesh, Punjab, Gujarat and Rajasthan.It mainly contains withanolides, glycowithanolides and withaferine.[8-10] Stress is defined as any sort of physical, emotional, or psychological change. it's your body's response to anything that needs attention or action. The events which increase stress are referred to as stressors.[11]

II. MATERIALS AND METHODS:

Drug Selection and Collection:

The plant has been selected on the basis of reason like the traditional use of this plant as anti - stress agent, Availability of plant material and its wide geographical distribution globally, Economic plant.The plant was collected From Unijules Life Sciences Ltd. Company, Nagpur.

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Ashwagandha Root

Extraction by microwave method:
10g. of Withania somnifera dried roots were exhaustively extracted with various solvent (alcohol, water, hydro - alcohol (50:50) and using different drug - solvent ratios (1 : 6 , 1 : 8 and 1:10) using microwave assisted extraction for 1 -2 min. and the micro-power is used for extraction is 800 MHz. The extracts were evaporated above their boiling points. Finally, the % yields were calculated of the dried extracts. [12,13]

Extraction by ultrasonication method:
10g. of Withania somnifera dried roots were exhaustively extracted with various solvent (alcohol, water, hydro - alcohol (50:50) and using different drug - solvent ratios (1 : 6 , 1 : 8 and 1:10)using ultrasonication for Ihr . and the temperature used for extraction is 60^0 C. The extracts were evaporated above their boiling points. Finally, the % yields were calculated of the dried extracts.[14]

Dried Extracts

Chromatographic Analysis:
Thin layer chromatography (TLC) of different ashwagandha extracts was performed to identify the constituents which are present in different extracts. Different solvent systems such as acetonitrile: water (75:25), toluene: ethyl-acetate: acetic acid (65:33:2) and Chloroform: Methanol (90:10). The detecting chamber used for detection was Iodine chamber.[15,16]

TLC Performed

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Pharmacological Screening

Acute Toxicity Study:

Acute toxicity study was done according to OECD (Organization for Economic Cooperation and Development) Guidelines on female mice (20-25gm). Animals were acclimatized to laboratory condition for five days prior to the experiment. Body weight of animals were recorded and individual identification was done, fixed dose method as in Annex 2d: Test procedure with a starting dose of 2000 mg / Kg Body weight for withania somnifera was adopted. Starting dose of extract was given to 5 animals following p.o. route, and animals were observed for behavioural changes and death. No animals were found dead after 14 days. [17]

Anti - stress activity

Animals

Mice (20-25g.) were selected and housed under standard laboratory condition for a period of 7 days prior to the experiment. Experimental protocols were approved by our Institutional animal ethical committee, which follows the guidelines of CPCSEA IAEC (Committee for the purpose of Control and Supervision of Experiments on Animals / Institutional Animal Ethics Committee), Reg. No. 918 / ac / 05 / CPCSEA. Anti-stress activity was determined by elevated plus maze method.[18,19] Mice (20–25g) were selected, weighed and divided into four groups of five animals each. **Group 1** was kept as normal control, received drinking water, **Group 2** was standard control, treated with 1 mg/kg of Alprazolam and remaining groups were treated orally with various extracts prepared using microwave and ultrasonication methods. After half-hour, the animals were kept 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 five minutes was recorded.[20]

Elevated Plus Maze

Statistical analysis

Standard evaluation was done using one - way analysis of variance (ANOVA) Statistical significance was set at $P < 0.0001$. Results are presented as mean + standard errors (S.E.).

III. RESULTS AND DISCUSSIONS:

Percentage Yield of Extracts:

The percentage yield of different extracts which are extracted by microwave and ultrasonication in three different ratios by using three different solvent which were described in table 1 and table 2. The highest yield was found in the extract which is prepared by the microwave method (1:10) drug solvent ratio.

