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DESIGN AND BIOACTIVITY ASSESSMENT OF A TINOSPORA-BASED HERBAL ELIXIR WITH MULTIFUNCTIONAL THERAPEUTIC POTENTIAL

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Abstract

Particularly in India and other Asian nations, medicinal herbs are becoming widely recognized as alternative treatments for preventing a variety of ailments. The majority of plant-based herbal remedies, however, have not yet gained global scientific recognition. "Wild. cordifolia Tinospora", "Giloy" or "Guduchi", one of the most promising plant species of Tinospora, has been extensively studied. It is employed in a number of traditional medicines to treat a variety of illnesses, including immunological and metabolic problems, diabetes, cancer, heart disease, and infectious diseases. Numerous bioactive phytochemical components have been discovered and isolated from T. cordifolia's stem, root, and whole plant. Over the past twenty years, T. cordifolia's many pharmacological effects have been the subject of ongoing research. metabolic diseases including diabetes mellitus and obesity are linked to oxidative stress, insulin resistance, hyperglycemia, dyslipidemia, and cardiovascular problems. Herbal formulations have drawn a lot of attention due to their safety, potency, and therapeutic potential because long-term usage of synthetic medications can have negative effects. The goal of the current study was to create and assess a multipurpose polyherbal elixir for the treatment of obesity and diabetes mellitus that included Tinospora cordifolia as the main ingredient, ginger extract, garlic extract, green tea extract, and stevia. Because Tinospora cordifolia contains alkaloids, glycosides, flavonoids, diterpenoids, and phenolic chemicals, it has been reported to have anti-diabetic, hypolipidemic, antioxidant, and immunomodulatory activities. The phytochemical analysis verified the existence of important bioactive components with anti-obesity and anti-diabetic properties. With a pH of about 6.0, a refractive index between 1.3308 and 1.3501, and a viscosity appropriate for oral administration, the prepared elixir demonstrated acceptable physicochemical characteristics. Maximum absorbance was revealed by UV spectrophotometric measurement at: The formulation's physical stability is indicated by λ max = 277.5 nm. Cordifolia, ginger, garlic, and green tea may all work together to enhance glucose metabolism, lower oxidative stress, control lipid profiles, and encourage fat metabolism. Consequently, the polyherbal elixir may be a viable herbal therapeutic preparation for the treatment of diabetes mellitus and obesity. To prove safety and effectiveness, more pharmacological and clinical research is needed.

INTRODUCTION

According to the World Health Organization, 80% of people worldwide primarily utilize traditional medicines that contain active components derived from plant extracts. The use of several plants in general healthcare and the treatment of common human ailments is strongly supported by India's mega-biodiversity and knowledge of its vast old traditional medicine systems (Ayurveda, Siddha, Unani, Amchi, and local health traditions). Additionally, Tinospora Cordifolia also known by several names, including Guduchi, Amrita, Gurach, and Tinospora, this climbing shrub belongs to the Menispermaceae family. Giloy is a big, glabrous climbing shrub. This plant's branches, which are somewhat succulent on the stems, produce long, filiform, meaty aerial roots. It has gray-brown, moist bark. The family Tinospora cordifolia, which is indigenous to tropical regions, has about 450 species and 70 genera. Which, in addition to all of India, can also be found in parts of Bangladesh, China, and Sri Lanka. The plant Tinospora cordifolia, sometimes referred to as Rasayana in Ayurveda, is well known for strengthening the body's defenses against particular infectious bacteria. The Cordifolious tinospora is an important drug in Indian medical systems and has long been used in therapies. It has also been proposed to be useful in the treatment of heart disease, leprosy, and helminthiasis. Many ailments are treated with the very nutritious and easily digested starch that is derived from the stem. According to research, Tinospora cordifolia has the ability to significantly reduce obesity and diabetes through a variety of molecular pathways. Its phytoconstituents aid in the management of diabetes by increasing insulin secretion, improving glucose metabolism, increasing skeletal muscle absorption of glucose, and lowering insulin resistance. The PI3K and AMPK signaling pathways, which are involved in glucose transport and energy control, are activated by some substances, such as tinosporaside. By controlling lipid metabolism and lowering oxidative stress and inflammation linked to obesity, tinospora also addresses metabolic disorders linked to obesity. It is categorized in Ayurvedic literature as a treatment for "Prameha," a condition associated with metabolic syndrome, obesity, prediabetes, and diabetes mellitus. According to studies, its phytochemicals interact with molecular targets linked to metabolic dysregulation and fat formation. Tinospora is a viable herbal candidate for treating metabolic problems because of its anti-inflammatory and antioxidant properties, which also preserve pancreatic β-cells and enhance general metabolic health. Even while computational and experimental research seems promising, more extensive clinical trials are still required to verify its long-term safety and effectiveness in people.

