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RESEARCH ARTICLE

Evaluation of the *in vitro* phytochemical components of the commercialized traditional medication Trasina®

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ABSTRACT

Background: Plants are the rich sources of secondary metabolites such as alkaloids, phenols, flavonoids, tannins, saponins, glycosides, terpenoids etc. that possess a wide array of biological properties. Herbal formulation is the therapeutic medicine composed of medicinal plants having lots of phytochemical with pharmacological action. Quantitative analysis of these phyto-constituents is essential for the preparatory medicine. Trasina is a preparatory medicine extensively used to reduced stress and helpful to maintain body's immunity. To create polyherbal compounds, the active ingredients of plants must first be extracted using a suitable solvent, the solution must then be evaporated, and the residue must then be adjusted to a specific standard. The Indian herbs *Withania somnifera*, *Tinospora cordifolia*, *Eclipta alba*, *Ocimum sanctum*, and *Picrorrhiza kurroa*, which are excellent under stressful circumstances, are the major ingredients in Trasina®, which is marketed as a multi-herbal capsule. Disease-curing phytochemicals included in natural therapies include flavonoids, glucosinolates, saponins, amino acids, monoterpenes, and others. Targets and goals: The purpose of this investigation was to find out if Trasina® included any phytochemical components. **Methods:** The material was extracted using ethanol, methanol, and aqueous solutions. Several phytochemical components were screened using a recognized technique. **Results:** In our study the multi herbal formulation (Trasina®) contained various phytochemicals like saponins, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, sugars, anthraquinone, phenolic compounds, and glycosides. **Conclusion:** The phytochemical constituents of the material were found to include saponins, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, carbohydrates, anthraquinone, phenolic compounds, and glycosides. The pharmacopoeial limitations were met for analytical research parameters such loss on drying, total ash, acid-soluble ash, and pH. The presence of active components may have therapeutic qualities significant for pharmacological activity, according to preliminary phytochemical screening.

Keywords: Trasina®, Indian medicinal plants, Solvent extraction, Phytochemicals

INTRODUCTION

Each herbal material used to create ayurvedic and herbal medications possesses a unique chemical composition that, when combined, can have the desired effect. Ayurveda[1,2] is expanding quickly on the market as a result of rising demand for herbal remedies. Ayurvedic literature Sarangdhar Samhita established the idea of multi-herb for greater therapeutic efficiency. The multi-herbal mixture has been utilized all over the world for medical and therapeutic purposes.[3] It is also referred to as multi-herb therapy or herb-herb combination therapy. Individual plants' active phytochemical components are insufficient to produce the necessary therapeutic effect.[4] The therapeutic impact increases and negative effects reduce when numerous herbs are mixed in a careful ratio with herbal and herbal-mineral combinations.[5]

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Although many people believe that herbal remedies are safe, they are frequently combined and derived from plants, each of which has a distinct variety of species, growing environments, and biologically active components. A significant potential benefit over conventional single-component medications is the availability of many active components in herbal compounds, which when combined can provide boosting effects that might not be feasible with a single ingredient. Due to their many active components, the herbal pharmacological substances found in polyherbal formulations can generate synergistic, potentiating, agonistic, and antagonistic actions.[6]

The traditional Indian medicinal system of Ayurveda classifies some Indian plants as "Medhyarasayan," or "medicines that improve memory and intelligence." The main Indian herbs that go into the formulation of Trasina, which is advertised as a multi-herbal capsule, include *Withania somnifera*, *Tinospora cordifolia*, *Eclipta alba*, *Ocimum sanctum*, and *Picrorrhiza kurroa*, all of which are helpful in stressful conditions. According to a 1997 investigation by Bhattacharya and Kumar,[7] the dose form had memory-improving effects. In two animal models, subchronic injection of Trasina for 21 days was found to imitate key biochemical characteristics linked to Alzheimer's disease (AD).[8] Our earlier research has demonstrated the safety of Trasina® as a medication[9] and the lack of any unfavorable effects on animals. Another recent study discovered that Trasina significantly reduced stress and preserved healthy homeostasis.[10] According to an *in vitro* stability study, the ingredients used to make Trasina capsules are long-term stable.[11]