Table 1 Percentage Yield of different extract by microwave (%w/w)

S.No.	Ratios	A	B	C
1	1:6	11.4	3.3	7.9
2	1:8	15.8	3.7	11.08
3	1:10	19.8	4.3	11.70

A- Water, B- Alcohol, C- Hydroalcohol

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Table 2 Percentage Yield of different extract by ultrasonication (%w/w)

S.No.	Ratios	A	B	C
1	1:6	15.08	3.48	11.88
2	1:8	16.18	4.80	9.83
3	1:10	11.88	6.62	12.30

A- Water, B- Alcohol, C- Hydroalcohol

Chromatographic Analysis:

Thin layer chromatography (TLC) was performed on different extracts of ashwagandha to identify the different constituents which are present in the extracts. The results are shown in table 3 and table 4.

Table 3 TLC of Microwave Extract

S.No.	Extracts	Toluene:Ethyl acetate: acetic acid (65:33:2)	Acetonitrile:Water (75:25)	Chloroform:Methanol (90:10)
1	Water (1:6)	No Spot	No Spot	0.54
2	Water (1:8)	No Spot	0.45	0.47
3	Water (1:10)	No Spot	0.67	0.51
4	Alcohol (1:6)	0.50 & 0.76	0.76	0.46
5	Alcohol (1:8)	0.50 & 0.74	0.76	0.52
6	Alcohol (1:10)	0.40 & 0.80	0.64	0.64
7	Hydro Alcohol (1:6)	No Spot	0.64 & 0.77	0.61
8	Hydro Alcohol (1:8)	0.64	0.64 & 0.77	0.65
9	Hydro Alcohol (1:10)	0.46 & 0.66	0.73 & 0.76	0.67 & 0.80

Table 4 TLC of Ultrasonic Extract

S.No.	Extracts	Toluene:Ethyl acetate: acetic acid (65:33:2)	Acetonitrile:Water (75:25)	Chloroform:Methanol (90:10)
1	Water (1:6)	No Spot	0.42	0.49
2	Water (1:8)	No Spot	0.44	0.49
3	Water (1:10)	No Spot	0.42	0.53
4	Alcohol (1:6)	0.50 & 0.67	0.84	0.50
5	Alcohol (1:8)	0.46 & 0.69	0.83	0.47
6	Alcohol (1:10)	0.46 & 0.71	0.84	0.56
7	Hydro Alcohol (1:6)	0.61 & 0.78	0.71	0.25 & 0.88
8	Hydro Alcohol (1:8)	0.59 & 0.74	0.53 & 0.71	0.24, 0.58 & 0.79
9	Hydro Alcohol (1:10)	0.55 & 0.74	0.19 & 0.69	0.26, 0.58 & 0.79

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Acute toxicity study:

The acute toxicity study showed that the aqueous, alcoholic and hydro alcoholic extracts of Withania somnifera was safe up to 2000 mg/kg body weight. Therefore 2000mg/kg dose was considered as a safe dose for withania somnifera. 1/10th of this safe dose as selected for all in vivo experiments as maximal dose.

Anti-Stress Activity:

The reference drug alprazolam (1.0 mg/kg po), water extract by microwave (1:8, 200 mg/ kg p.o.), alcoholic extract by ultrasonication (1:10, 100 mg/ kg po) significantly increased duration of stay in the open arms, indicating anti-stress activity. The results are shown in Tables 5 & 6. Withanolides are the main active constituents which is responsible to produce antistress activity. Comparatively the activity was found best in extracts prepared by microwave method.

