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In Vitro Evaluation of the Phytochemical Components of New Multi-Herbal Medicine LIV01-SSA-23

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ABSTRACT
The active ingredients of plants ware identified using a suitable solvent, the solutions were evaporated, and the residues were then adjusted to a set as standard to create polyherbal formulations, which were concentrated herbal compounds. The main components of LIV01-SSA-23 were the Indian herbs *Andrographis paniculata*, *Moringa oleifera*, *Ocimum Sanctum*, *Withania somnifera*, *Tinospora cordifolia*, *Picrorrhiza kurroa*, and *Terminalia chebula*, which was excellent for treating a variety of liver problems and preventing oxidative cellular damage. Phytochemicals that have the potential to treat diseases and were used in natural therapeutics include flavonoids, glycosylates, saponins, amino acids, monoterpenes, and others. The goal of this inquiry was to identify the many phytochemicals that were present in the extracts included in LIV01-SSA-23. Several phytochemical components were examined using previously standardized procedures. Saponins, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, sugars, anthraquinone, phenolic compounds, and glycosides were all present in the phytochemical sample. Using ethanol, methanol, and aqueous solutions, the substances ware extracted. Saponins, flavonoids, steroids, terpenoids, triterpenoids, alkaloids, carbohydrates, anthraquinone, phenolic compounds, and glycosides were found to be the phytochemical constituents of the formulation. For analytical study, criteria such as loss on drying, total ash, acid-soluble ash, and pH, the pharmacopeial constraints were satisfied. According to preliminary phytochemical screening of LIV01-SSA-23, the presence of active components was found. These active group of compounds which were found responsible for the therapeutic properties important for pharmacological action of the formulation.

Keywords: LIV01-SSA-23, Indian Medicinal plants; Bioactive compounds; solvent extraction; phytochemicals

INTRODUCTION

Ayurvedic and herbal medicines are made from a variety of natural ingredients, each of which has a special chemical makeup that, when combined, can provide the desired result. The market for Ayurvedic medicine [1,2] is growing significantly due to the increase in the demand for herbal medicines. The Sarangdhar Samhita, a work of Ayurvedic literature, introduced the concept of polyherbal for more effective treatment. The polyherbal blend has been used for medicinal and therapeutic purposes all over the world [3]. It is also known as herb-herb combination therapy or multi-herb therapy. Active phytochemical components found in individual plants are insufficient to have the desired therapeutic impact [4]. When a variety of herbs are combined with herbal and herbal-mineral combinations in a carefully made ratio, the therapeutic effectiveness grows and side effects are reduced [5].

Herbal treatments are frequently mixed kind of, and use extracts from plants, each of which has a unique diversity of species, growing habitats, and physiologically active components [6]. The availability of numerous active components in herbal substances, which when combined might create stimulating effects that might not be conceivable with a single ingredient, represents a huge potential advantage over traditional single-component pharmaceuticals. The herbal pharmacological ingredients present in polyherbal formulations might have synergistic, potentiating, agonistic, and antagonistic effects as a result of their numerous active components [7,8].

Indian traditional medicine is very useful to maintain normal liver function. It also prevents hepatic oxidative stress and fight against infection. Due to its herbal base the medicine is safe, non-toxic and sometimes have better therapeutic efficacy against allopathic medicine [9-11]. With this keeping in mind, we developed an herbal composite based on seven Indian medicinal plants namely *Andrographis paniculata*, *Moringa oleifera*, *Ocimum Sanctum*, *Withania somnifera*, *Tinospora cordifolia*, *Picrorrhiza kurroa*, and *Terminalia chebula* useful to maintain normal liver function. The present study was carried out for qualitative and quantitative phytochemical analysis of LIV01-SSA-23 using alcoholic (methanol and ethanol) and aqueous extracts.

MATERIALS AND METHODS

Reagents &Chemical

The following substances were obtained from Sisco Research Laboratories Pvt. Ltd. (SRL), Kolkata - HCl, NaOH, NH_4, Chloroform, Ferric Chloride, and H_2SO_4. We bought ethanol and methanol from Merck in India. Analytical grade chemicals and reagents were employed in the rest of the investigation.