MATERIALS AND METHODS

Reagents and chemical

HCl, NaOH, NH_4, chloroform, ferric chloride, and H_2SO_4 were taken from Sisco Research Laboratories Pvt Ltd., – Kolkata. Methanol and Ethanol were purchased from Merck, India. Others reagents and chemicals those used in this study were analytical grade.

Content of trasina®

Trasina® is an herbal medicine mainly made up of five Indian common medicinal plants [Figure 1] like *O. sanctum* 190 mg; *W. somnifera* 80 mg; *T. cordifolia* 10 mg; *P. kurroa* 10 mg; and *E. alba* 10 mg [Table 1]. All the plants have various pharmacological properties and very useful for curing diseases.

Qualitative phytochemicals screening

In this study, we prepare three types of extracts of Trasina®, namely, methanolic extract, ethanolic extract and water extract. Different plant-based constituents were measured by using commonly accepted procedures.[12-16] Details of the testing procedure are as follows:

Determination of alkaloids

2 mL of plant extract was taken in a glass beaker and the 2 mL of concentrated HCl was added within it. After shaking added few drops of Mayer's reagent within it. Appearing of green color or a white precipitate indicates that alkaloids were present within the extract.

Determination of flavonoids

2 mL of plant extract was taken in a glass beaker and 1 mL of 2 N sodium hydroxide was added within it. Appearing of yellow color indicates the presence of flavonoids.

Determination of anthraquinones

A conical flask containing 1 mL of plant extract mixed with few drops of 10% ammonia solution. If a pink precipitate arrives, it indicates the presence of anthraquinones.

Determination of terpenoids

Few drops of con. HCl were added on 2 mL of chloroform. Add 0.5 mL of the extract to obtained reddish-brown color which indicates the presence of terpenoids.

Determination of carbohydrates

A few drops of concentrated H_2SO_4 and 1 mL of Molisch's reagent were taken in a conical

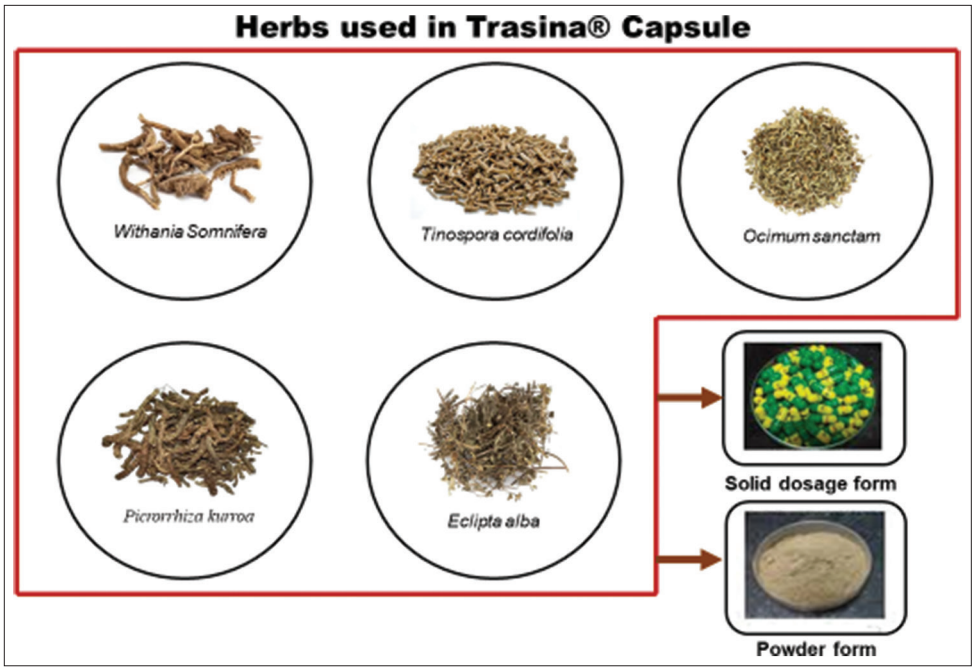

Figure 1: Composition of Trasina®, a unique polyherbal formulation

Table 1: Details composition of Trasina® (polyherbal formulation)

