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Phyto-Pharmacognostic Experimental Explication of Herbs like Tribulus Terrestris and Withania Somnifera Demonstrating Therapeutic Efficacy against Hypogonadism

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ABSTRACT

The pharmacognostic research on Tribulus terrestris and Withania somnifera is the subject of this publication. Physical-chemical characteristics including ash and extractive values as well as fluorescence analysis have been carried out. The various extracts have also undergone preliminary phytochemical investigation and thin layer chromatographic behaviour. According to studies, Withania somnifera and Tribulus terrestris have anti-inflammatory, anticancer, antiparkinsonian, adaptogen, memory-enhancing, antioxidant, and anxiolytic effects. There have also been studies on a number of additional effects, including immunomodulation, hypolipidemia, antimicrobial, cardiovascular protection, sexual behaviour, tolerance, and dependence. This plant should be further researched in order to confirm these findings and elucidate further possible therapeutic properties, according to these very positive results. There should also be clinical studies employing ashwagandha and Tribulus terrestris to treat a range of ailments.

Keywords: Tribulus terrestris; Withania somnifera; Testosterone; Adaptogen; Phytochemical screening; Physicochemical analysis

INTRODUCTION

Tropical regions around the world are home to Tribulus terrestris. It is primarily found in Australia, America, and India. Withania somnifera is referred to as "Indian Winter cherry" or "Indian Ginseng" in Ayurveda, whereas Tribulus terrestris is known as Chhota Gokhru. It has been used for millennia as a Rasayana for its extensive range of health effects, making it one of the most significant herbs in Ayurveda (India's traditional medical system) [1]. The Tribulus terrestris plant, often known as "Puncture Vine," has long been used around the world to treat a variety of illnesses, and its fruits have gained widespread popularity as having the ability to treat human sexual dysfunction. It has long been used to address issues with libido and infertility [2]. Withania somnifera, commonly referred to as ashwagandha, Indian ginseng, or winter cherry, has been a crucial herb in the Ayurvedic and indigenous medical systems for more than three thousand years. The plant's roots are classified as rasayanas, which are believed to enhance immunity to disease, stop the ageing process, revitalise the body in weakened states, increase a person's ability to withstand harmful environmental elements, and foster mental wellbeing. It is in use for a very long time for all age groups and both sexes and even during pregnancy without any side effects [3]. Historically, Tribulus terrestris and Ashwagandha [4,5] plant has been used for various disease [6] like as an anthelminthic [7, 8], antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, astringent and more recently to treat ulcers, bacterial infection, venom toxins and senile dementia. The decoction of the root boiled with milk and ghee is recommended for curing sterility in women [9]. The roots are also used in constipation, senile debility, rheumatism, general debility, nervous exhaustion, loss of memory, loss of muscular energy and spermatorrhoea [10]. Withanolides that contribute to most of the biological activity of Withania somnifera [11]. Numerous groups of chemical components found in the leaves, including steroidal lactones, alkaloids, and tannin, have been researched in depth with regard to this plant's chemistry [12]. From aerial portions, more than 12 alkaloids, including 40 withanolids with a glucose molecule at carbon, have been extracted and described. The primary chemical components of these plants, withanolids, are widely distributed in the leaves and typically range in concentration from 0.001 to 0.5 % dry weight [13]. The use of Withania somnifera for anxiety, neurological diseases, inflammation, hyperlipidaemia, and Parkinson's disease is supported by clinical trials and animal research. Because of its ability to prevent chemotherapy, Withania Somnifera may be a helpful supplement for people receiving radiation and chemotherapy. Withania somnifera has recently been used to prevent continuous use of certain psychotropic medicines from leading to the development of tolerance and dependency. As a result, Tribulus terrestris and Withania somnifera have been the subject of a thorough pharmacognostic study.

MATERIALS AND METHODS

Collection of the plant and Authentication

In the month of November 2022, fresh Tribulus terrestris fruit was collected from the herbal garden of Vishveswaraya College of Pharmacy Dadri in the G. B. Nagar District and Withania somnifera root was collected from the Gurukul Kangri University Haridwar, India. Professor Dr. R. K. Shukla of Gurukul Kangri Vishwavidyalaya in Haridwar, India, recognised the specimens.

