Section A-Research paper

PHYTO-PHARMACOGNOSTIC EXPERIMENTAL EXPLICATION OF HERBS LIKE TRIBULUS TERRESTRIS AND WITHANIA SOMNIFERA DEMONSTRATING THERAPEUTIC EFFICACY AGAINST HYPOGONADISM

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ABSTRACT:

This article discusses the pharmacognostic study on Tribulus terrestris and Withania somnifera. Fluorescence analysis and physical-chemical properties, such as ash and extractive values, have been performed. Additionally, preliminary phytochemical analysis and thin layer chromatographic behavior have been performed on the different extracts. Withania somnifera and Tribulus terrestris have been shown in research to have anti-inflammatory, anti-cancer, anti-parkinsonian, adaptogen, memory-enhancing, antioxidant, and anxiolytic properties. This article discusses the pharmacognostic study on Tribulus terrestris and Withania somnifera. Fluorescence analysis and physical-chemical properties, such as ash and extractive values, have been performed. Additionally, preliminary phytochemical analysis and thin layer chromatographic behavior have been performed on the different extracts. Withania somnifera and Tribulus terrestris have been shown in research to have anti-inflammatory, anti-cancer, anti-parkinsonian, adaptogen, memory-enhancing, antioxidant, and anxiolytic properties. Numerous other effects, such as immunomodulation, hypolipidemia, antibacterial, cardiovascular protection, sexual behavior, tolerance, and dependence have also been studied. These highly encouraging results call for further investigation of this plant to confirm these findings and clarify additional potential medicinal characteristics. Ashwagandha and Tribulus terrestris should be used in clinical research to treat a variety of diseases.

KEYWORDS: Tribulus terrestris, Withania somnifera, testosterone, adaptogen, phytochemical screening, physicochemical analysis.

INTRODUCTION:

Tribulus terrestris is a tropical plant that may be found all over the world. Australia, the United States, and India are its main habitats. In Ayurveda, Withania somnifera and Tribulus terrestris are known as "Indian Winter cherry" and "Indian Ginseng," respectively. It is one of the most important herbs in Ayurveda (India's traditional medical system), having been used for millennia as a Rasayana for its wide variety of health effects [1]. The Tribulus terrestris plant, also referred to as "Puncture Vine," has long been used to treat a number of diseases around the world, and its fruits are now widely believed to be able to treat human sexual dysfunction. It has been used for a long time to treat libido and reproductive problems [2]. For more than three thousand years, the Ayurvedic and indigenous medical systems have used withania somnifera, also known as ashwagandha, Indian ginseng, or winter cherry. The plant's roots are known as rasayanas, which are said to improve immunity against disease, slow down the ageing process, revitalize the body in weaker conditions, boost resistance to hazardous environmental components, and promote mental wellbeing. There is a use for it [3]. Tribulus terrestris and Ashwagandha [4,5] are plants that have historically been used to treat a variety of diseases [6] such as anthelminthic [7, 8], antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, and astringent, as well as more recently to treat ulcers, bacterial infection, venom toxins, and senile dementia. For the treatment of female infertility, the decoction of the root that has been boiled in milk and ghee is advised [9]. Additionally, the roots are used to treat spermatorrhoea, senile debility, rheumatism, general debility, nervous weariness, memory loss, and rheumatism [10]. Withanolides that contribute to most of the biological activity of Withania somnifera [11]. This plant's chemistry has been extensively studied in relation to a variety of classes of chemical constituents present in the leaves, including as steroidal lactones, alkaloids, and tannin [12]. More than 12 alkaloids have been isolated and characterized from aerial parts, including 40 withanolids with a glucose molecule at carbon. These plants' main chemical constituents, withanolids, are extensively dispersed in the leaves and typically have concentrations between 0.001 and 0.5 percent dry weight [13]. Clinical studies and animal studies support the use of Withania somnifera for anxiety, neurological disorders, inflammation, hyperlipidemia, and Parkinson's disease. Withania Somnifera may be a beneficial supplement for persons experiencing radiation and chemotherapy due to its capacity to avoid chemotherapy. Recently, withania somnifera has been utilized to prevent tolerance and dependency from forming from long-term usage of several psychotropic medications. As a result, much pharmacognostic research has been done on Tribulus terrestris and Withania somnifera.