Composition of LIV01-SSA-23

Seven commonly used Indian medicinal herbs, including *Andrographis paniculata*, *Moringa oleifera*, *Ocimum Sanctum*, *Withania somnifera*, *Tinospora cordifolia*, *Picrorrhiza kurroa*, and *Terminalia chebula*, make up the majority of the herbal medication LIV01-SSA-23 (Fig. 1) (Table 1). Each plant has unique pharmacological qualities that make them all effective at treating disease.

All the required medicinal plants and species were procured from registered suppliers and authenticated by renowned taxonomist, department of Pharmaceutical Technology, Jadavpur University, Kolkata, India. The samples are deposited to the institutional collection.

Qualitative Phytochemicals Screening:

Three different LIV01-SSA-23 extracts—methanolic, ethanolic, and water—have been prepared for this study. Standard methods were used to study a variety of Phyto-constituents [12–16]. The testing processes are as follows:

Determination of alkaloids

A glass beaker containing 2 ml of extract was then filled with 2 ml of strong HCl. Add a few drops of Mayer's reagent after shaking. Alkaloids were found in the extract if it appeared green or as a white precipitate.

Identifying flavonoids

A glass beaker was filled with 2 ml of extract and 1 ml of 2 N sodium hydroxide. The presence of flavonoids is indicated by a yellow appearance.

Analysis of Anthraquinones

A conical flask containing a little amount of 10% ammonia solution and one milliliter of extract. The presence of anthraquinones is shown by the arrival of pink precipitate.

Terpenes determination

After adding 2 ml of chloroform, a few drops of con. HCl were added. A reddish-brown colour was obtained by adding 0.5 ml of the extract, indicating the presence of terpenoids.

Calculating carbohydrates

A conical flask was filled with a few drops of concentrated H_2SO_4 and 1 ml of Molisch's reagent. Within it, 2 ml of extract was put. The presence of carbohydrates is indicated by a purple or reddish color.

Measurement of phenols

The correct mixture of 1 ml of extract and 2 ml of distilled water was used. Then, a few drops of chloride heated to 10°C were applied to them. The presence of phenols could be determined by their blue or green appearance.

Analysis of Phlobatannins
In a glass beaker, 1 ml of extract was added along with a few drops of 2% HCl. Phlobatannins are present when a precipitate of a red color forms.

Measurement of tannins
A clean glass container containing 1 ml of extract was filled with 2 ml of 5°C chloride. The development of a deep blue or green-black hue indicated the presence of tannins.

Measurement of saponins
In a glass beaker, 2 ml of plant extract was added before 2 ml of distilled water. Shaken lengthwise in a measuring cylinder for 15 minutes. The development of a 1 cm foam layer is a sign that saponins are present.

Identifying glycosides
2 ml of extract were combined with 3 ml of 10% NH_4 solution and 3 ml of chloroform. The development of a pink color suggests the presence of glycosides.

Amino acid determination
In a glass conical flask that had been gently heated, 0.5 mg of extract was carefully put on top of 2-3 drops of freshly made 0.2% ninhydrin reagent. Proteins, peptides, or amino acids are present when purple or pink color formation occurs.

Terpenes determination
In a clean glass beaker, 0.5 ml of the extract was placed before being carefully mixed with strong sulfuric acid and 2 ml of chloroform.

Steroid and phytosterol determination
One milliliter of extract is combined with the same amount of chloroform and subjected to a few drops of strong sulfuric acid. The presence of steroids is indicated by the presence of a brown ring, while the presence of phytosterols is indicated by the presence of a blue-brown ring.