Standardization of raw material

The Organoleptic evaluation and determination of Foreign organic matter of raw materials were carried out as per the Ayurvedic Pharmacopoeia of India [14].

Physicochemical studies

The ash values, extractive values and loss on drying were performed according to the official methods prescribed in Ayurvedic Pharmacopoeia of India [14].

Preliminary Phyto-chemical screening

Tribulus terrestris (Seed and leaves) and Withania somnifera (Root) extracts, each weighing one gramme, were dissolved in 100 ml of their respective mother solvents to produce a stock containing 1% w/v of the substance. This stock was then tested for the presence of alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, carbohydrate and phenolic compounds, and saponin [15].

Fluorescence evaluation

Under natural and ultraviolet light, the crude medication was examined for any fluorescence. In order to study samples, they were treated with 50% HCl and 50% NaOH before and after, and the findings were tabulated [16].

Studies on safety profiles

The safety profile parameters like heavy metal analysis, pesticide residual analysis and microbial load analysis were studied according to the official methods prescribed in Ayurvedic Pharmacopoeia of India.

Quantitative estimation of heavy metals

Quantitative estimation of heavy metals was done for the detection of arsenic, lead, cadmium and mercury as per the Ayurvedic pharmacopoeia procedures [17].

Quantitative estimation of pesticide residues

Quantitative estimation of pesticide was done for the detection of Organochlorine compound, Organophosphorus compound and Carbamates as per the Ayurvedic pharmacopoeia procedures [18].

Microbial load analysis

Escherichia coli, Salmonellae, Pseudomonas Staphylococcus, and Shigella were tested as well as the total aerobic viable count, yeasts, and moulds to ensure that the raw material for the Bi-herbal capsules was safe to use [19].

PREPARATION OF EXTRACT

The materials from the chosen plants were sun-dried and kept in an airtight container. Each substance was then coarsely ground and extracted with hydro-alcoholic (30:70) using the Soxhlet device. The produced hydro-alcohol extracts were concentrated using a rotary vacuum evaporator at a temperature of 40^{o}C while under vacuum (removal of alcohol). At -20^{o}C, the concentrated extracts were freeze dried. The powders were kept in the desiccator until further use in an airtight container.

FORMULA OF MIXED HERBAL FORMULATION

The herbal formulation contained the hydro-alcoholic extracts of Tribulus terrestris (Seed and leaves) and Withania somnifera (root) in the ratio of 1:1.

PREPARATION OF FORMULATION BY WET GRANULATION METHOD

Trials were conducted to prepare the formulation by choosing the quantity of lubricants and preservatives and adding different ratios of binders before the process was finally optimised. Withania somnifera and Tribulus terrestris extracts were powdered (sieve 40), blended in a 1:1 ratio, and used to make capsules using the wet granulation method with 5% starch paste as a binder. To obtain granules, the moist bulk was run through sieve number 22. In a tray, the granules were dried at 45°C [20].

PRE-FORMULATION STUDIES

The obtained herbal granules were subjected to pre-formulation characteristics such as bulk density, tap density, compressibility index, Hausner's

ratio, and angle of repose, and the best trial batch was chosen for capsule filling and additional research [21,22].

Standardization of herbal formulation

Capsule evaluation: The herbal capsules were assessed in accordance with Indian pharmacopoeia standards for their description, average weight, weight variation, moisture content, disintegration time, pH, and microbiological load [23]. Phyto-constituent quantitative estimation and preliminary screening of Phyto constituents were also conducted.

Average weight: Twenty capsules were individually weighed and the average weight of the capsule was calculated.

Weight variation: The individual weights of each capsule should be within the limits of 90% and 110% of the average weight.

Moisture content: Moisture content was determined by using automatic Karl Fischer titration apparatus.

Disintegration time: Disintegration testing was done utilising a digital microprocessor-based disintegration test device. Individually tube received a disc and a capsule, which were each added. In a 1000 ml beaker, the assembly was submerged in water. The volume of water at its highest position was at least 25 mm below the water's surface, and at its lowest point, it was at least 25 mm above the beaker's bottom. A temperature of 37°C with an accuracy of 2°C was used to run and maintain the device.

pH value: pH of 1% solution was determined by using a digital pH meter.