Each capsule contains powder of					
S. No.	**Scientific Name**	**Common Name**	**Family**	**Quantity**	**Parts Used**
1.	*Withania somnifera*	Ashwagandha	Solanaceae	80 mg	Root
2.	*Ocimum sanctum*	Tulasi	Cupressaceae	190 mg	All Parts
3.	*Tinospora cordifolia*	Guduchi	Menispermaceae	10 mg	Stem
4.	*Picrorrhiza kurroa*	Katuka, Kutki	Scrofulariaceae	10 mg	Root
5.	*Eclipta alba*	bhringaraj	Asteraceae	10 mg	Flower

flask. 2 mL of plant extract was added within it. Appearing purple or reddish color indicates the presence of carbohydrates.

Determination of phenols

1 mL of extract and 2 mL of distilled water were mixed properly. After that a few drops of 10°C chloride was added on them. Appearing blue or green color established the presence of phenols.

Determination of phlobatannins

1 mL of plant extract was taken in a glass beaker and then added few drops of 2% HCl within it. Appearance of a red color precipitate indicates the presence of phlobatannins.

Determination of tannins

1 mL of plant extract was taken in a clean glass container and then added 2 mL of 5°C chloride on it. Formation of dark blue or green-black color established the presence of tannins.

Determination of saponins

2 mL of plant extract was taken in a glass beaker and then 2 mL of distilled water added on it. Shaken lengthwise for 15 min in a measuring cylinder. The formation of a 1 cm foam layer indicates the presence of saponins.

Determination of glycosides

A mixture of 3 mL of chloroform and 10% NH_4 solution were added to 2 mL of plant extract. Pink color developed indicates the presence of glycosides.

Determination of amino acid

2–3 drops of freshly prepared 0.2% ninhydrin reagent were carefully added on 0.5 mg of extract

in a glass conical flask heated gently. Formation of purple or pink color indicates the presence of proteins, peptides, or amino acids.

Determination of terpenoids

0.5 mL of the plant extract was taken in a clean glass beaker and then added 2 mL of chloroform and concentrated sulfuric acid very carefully.

Determination of steroids and phytosterols

The same amount of chloroform is added to 1 mL of plant extract and exposed to a few drops of concentrated sulfuric acid. The appearance of a brown ring indicates the presence of steroids and the appearance of a blue-brown ring indicates the presence of phytosterols.

Quantitative phytochemicals screening determination of total phenol

The phenolic component was extracted from the fat-free sample by boiling it in 50 mL of ether for 15 min. A 50 mL flask was filled with 5 mL of the extract and 10 mL of distilled water. In addition, 5 mL of concentrated amyl alcohol and 2 mL of ammonium hydroxide solution were added. The samples were placed exactly to the mark in a 50 mL vial and given 30 min to react and take on color. Its wavelength was 505 nm.[17]

Determination of total flavonoids

10 g of plant sample was repeatedly extracted with 100 mL of 80% of aqueous methanol at room temperature. The entire solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was then transferred to a crucible and evaporated to dryness on a water bath and weighed to constant mass.[18]

Examination of color, odor, and taste

Color: 5 g of Trasina were placed in watch glasses and placed in a white tube light against a white background. Their color was visible to the naked eye.

Scent: 2 g of Trasina scent.