Quantitative Phytochemicals Screening Determination of total phenol
The fat-free sample was boiled in 50 mL of ether for 15 minutes to extract the phenolic component. 5 ml of the extract and 10 ml of distilled water were added to a 50 ml flask. Additionally, 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were added. After being precisely positioned in a 50 mL vial, the samples were given 30 minutes to react and change colour. It had a 505 nm wavelength. [17]

Determination of total flavonoids
100 cc of 80% aqueous methanol was repeatedly used to extract 10 g of material while it was still in room temperature. Filtered via Whatman filter paper No. 42 (125 mm) the entire solution. The filtrate was then put into a crucible, dried out on a water bath, and mass-weighed to a constant value. [18]

Examination of color, odor and taste
Color: Five grams of LIV01-SSA-23 were placed in watch glasses and placed in a white tube light against a white background. The color was visible to the naked eye.
Scent: Two grams of LIV01-SSA-23 was taken and the herbal medicine's scent was examined.
Taste: A pinch of LIV01-SSA-23 was taken and its taste was explored with the tongue taste.

Calculating the loss after drying
2 g of material were weighed into a dry petri dish (a tar evaporating dish) to quantify loss on drying. The extract was dried at $105\text{-}110^0$ C until two subsequent weights did not deviate by more than 5 mg. After drying, the weight was measured, and the drying loss was computed. The proportion was given in weight percent for the air-dried sample [13].

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Measurement of the overall ash

In a pre-weighed crucible, 1 g of ground, air-dried material was utilized, and its ash content was determined by gradually heating it to between 500 and 600^{0}C until it was carbon-free. After cooling off, it was dried and weighed. The total ash was calculated as a mass percent of the mass of air-dry material [13].

Calculating the extractive value of water

5 g of LIV01-SSA-23 that had been accurately weighed were immersed in an Erlenmeyer flask with a glass lid. 100 cc of chloroform water was added, and the mixture was soaked and agitated continuously for 6 hours. It was quickly filtered after standing for 18 hours, and 20 ml of the filtrate was added to a plate with a flat bottom and tar on top. The dish was dried out on a boiling water bath after 24 hours. The dish was dried at 105°C for 6 hours, and then it was cooled and weighed. When compared to the air-dried sample, the weight percentage of the water-soluble extract in the residue was calculated [13].

Microbiological Test

We follow standard recommended microbiological technique to determine the microbial burden with a little modification. We adhere to the accepted Indian Pharmacopoeia (IP) procedure [14].

Calculation of pH

A 100 ml volumetric flask was filled with distilled water and 1 g of LIV01-SSA-23 powder. For around ten minutes, sonicated the solution. We used a digital pH meter to measure pH.

RESULTS

Physical observation:

LIV01-SSA-23 a multi-herbal capsule mainly consists of seven Indian medicinal plants those are used in Ayurveda. *Andrographis paniculata*, *Moringa oleifera*, *Ocimum Sanctum*, *Withania somnifera*, *Tinospora cordifolia*, *Picrorrhiza kurroa*, and *Terminalia chebula*, (Figure 1). Each LIV01-SSA-23 pill contains 100mg *Andrographis paniculata*, 50mg *Moringaoleifera*, 190mg *Ocimum Sanctum*, 80mg *Withanias omnifera*, 10mg *Tinospora cordifolia*, 10mg *Picrorrhiza kurroa*, and 10mg *Terminalia chebula* (Table 1).