Phytochemical screening

Standard methods of phytochemical screening, such as the mayers, dragendroffs, and borntragers tests, were used to conduct the preliminary phytochemical investigation of the ethanolic areal components extract. The alkaline test, the lead acetate test, the foam test, and the lead acetate test [24- 26]. Therefore, in the current investigation, preliminary phytochemical analysis, thin layer chromatographic studies, extractive values, weight loss on drying, moisture content, total ash, acid insoluble ash, water soluble ash, residue on ignition, and fluorescence analysis have all been performed on the two medicinal plants. Fruit and root samples, as well as their extracts in various solvents, were subjected to fluorescence examination. The Pharmacopoeia of India's procedures were used to determine the ash levels and extractive values of the fruit and root samples [27]. The powdered air-dried fruit and root samples were subjected to a series of extractions using Petroleum ether (60-800C), benzene, chloroform, ethanol, and water. The extracts were then utilised to conduct phytochemical testing.

Fluorescence analysis

Under both conventional and UV light, the crude medication was examined for any colour changes. Samples were examined in the same manner after being treated with a mixture of 50% HCl and 50% NaOH, and the findings were tabulated. The samples were submitted to fluorescence examination using 365 nm light (UV region).

Quantitative estimation of phytoconstituents

The Bi-herbal formulation's total alkaloids, phenolic compounds, flavonoids, and tannin content were estimated [28].

Microbial load analysis

The total aerobic viable count, yeasts, and moulds, as well as the bacteria Escherichia coli, Salmonellae, Pseudomonas Staphylococcus, and Shigella, were measured to ensure that the raw material for the Bi-herbal capsules could be used safely.

RESULTS

The standardisation process, which guarantees the formulation's quality, safety, and reproducibility, is its most crucial component. It involves the entire bio-prospecting process, from the gathering of raw materials through the creation of the finished product. To substitute the conventional liquid dose form in the current trial, a standardised bi-herbal mixture was created in hard gelatine capsules. Two ingredients make up this Bi-herbal mixture, and they come from two different families, morphological plant sections, and phytoconstituents.

The petroleum ether and benzene extract fluorescence were seen in the long-UV region. Under UV light, Tribulus terrestris aqueous and ethanol extracts exhibit yellowish brown fluorescence. The crude medicines exhibit brown fluorescence when exposed to UV light (365 nm), and brown fluorescence when treated with 1N NaOH and 1N HCl and in benzene and ethanol glow Red and yellow in the long-UV region, while extracts in petroleum ether fluoresce red orange. The sample, weight loss during drying ranged from 03 to 07%. Mixture has the highest physicochemical characteristics, such as ash levels (16.57-6.03 percent). These medications have acid-insoluble ash contents of less than 1.61%, water-soluble ash contents of less than 15%, and residual on ignition contents of less than 7.43%. As the solvent's polarity rises, the extractive values rise as well. The water extract value is higher than the other extractive values. Commonly, the extracts from both samples demonstrate the presence of saponins, reducing sugars, triterpenoids, steroids, tannins, and alkaloids in the preliminary physiochemical study of crude pharmaceuticals. Flavonoids are present in the petroleum ether and chloroform extract of sample. The thin layer chromatographic behaviour of the numerous plant extracts used in the current experiment yields some incredibly intriguing findings. The maximum spots in the ethyl acetate: benzene (1:9) solvent system may be seen in the benzene extracts of sample. All of the pharmacognostic characteristics can be tested for adulteration, if any, and utilised as a diagnostic tool for accurately identifying the drug.

Fluorescence Analysis of Raw Materials (Tables 1-5)

The fluorescence analysis of raw materials was carried out and the results were recorded and detailed in Table 1.

Table 1: Fluorescence analysis.