Taste: A pinch of Trasina was taken and its taste was explored with the tongue taste.

Determination of loss on drying

To measure loss on drying, 2 g of soil material was weighed into a dry petri dish (tar evaporating dish) and dried at 105–110°C until two consecutive weights did not differ by more than 5 mg. The weight after drying was recorded and the loss on drying was calculated. For the air dried sample, the percentage was reported in wt%.[13]

Determination of total ash

About 1 g of ground, air-dried material was used in a pre-weighed crucible and its ash content was calculated by firing at 500–600°C gradually until carbon-free. It was then dried to cool and weighed. Calculated as a percentage of the mass of air-dry material, the total ash was in mass percent.[13]

Determination of water soluble extractive value

5 g of correctly weighed Trasina were soaked in an Erlenmeyer flask with a glass lid. Following the addition of 100 cc of chloroform water, the mixture was soaked for 6 h while being constantly stirred. After 18 h of standing, it was swiftly filtered, and 20 mL of the filtrate was put to a flat-bottomed dish that had been covered with tar. After 24 h, the dish was dried out on a boiling water bath. The dried dish was then chilled and weighed after being dried at 105°C for 6 h. The amount of water-soluble extract in the weight of the residue was determined and shown as a weight percentage[13] in comparison to the air-dried sample.

Microbial load

Standard recommended described protocol with slight modification was used for determination of Microbial load. We follow the standard Indian Pharmacopoeia method.[14]

Determination of pH

1 g of Trasina powder was taken in a 100 mL volumetric flask and make up the volume by distilled water. Sonicated the solution for about 10 min. pH was measured using a digital pH meter.

RESULTS

Physical observation

Trasina, a multi-herbal capsule, mainly consists of five Indian medicinal plants those are used in Ayurveda. *W. somnifera*, *T. cordifolia*, *E. alba*, *O. sanctum,* and *P. kurroa* are the main ingredients present in Trasina [Figure 1]. Each Trasina® pill contains 80 mg of *W. somnifera*, 190 mg of *O. sanctum*, 10 mg of *T. cordifolia*, *P. kurroa,* and *E. alba* [Table 1].

Phytochemical screening of Trasina®

Table 2 lists several phytochemicals found in Trasina®. Secondary metabolites include steroids, saponins, triterpenoids, alkaloids, carbohydrates, flavonoids, amino acids, tannins, polyphenols, and glycosides, which have been identified with positive results in Trasina®. Phlobatannins were not present in any of the extracts produced. Water, ethanol, and methanol extracts of Trasina® contained bioactive agents such as saponin, flavonoids, steroids, terpenoids, alkaloids, carbohydrates, anthraquinone, polyphenols, and glycosides.

Table 2: Phytochemical screening of different extracts of Trasina® (polyherbal formulation)

S. No.	Secondary Metabolites	Extracts		
		Methanol	Ethanol	Water
1.	Alkaloids	+	+	++
2.	Flavonoids	++	+	++
3.	Anthraquinone	+	+	+
4.	Terpenoids	+	+	+
5.	Carbohydrate	++	++	+++
6.	Polyphenol	++	+	++
7.	Phlobatannins	−	−	−
8.	Tannin	+	+	+
9.	Saponin	+	+	+
10.	Glycoside	+	++	+
11.	Amino acid	+	+	+
12.	Triterpenoids	+	+	+
13.	Steroids and Phytosterols	+	+	+

(+) Presence, (−) Absence, (++) High concentrations

Results for total phenol and total flavonoid contents

Figure 2 presents information on the concentrations of flavonoids and phenols. It was found that the total phenol content of Trasinan® was 131.58 mg/g and the total flavonoid content was 69.37 mg/g.

Determination of analytical parameters

The physical parameters like order, colour, test and texture of the sample (Trasina®) compiles with the prescribed standard IP specification [Table 3]. All chemical parameters such as loss on drying, total ash, acid-soluble ash, and pH were within the range of lime [Table 4]. The disintegration times of the preparation (Trasina®) were also within the limits of the pharmacopoeia [Table 4].