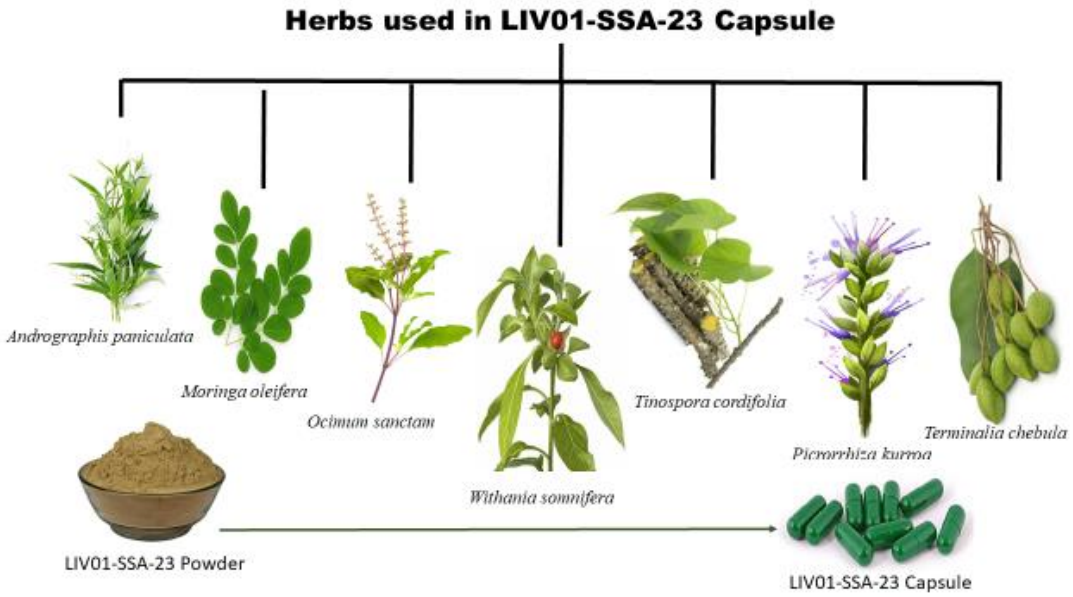

Figure 1: Composition of LIV01-SSA-23 (multi-herbal formulation)

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Table 1: Details composition of LIV01-SSA-23(multi-herbal formulation) Each capsule contains powder of:

Sl. No.	Scientific Name	Common Name	Family	Quantity	Parts Used
1.	*Andrographis paniculata*	Kalmegh	Acanthaceae	100 mg	Leaf
2.	*Moringa oleifera*	Shajna	Moringaceae	50 mg	Leaf
3.	*Ocimum Sanctum*	Tulasi	Cupressaceae	190 mg	All Parts
4.	*Withania somnifera*	Ashwagandha	Solanaceae	80 mg	Root
5.	*Tinospora cordifolia*	Guduchi	*Menispermaceae*	10 mg	Stem
6.	*Picrorrhiza kurroa*	Katuka, Kutki	Scrofulariaceae,	10 mg	Root
7.	*Terminalia chebula*	Haritaki	*Combretaceae*	10 mg	Seed

Phytochemical screening:

Table 2 lists a number of phytochemicals present in LIV01-SSA-23. Steroids, saponins, triterpenoids, alkaloids, carbohydrates, flavonoids, amino acids, tannins, polyphenols, and glycosides are examples of secondary metabolites that have been positively found in LIV01-SSA-23. There were no phallobatannins in any of the extracted materials. Bioactive substances like saponin, flavonoids, steroids, terpenoids, alkaloids, carbohydrates, anthraquinone, polyphenols, and glycosides were present in the water, ethanol, and methanol extracts of LIV01-SSA-23.

Table. 2: Phytochemical screening of different extracts of LIV01-SSA-23(multi-herbal formulation)

S. No.	Secondary Metabolites	Extracts		
		Methanol	Ethanol	Water
1.	Alkaloids	+	+	+ +
2.	Flavonoids	+ +	+	+ +
3.	Anthroquinone	+	+	+
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

Examination of color, odor and taste:

The color, odor, texture and taste as observed are shown in Table-3

Table no. 3: Organoleptic Parameters of LIV01-SSA-23(multi-herbal formulation)

Organolepticcharacters	Observation
Odour	Typical Herbal dust smell
Colour	Light pale brown
Test	Characteristic
Texture	Soft Powder

Total phenol and total flavonoid content findings:

The amounts of phenols and flavonoids are shown in Figure 2. It was observed that LIV01-SSA-23 had 131.58 mg/g of total phenol and 69.37 mg/g of total flavonoids.