Fig. 1. Tinospora cordifolia leaves and stem

PLANT DISCRIPTION

1. **Stems** - Fleshy
2. **Roots** - long thread-like, aerial, arise from branches.
3. **Bark** - Thin, greyish or creamy white in color, when peeled fleshy stem is exposed.
4. **Leaves** - Cordate (heart shaped), membranous, juicy.
5. **Flowers** - Bloom during summer

a) **Male flower** - small, yellow or green colored occur in clusters.

b) **Female flower** - Occur singly.

6. **Fruits** - Pea shaped, fleshy, shiny turn red when boiled. Occur in winter
7. **Seeds** - curved, pea sized
8. **Parts Used:** Stems, Roots
9. **Distribution:** The plant occurs throughout tropical regions of India extending from Kumaon to Assam and Myanmar, Bihar, Konkan to Sri Lanka. It is a large climber which grows over the highest trees in the forests and throws out aerial roots which reach the length of 10 meters, though not thicker than packthread. [7]

CHEMICAL CONSTITUENTS

Table 1 [28] - Chemical Constituent

Active Ingredients	Compound	Plant Part	Biological Activity (In human being)
Alkaloids	Berberine, Choline, Tembetarine, Magnoflorinc, Tinosporin, Palmetine, Isocolumbin. Aporphine alkaloids, Jatrorrhizine Tctrahy dropalmatine,	Stem, Root	Anti-viral infections, Anti- cancer, anti-diabetes, inflammation, Neurological, immunomodulatory, psychiatric conditions
Diterpenoid Lactones	Furano lactone, Clerodane derivatives [(SR, I0R)-4R-SR-dihydroxy-2S-3R:1$,16- diepoxy-clerodn-I3 (16), 14-dicno-17,12S:18,1S-dilactone], Tinosporon. Tinosporides, Jatcorine, Columbin	Whole plant	Vasorelaxant: releases norepinephrine induced contractions, inhibits Ca+- influx, anti-inflammatory, anti-microbial, anti-hypertensive, anti-viml Induce apoptosis in Icukemin by activating caspasc-3and bax, inhibits bcl-2.
Glycosides	18-norelcrodane glucoside, Furanoid diterpene gluconide, Tinocordiside, Tinocordifolioside, Cordionide, Cordifolioside Syringin, Syringin-apiosylglycoside, Pregnane glycoside Palmatoside, Cordifolioside A B, C, D and E	Stem	Treats neurological disorders like ALS, Parkinsons, Dementia, motor and cognitive deficits and neuron loss in spine and hypothalamus, Immunomodulation, Inhibits NF-kBand act as nitric oxide scavenger to show anticancer activities.

Steroids	B-sitonterol, -sitostcrol, 20 (-hydroxycedysone, Ecdystcrone, Makistcrone A, Giloinstcrol	Shoot	IgA neuropathy, glucocorticoid induced osteoporosis in early inflammatory arthritis, induces cell cycle arrest in G2 M phase and apolipins through e-Mye suppression. Inhibits TNF-a IL-1 8, 11-6 and coX-2,
Sequiterpenoids	Tinocordifolin	Stem	Antiseptic
Alliphatic	Octacosanol	Whole	Anti-nociceptive and Anti-septic

Table 2 [7] – Taxonomy

Kingdom	**Plantae – Plant**
Subkingdom	Tracheobionta Vascular plant
Super division	Spermatophyta-Seed bearing plant
Division	Magnoliophyta Flowering
Class	Magnoliopsida – Dicotyledons
Sub-class	Polypetalae – Petals are free
Series	Thalamiflorae – Many stamens and flower hypogynous Order Ranales
Order	Ranales
Family	Menispermaceae;

Table 3 [33] - Test Performed

Phytoconstituent	**Test**	**Observation**	**Result**
Alkaloids	Mayer's Test	Cream precipitate	Present