Sample	Light	Before Treatment	1N NaOH	1N HCl	1:1 H_2SO_4	1:1 HNO_3	Name of the extract				
							Ether	Benzene	CCl_4	Ethanol	Water
Tribulus Terrestris	Ordinary	Yellowish brown	Dark Yellow	Brown	Dark brown	Dark yellow	Dark yellow	Dark brown	Yellowish black	Yellowish green	Dark yellow
	Long-UV (366 nm)	Green	Dark green	Dark green	Black	Black	Red	Red Orange	Orange	Yellowish blue	Greenish yellow
	Short-UV (254 nm)	Yellowish Green	Green	Green	Yellowish black	Dark green	Dark yellow	Yellowish green	Yellowish green	Yellowish green	Yellowish green
Withania Somnifera	Ordinary	Crimson to dark brown	Light brown	Brown	Dark brown	Orange yellow	Dark yellow	Dark brown	Yellowish brown	Light brown	Yellowish brown
	Long-UV (366 nm)	Light Brown	Light brown	Dark brown	Black	Fluorescent green	Light brown	Red orange	Green	Yellow	Light Brown
	Short-UV (254 nm)	Green	Dull Brown	Brown	Greenish black	Black	Dark yellow	Yellowish green	Yellowish brown	Yellow	Yellowish brown

Physicochemical Parameters

For the herbal medications included in the bi-herbal formulation, a number of physicochemical properties were computed. The report of several physicochemical characteristics is shown in Table 2.

Table 2: Physicochemical parameters.

Particulars	Tribulus Terrestris	Withania Somnifera
Loss of weight on drying	3.63%	6.23
Moisture content	10.68%	2.05%
Total ash	16.57%	6.03%
Water soluble ash	15.00%	1.48%
Acid-insoluble ash	1.61%	0.34%
Residue on ignition	7.43%	4.24%

Table 3: Extractive values.

Solvents	Tribulus terrestris	Withania Somnifera
Petroleum ether (60-800C)	4.18%	4.25%
Benzene	4.66%	4.70%
Chloroform	5.50%	5.18%
Ethanol	6.46%	8.05%
Water	10.52%	22.46%

Table 4: Thin layer chromatographic behaviour of the fruit of Tribulus terrestris in Ethyl acetate: Benzene (1:9) Solvent System.

Name of the Extract	Rf value under UV light		Rf value in iodine chamber
	Long – UV 365nm	Short – UV 254nm	
Petroleum ether (60 – 800C)	*0.65	--	*0.65, @ 0.52
Benzene	@0.55, @ 0.48, * 0.89	--	*0.55, @ 0.48, * 0.79, *0.84, * 0.89,
Chloroform	--	--	*0.90, @ 0.57
Ethanol	@ 0.74	*0.62	@0.62, * 0.74, *0.84,
Water	*0.83	*0.83	*0.83, * 0.52

Table 5: Thin layer chromatographic behaviour of the fruit of Withania somnifera in Ethyl acetate: Benzene (1:9) Solvent System.

Name of the Extract	Rf value under UV light		Rf value in iodine chamber
	Long – UV 365nm	Short – UV 254nm	
Petroleum ether (60 – 800C)	*0.60	--	*0.75, @0.67, *0.82
Benzene	*0.70, @ 0.42, * 0.90	--	@0.68, @ 0.48, * 0.81, *0.80, * 0.90, * 0.92
Chloroform	--	--	*0.90, @ 0.60
Ethanol	@ 0.78	*0.68	*0.84, @ 0.80, *0.85, *0.90
Water	*0.64	*0.73	*0.65, @ 0.98
@-Less intence *-more intense			

Phytochemical analysis

The chemical tests for various Phyto constituents in the raw materials were carried out and the results were recorded and detailed in Table 6a. (Tables 6-8).

Table 6a: A Preliminary phytochemical screening of Tribulus terrestris.

Extracts	Steroids	Triterpenoids	Reducing sugars	Alkaloids	Saponins	Tannins	Flavonoids
Pet. ether	+	+	+	+	+	+	+
Benzene	+	+	+	-	+	+	-
Chloroform	+	+	+	+	+	+	+
Ethanol	+	+	+	+	+	-	+
Water	-	-	+	-	+	+	-

Table 6b: Preliminary phytochemical screening of *Withania somnifera.*

Extracts	Steroids	Triterpenoids	Reducing sugars	Alkaloids	Saponins	Tannins	Flavonoids
Pet. ether	+	+	+	+	+	+	-
Benzene	+	+	+	+	-	+	-
Chloroform	+	+	+	-	+	+	-
Ethanol	-	-	+	+	-	-	+
Water	-	-	+	+	+	-	+

Safety Profile Parameters Studies

Heavy metal analysis Quantitative estimation of heavy metals Quantitative estimation of heavy metals in the raw materials was carried out and the results were recorded and detailed in Table 6.