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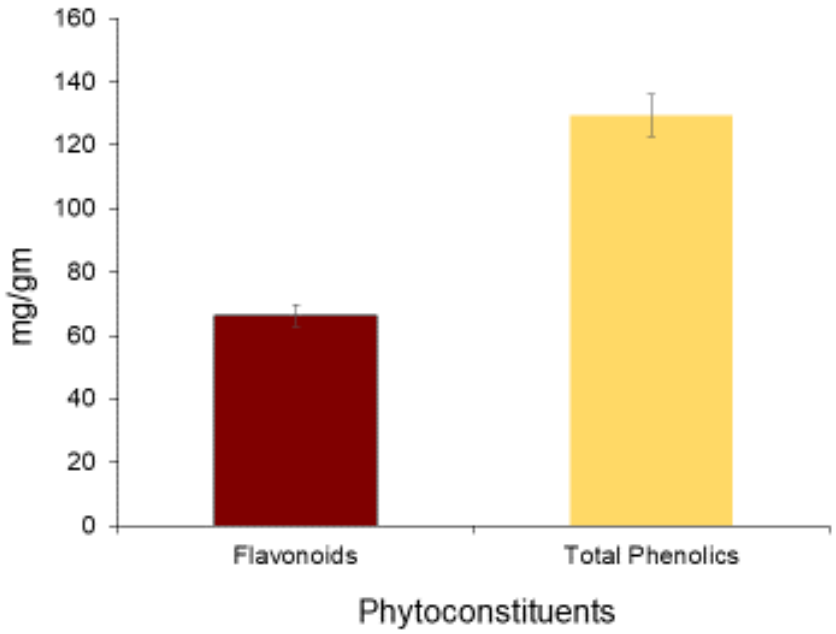

Figure 2: Quantitative analysis of total phenols and flavonoids in LIV01-SSA-23 (multi-herbal formulation) Values were expressed as Mean ± SD for triplicates

Choosing analytical parameters:
All the chemical parameters, including loss on drying, total ash, acid-soluble ash, and pH showed in Table 4. The preparation's (LIV01-SSA-23) disintegration times fell within acceptable pharmacopoeia bounds as well (Table 4).

Table 4: Different analytical parameters of LIV01-SSA-23(multi-herbal formulation)

Parameters	Specification	Results
Disintegration	NMT 30 min.	11.55
Loss on drying at 105°C	NMT 10% w/w	1.97% w/w
Total Ash at 450°C	NMT 10% w/w	12.48% w/w
Acid soluble ash	NMT 10% w/w	1.86% w/w
pH	6.0 – 8.0	6.05
Total Bacterial Count	NMT $1x10^5$cfu/gm	9.75

DISCUSSION
Plant extracts were subjected to phytochemical analysis in order to identify components with demonstrated physiological and therapeutic effects [19]. Phytochemical analysis revealed that plant extracts contain phenols, tannins, flavonoids, glycosides, steroids, terpenoids, and carbohydrates. One of the largest and most common categories of plant metabolites are phenolic compounds [20]. Apoptosis, anti-aging, anti-cancer, anti-inflammatory, anti-atherosclerosis, cardiovascular protection, strengthening of endothelial function, and prevention of angiogenesis and cell proliferation are a few of their biological characteristics [21]. According to a recent study [22], phenolic components have a considerable impact on antioxidant levels present in almost all extracts. Plants can produce flavonoids, which are hydroxylated phenolic compounds, in response to microbial infection. Testing in vitro on various pathogens reveals flavonoids have antibacterial properties. Their ability to interact with soluble and extracellular proteins as well as the bacterial cell wall helps to explain a large portion of their action [23]. They work well as antioxidants and have strong anticancer properties [24–26]. In the current study, it was found that flavonoids can be independently eluted with polar solvents. Steroids are antimicrobial, according to certain authorities [27]. Recent investigations have shown that the majority of plant extracts include steroids and phytosterols. From the results of the study, it may be inferred that the discovered phytochemicals are bioactive compounds. According to the analytical examination, the product satisfies each of the pharmacopoeia's test requirements.

CONCLUSION

The findings of this investigation demonstrated that the aqueous extract of LIV01-SSA-23 includes considerable levels of crucial phytochemicals. Although LIV01-SSA-23's ethanolic and methanolic extracts contain phytochemicals, their concentration is a little bit lower than it is in the aqueous extract. The phenolic and flavonoid content of the methanolic extract may be the cause of the activity that was found. The newly developed formulation successfully complies with all of the pharmacopoeia's test requirements, according to the analytical investigation.

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Conflict of Interest

We declare that we have no conflict of interest.

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