MATERIALS & METHODS:

A. Plant collection and authentication

Fresh Tribulus terrestris fruit and Withania somnifera root were procured in November 2022 from the herbal garden of the Gurukul Kangri University in Haridwar, India, and the Vishveswaraya College of Pharmacy in Dadri, G. B. Nagar District, respectively. The specimens were recognised by Professor Dr. R. K. Shukla of Gurukul Kangri Vishwavidyalaya in Haridwar, India.

B. Uniformity of raw materials

The Ayurvedic Pharmacopoeia of India was followed for the organoleptic evaluation and determination of Foreign Organic Matter of Raw Materials [14].

C. Physicochemical studies

The Ayurvedic Pharmacopoeia of India's approved procedures was followed to perform the ash values, extractive values, and loss on drying test.

D. **Preliminary Phyto-chemical screening**

One gm. of each of the extracts from Tribulus terrestris (Seed) and Withania somnifera (Root) were dissolved in 100 ml of their respective mother solvents to create a stock that contained 1 percent by weight of the material. The presence of alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, carbohydrate and phenolic compounds, and saponin were then determined in this stock [15].

E. Fluorescence evaluation

The unfinished medicine was checked for fluorescence under both natural and UV light. Samples were treated with 50% HCl and 50% NaOH both before and after the investigation, and the results were tabulated [16].

F. Research into safety profiles

According to the official procedures outlined in the Indian Ayurvedic Pharmacopoeia, the safety profile characteristics such as heavy metal analysis, pesticide residual analysis, and microbiological load analysis were investigated.

G. Heavy metals' quantitative estimation

According to the Ayurvedic pharmacopoeia methods, quantitative heavy metal estimate was carried out for the detection of arsenic, lead, cadmium, and mercury [17].

H. Calculating the amount of pesticide residues

According to the Ayurvedic pharmacopoeia protocols, quantitative pesticide assessment was carried out for the detection of organochlorine compound, organophosphorus compound, and carbamates [18].

I. Microbial load analysis

To guarantee that the raw material for the Bi-herbal capsules was safe to use, Escherichia coli, Salmonellae, Pseudomonas Staphylococcus, and Shigella were examined along with the total aerobic viable count, yeasts, and moulds [19].

PREPARATION OF EXTRACT

The components from the selected plants were dried in the sun and stored in an airtight container. Then, using the Soxhlet apparatus, each material was coarsely powdered before being extracted with hydro-alcoholic (30:70). The generated hydro-alcohol extracts were concentrated in a rotating vacuum evaporator under vacuum at a temperature of 40^{o}C (removal of alcohol). The concentrated extracts were freeze dried at -20 ^{o}C. The powders were stored in an airtight container in the desiccator until further usage.

FORMULA OF MIXED HERBAL FORMULATION

The hydro-alcoholic extracts of Tribulus terrestris (Seed and leaves) and Withania somnifera (Root) were present in the herbal formulation in a 1:1 ratio.

FORMULA PREPARATION USING THE WET GRANULATION METHOD

Before the procedure was completely optimised, tests were done to produce the formulation by selecting the quantity of lubricants and preservatives and adding various ratios of binders. Wet granulation was utilised to create capsules utilising the extracts of Withania somnifera and Tribulus terrestris in a 1:1 ratio, with 5% starch paste serving as a binder. The moist bulk was sent through sieve number 22 to produce granules. The granules were dried at 45 °C in a tray [20].

PRE-FORMULATION STUDIES

The resulting herbal granules were tested for pre-formulation qualities like bulk density, tap density, compressibility index, Hausner's ratio, and angle of repose, and the best trial batch was chosen for capsule filling and further investigation [21, 22].

A. Standardization of herbal formulation

1. **Capsule evaluation:** The herbal capsules were evaluated for their description, average weight, weight variation, moisture content, disintegration time, pH, and microbiological load in accordance with Indian pharmacopoeia requirements [23]. Additionally, quantitative estimation of phytoconstituents and a preliminary screening of phytoconstituents were carried out.
2. **Average weight:** Each of the twenty capsules was weighed separately, and the average weight of the capsule was determined.
3. **Weight variation:** Each capsule's unique weight should fall between 90 and 110 percent of the average weight.
4. **Moisture content:** Karl Fischer titration equipment that is automatic was used to calculate moisture content.
5. **Disintegration time:** A digital microprocessor-based disintegration test device was used for the disintegration testing. A disc and a capsule were inserted to each tube individually. The assembly was placed in a 1000 ml beaker of water. At its highest point, the volume of water was at least 25 mm below the water's surface, and at its lowest, it was at least 25 mm above the bottom of the beaker. The apparatus was operated and maintained at a temperature of 37°C with a 2°C precision.
6. **pH value:** A digital pH metre was used to determine the pH of a 1 percent solution.