Table 7: Test for heavy metals.

OBSERVATION (in ppm/ml)				
Plant name	**Arsenic (NMT 5)**	**Lead (NMT 10)**	**Cadmium (NMT 0.3)**	**Mercury (NMT 0.5)**
Tribulus terrestris	0.005	0.077	0.005	0.006
W. somnifera	0.008	0.081	0.025	0.004

Table 8: Microbial load analyses.

Parameters	**Tribulus terrestris**	**W. somnifera**
Total aerobic count (NMT 1000 cfu/g)	650cfu/g	650cfu/g
Yeast and mould count (NMT 100 cfu/g)	NIL	NIL
E. coli (To be absent)	absent	absent
Salmonella (To be absent)	absent	absent
Pseudomonas (To be absent)	absent	absent
Staphylococcus (To be absent)	absent	absent
Shigella (to be absent)	absent	absent

Table 9: Evaluation of capsules.

Parameter	**Observation**
Average weight	574.31 ±4.5mg
Weight variation	Within I.P. Limit
Moister content (LOD)	2.51±0.1 %w/w
Disintegration time	10.9±0.5(min)
pH (1% aqueous solution)	5.52± 0.68

Table 10: Fluorescence Analysis of Bi-herbal Capsule.

Sample	**Before treatment**			**After treating with 50 % HCl**			**After treating with 50% NaOH**		
	Ordinary light	**Short UV**	**Long UV**	**Ordinary light**	**Short UV**	**Long UV**	**Ordinary light**	**Short UV**	**Long UV**
Bi-herbal formulation	Greenish brown	Green	Green	Green	Greenish brown	Brown	Greenish yellow	Greenish yellow	Dark brown

DISCUSSION

The standardisation process, which guarantees the formulation's quality, safety, and reproducibility, is its most crucial element. From procuring raw materials to creating the completed product, it covers every stage of the bio-prospecting process. In the current study, hard gelatine capsules were filled with a normal bi-herbal mixture. There are only two components in this bi-herbal mixture, and they come from two different families, morphological plant parts, and phytoconstituents. Herbal preparations are widely eaten by people without a prescription because they are historically thought to be harmless. Yet some of them can harm your health, others don't work, and some might interact with other medicines. In order to evaluate the quality and purity of medicines based on the concentration of their active components; standardisation of herbal formulations is crucial [29]. Standardization is a crucial tool for determining the quality, purity, and identification of samples. For the materials to be correctly identified, it is crucial. An essential factor in the quality assessment of herbal medicines is the ash value. A high ash value suggests adulteration, contamination, substitution, or negligence in the preparation of the medicine or drug combination for marketing. The portion of the total ash content that is soluble in water is known as water-soluble ash. It is a reliable sign of either improper preparation or earlier extraction of the drug's water-soluble components. As a result, it is the weight difference between total ash and the residue that results from treating total ash with water. In plant medications, high moisture leads to hydrolysis of components, bacterial and fungal development, and biochemical reactions. The pharmacopoeial monographs require water content limits, especially for medications with hygroscopic natures or for which an excessive amount of water results in product deterioration [30]. In plant medications, high moisture leads to hydrolysis of components, bacterial and fungal development, and biochemical reactions. . The pharmacopoeial monographs require water content limits, notably for medications with hygroscopic natures or for which too much water results in deteriorated goods. A formulation with less moisture can be anticipated to be secure for a longer period of time.

CONCLUSION

The diagnostic traits developed from this work will be helpful in the accurate identification of the crude drug derived from Tribulus terrestris (Seed) and Withania somnifera (Root) and they will also aid in quality assurance of it. A wide variety of therapeutically significant phytochemical groups have been found in medicinal herbs, supporting their traditional applications for a number of medical problems, such as spermatorrhoea and low libido power by increasing testosterone levels. Additionally, aqueous and alcoholic extracts demonstrated the highest extractive values for both plant sections, indicating that hydro-alcoholic extract can be exploited in the search for novel bioactive chemicals and in the study of their biological activity. Tribulus terrestris (Seed) and Withania somnifera (Root) chosen extract fractions may be further studied scientifically to generate novel drugs and establish this important plant as a potential source of phytomedicines. The Indian Ayurvedic Pharmacopoeia is used to standardise the job.

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