Table 5 Anti stress activity of Microwave Extracts

Groups	Dose	Stay in open arm (Mean±SEM)	Stay in closed arm (Mean±SEM)
Normal Control	-	58.333 ± 1.667	241.67 ± 1.667
Standard	1mg/kg	133.33 ± 16.667 *	59.000 ± 30.238*
Water (1:6)	100mg/kg	50.000 ±2.887	133.33 ± 22.048
Water (1:8)	100mg/kg	197.67 ± 12.46**	102.33 ± 12.468
Water (1:10)	100mg/kg	133.33 ± 22.048*	133.00 ± 17.578*
Water (1:6)	200mg/kg	166.67 ± 22.048**	250.00 ± 2.887**
Water (1:8)	200mg/kg	256.33 ± 4.096**	43.667 ± 4.096**
Water (1:10)	200mg/kg	157.00 ±21.656**	133.00 ± 17.578**
Alcohol (1:6)	100mg/kg	140.67 ± 29.672*	159.33 ± 29.672*
Alcohol (1:8)	100mg/kg	211.33 ± 5.783**	122.00 ± 29.816**
Alcohol (1:10)	100mg/kg	147.33 ±3.180*	152.67 ±3.180*
Alcohol (1:6)	200mg/kg	75.000 ± 5.774	245.00 ± 5.774
Alcohol (1:8)	200mg/kg	198.67± 2.404**	101.00 ± 2.082**
Alcohol (1:10)	200mg/kg	176.67 ± 8.819**	123.33 ± 8.169**
Hydroalcohol (1:6)	100mg/kg	143.33 ± 4.410*	156.67 ± 4.410*
Hydroalcohol (1:8)	100mg/kg	199.33 ± 2.333**	100.67 ± 2.333**
Hydroalcohol (1:10)	100mg/kg	121.00 ± 51.160	179.00 ± 51.160
Hydroalcohol (1:6)	200mg/kg	217.33 ± 10.745**	82.667 ± 10.745**
Hydroalcohol (1:8)	200mg/kg	227.00 ± 5.774**	73.000 ± 5.774**
Hydroalcohol (1:10)	200mg/kg	78.000 ± 6.429	222.00 ± 6.429

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

Table 6 Anti stress activity of Ultrasonic Extracts

Groups	Dose	Stay in open arm (Mean±SEM)	Stay in closed arm (Mean±SEM)
Normal Control	-	58.333 ± 1.667	241.67 ± 1.667
Standard	1mg/kg	133.33 ± 16.667 *	59.000 ± 30.238*
Water (1:6)	100mg/kg	159.00 ±17.231	210.33 ±17.231
Water (1:8)	100mg/kg	197.67 ± 46.090**	141.00 ± 46.090**
Water (1:10)	100mg/kg	117.67 ± 5.364	101.00 ± 5.364
Water (1:6)	200mg/kg	92.000 ± 4.163	208.00 ± 4.163
Water (1:8)	200mg/kg	156.67 ± 19.099**	143.33 ± 19.099**
Water (1:10)	200mg/kg	137.00 ± 3.512**	156.33 ± 8.762**
Alcohol (1:6)	100mg/kg	172.67 ± 3.712**	126.00 ± 3.055**
Alcohol (1:8)	100mg/kg	198.33 ± 1.667**	101.67 ± 1.667**
Alcohol (1:10)	100mg/kg	244.67 ± 3.930**	55.333 ± 3.930**
Alcohol (1:6)	200mg/kg	137.00 ± 3.512**	156.33 ± 8.762**

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Alcohol (1:8)	200mg/kg	80.000 ± 5.774	220.00 ± 5.774
Alcohol (1:10)	200mg/kg	209.33 ± 4.702**	91.000 ± 0 4.583
Hydroalcohol (1:6)	100mg/kg	155.33 ± 10.924 *	144.00 ± 10.263*
Hydroalcohol (1:8)	100mg/kg	230.00 ± 35.119**	700.000 ± 35.119**
Hydroalcohol (1:10)	100mg/kg	32.667 ± 6.489	261.33 ± 6.489
Hydroalcohol (1:6)	200mg/kg	68.000 ± 19.079	232.00 ± 19.079
Hydroalcohol (1:8)	200mg/kg	51.667 ± 4.910	248.33 ± 4.290
Hydroalcohol (1:10)	200mg/kg	50.000 ± 5.774	250.00 ± 5.774

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

IV. CONCLUSION

From this study, it was concluded that the highest yield was found in microwave assisted extraction in solvent water and ratio 1:10. was 19.8 % which is shown in table 1 and the anti-stress activities was found significantly in microwave assisted extraction method in water 1:8 ratio is 256.33 + 4.096 due to the presence of withanolides in highest concentration which is shown in table 3. Finally, we found that the microwave assisted extraction method is the best method from all these two methods like ultrasonic and microwave extraction methods.

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CONFLICT OF INTEREST - NIL

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