B. Phytochemical screening: The preliminary phytochemical analysis of the ethanolic areal components extract was carried out using traditional methods of phytochemical screening, such as the Mayers, dragendroffs, and borntragers tests. The lead acetate test, the foam test, the lead acetate test, and the alkaline test [24, 25, 26]. 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 carried out on the two medicinal plants in the current investigation. Fluorescence analysis was done on samples of fruits and roots as well as their extracts in different solvents. The ash content and extractive values of the fruit and root sample were determined using the methods outlined in the Pharmacopoeia of India [27]. A series of extractions were performed on the powdered, air-dried fruit and root samples using Petroleum ether (60-800°C), benzene, chloroform, ethanol, and water. Testing for phytochemicals was then done using the extracts.

C. Fluorescence analysis

We looked for any colour changes in the crude medicine under both conventional and UV light. Following treatment with a mixture of 50% HCl and 50% NaOH, samples were inspected in the same way, and the results were tabulated. Using 365 nm light, the samples were subjected to fluorescence analysis (UV region).

D. Quantitative estimation of phytoconstituents

Estimates were made of the amount of alkaloids, phenolic compounds, flavonoids, and tannin in the Bi-herbal formulation [28].

E. Microbial load analysis

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

RESULT:

In the long-UV range, fluorescence from petroleum ether and benzene extract could be visible. Tribulus terrestris ethanol and aqueous extracts glow a yellowish brown colour under UV light. The unprocessed medications glow brown when exposed to UV light (365 nm), and they glow brown when treated with 1N NaOH and 1N HCl as well as in benzene and ethanol. While extracts in petroleum ether glow red orange, colours in the long-UV region are red and yellow. The sample lost between 3 and 7 percent of its weight when drying. The highest physicochemical properties, such as ash levels, are present in the mixture (16.57-6.03 percent). These drugs have ash concentrations that are less than 1.61 percent acid-insoluble, less than 15 percent water-soluble and less than 7.43 percent residual on ignition. The extractive values increase together with the increasing polarity of the solvent. In comparison to the other extractive values, the water extract value is higher. In the initial physiochemical analysis of crude pharmaceuticals, the extracts from both samples typically show the presence of saponins, reducing sugars, triterpenoids, steroids, tannins, and alkaloids. The petroleum ether and chloroform extract of the sample contain flavonoids. Some really exciting results are obtained from the thin layer chromatographic behaviour of the various plant extracts used in the current experiment. The sample's benzene extracts may show the most spots in the ethyl acetate: benzene (1:9) solvent system. All of the pharmacognostic properties can be used as a diagnostic tool to precisely identify the medicine and checked for adulteration, if any.

A. Fluorescence Analysis of Raw Materials

Raw materials were subjected to a fluorescence analysis, and the outcomes were documented and described 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

B. Physicochemical Parameters

Numerous physicochemical parameters of the herbal drugs found in the bi-herbal formulation were calculated. Table 2 presents a summary of numerous physicochemical properties.

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 Tribulus terrestris fruit thin layer chromatographic behaviour in ethyl acetate: Benzene (1:9) system of solvents

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 intense *-more intense

C. Phytochemical Analysis

The raw materials underwent chemical testing for several phytoconstituents; the findings were documented and described in Table 6a.

Table 6.a Preliminary phytochemical screening of Tribulus terrestris

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

Table 6.b Preliminary phytochemical screening of Withania somnifera

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

D. Safety Profile Parameters Studies

Examination of heavy metal calculating the amount of heavy metals Heavy metals in the raw materials were quantitatively estimated, and the results are described 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 characteristics created as a result of this work will help in both the precise identification and quality control of the crude medication made from Tribulus terrestris (Seed) and Withania somnifera (Root). It has been discovered that medicinal herbs contain a wide range of therapeutically important phytochemical groups, supporting their traditional uses for a number of health issues such spermatorrhoea and poor libido power by raising testosterone levels. A hydro-alcoholic extract can be used in the search for novel bioactive compounds and in the research of their biological activity, as well as aqueous and alcoholic extracts showed the highest extractive values for both plant sections. The selected extract fractions from Tribulus terrestris (Seed) and Withania somnifera (Root) may be further researched scientifically to produce innovative pharmaceuticals and establish this significant plant as a potential source of phytomedicines. To standardise the work, reference is used to the Indian Ayurvedic Pharmacopoeia.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest regarding this investigation.

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