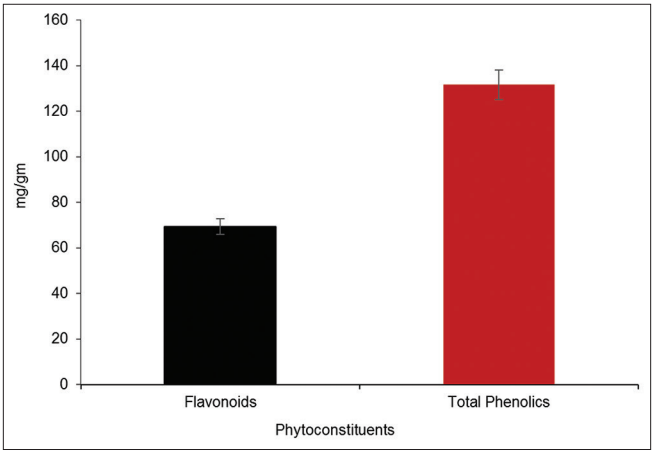

Figure 2: Quantitative analysis of total phenols and flavonoids in Trasina® (polyherbal formulation). Values were expressed as mean ± standard deviation for triplicates

Table 3: Organoleptic parameters of Trasina® (polyherbal formulation)

Organoleptic characters	Observation
Odor	Typical herbal dust smell
Color	Light pale brown l
Test	Characteristic
Texture	Soft powder

Table 4: Different analytical parameters of masura ghirta Trasina® (polyherbal formulation)

Parameters	Specification	Results
Disintegration	NMT 30 min.	10.36
Loss on drying at 105°C	NMT 10% w/w	2.07% w/w
Total Ash at 450°C	NMT 10% w/w	13.47% w/w
Acid soluble ash	NMT 10% w/w	1.99% w/w
pH	6.0–8.0	6.15
Total bacterial count	NMT 1×10^5 cfu/g	10.36

DISCUSSION

To find elements with proven physiological and therapeutic benefits, plant extracts were subjected to phytochemical analysis.[19] Plant extracts were discovered to contain phenols, tannins, flavonoids, glycosides, steroids, terpenoids, and carbohydrates after phytochemical examination. Phenolic chemicals are one of the biggest and most prevalent groups of plant metabolites.[20] Some of their biological features include apoptosis, anti-aging, anti-cancer, anti-inflammatory, anti-atherosclerosis, cardiovascular protection, strengthening of endothelial function, and prevention of angiogenesis and cell proliferation.[21] In practically all extracts, antioxidant levels are significantly impacted by phenolic components, according to a recent study.[22] In response to microbial infection, plants can create flavonoids, which are hydroxylated phenolic molecules. *In vitro* tests on different pathogens show that flavonoids have antibacterial effects. Much of their action is explained by their capacity to interact with soluble and extracellular proteins as well as the bacterial cell wall.[23] They are also effective antioxidants and have potent anticancer effects.[24-26] Flavonoids were discovered to be independently eluted with polar solvents in the current investigation. According to certain sources, steroids are antibacterial.[27] Most plant extracts contain steroids and phytosterols, according to recent studies. As a result, the study's findings and the presence of these plants suggest that the detected phytochemicals may be bioactive substances. The preparation satisfies all of the test requirements listed in the pharmacopoeia, according to the analytical analysis.

CONCLUSION

The results of this study showed that Trasina® aqueous extract contains significant amounts of important phytochemicals. Although there are phytochemicals in the ethanolic and methanolic extracts of Trasina®, their concentration is slightly lower than in the aqueous extract. The observed activity may be due to the phenolic and flavonoid content of the methanolic extract. According to the analytical study, the dosage form meets all the test requirements of the pharmacopoeia.

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CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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