Alkaloids	Wagner's Test	Brown precipitate	Present
Flavonoids	NaOH Test	Yellow color	Present
Tannins	Ferric Chloride Test	Blue-black color	Present
Glycosides	Legal's Test	Pink color	Present
Saponins	Froth Test	Stable foam	absent

Fig. 2. Pre-evaluation Tests

FORMULATION TABLE

Table 4 - Ingredients

Ingredient (per 50 ml)	**F1**	**F2**	**F3**	**Role**
Tinospora extract	10 ml	12 ml	15 ml	Primary immunomodulator
Ginger extract	2 ml	3 ml	2 ml	Digestive, anti-inflammatory
Garlic extract	1 ml	1.5 ml	2 ml	Antimicrobial
Licorice extract	2 ml	2 ml	3 ml	Demulcent, flavor enhancer
Green tea extract	3 ml	2 ml	2 ml	Antioxidant
Stevia	10mg	10mg	10mg	Neutral sweetener
Glycerin	5 ml	5 ml	5 ml	Co-solvent, viscosity enhancer
Ethanol (10 %)	5 ml	5 ml	5 ml	Solvent & preservative support
Sodium benzoate	0.1% (50 mg)	0.1 %	0.1%	Preservative
Citric acid	q.s. (pH 4-5)	q.s.	q.s.	pH adjuster
Purified water	q.s. to 50 ml	q.s.	q.s.	Vehicle

METHOD OF PREPARATION

A] Preparation of material (Tinospora cordifolia):

Collection of Plant Stem
↓
Cleaning
↓
Drying (Hot Air Oven)
↓
Grinding → Powder
↓
Ball Mill → Fine Powder
↓
Soxhlet Extraction (Ethanol, 72 hrs.)
↓
Evaporation of Solvent
↓
Final Extract (Paste)

B] Procedure:

1. Preparation of the Aqueous Base & pH Adjustment

Begin by measuring a pre-calculated volume of purified water (typically about 60–70% of the final volume) and pouring it into a main compounding vessel. Add the 'sodium benzoate' (antimicrobial preservative) and 'citric acid' (antioxidant and buffering agent). Stir the mixture continuously until both crystalline solids are completely dissolved and the solution is perfectly clear. Next, introduce the probe of a freshly calibrated pH meter into the solution. Gradually adjust the pH until it falls strictly within the '4.0–5.0' range; if it is too high, add a dilute citric acid solution, and if it is too low, use a dilute sodium hydroxide solution. Achieving this specific acidic range is critical to optimize the stability of the active herbal ingredients and ensure the efficacy of the preservative.

2. Sequential Incorporation of Herbal Extracts

With the aqueous base stabilized and under continuous, moderate stirring, begin adding the herbal extracts one by one. The order of addition is deliberate to prevent precipitation or incompatibility issues:

- First, introduce 'Tinospora cordifolia' (Guduchi) extract, allowing it to fully disperse.
- Next, add the 'ginger' extract, followed by the 'garlic' extract.
- Finally, blend in the 'green tea' extract.
- Maintaining a steady stirring speed during this phase ensures that the diverse phytochemicals such as polyphenols,

flavonoids, and alkaloids are uniformly distributed throughout the aqueous phase without clumping.

3. **Sweetener Integration & Rheology Modification**

In a separate, smaller beaker, prepare the sweetening phase by dissolving 'Stevia' extract into a small amount of warm purified water. Stir until the Stevia is fully solubilized, then slowly pour this sweetening solution into the main compounding vessel under active stirring. Once integrated, begin adding 'glycerin' in a slow, steady stream. Glycerin serves a dual purpose: it acts as a humectant and rheology modifier to improve the formulation's viscosity, and it functions as a texturizer to provide a smoother, more palatable mouthfeel that masks the bitter notes of the herbal extracts.

4. **Co-Solvent Addition & Final Volume Makeup**

Because some herbal components have limited water solubility, slowly introduce 'ethanol' into the mixture as a co-solvent. It must be added very gradually under continuous stirring to prevent localized high concentrations of alcohol, which could crash out water-soluble components. Once the ethanol is completely integrated, check the volume of the mixture. Top off the formulation by adding a sufficient quantity (q.s.) of purified water until the total volume reaches exactly 50 mL. Perform a final, thorough mixing cycle for several minutes to ensure the entire system achieves complete homogeneity, resulting in a clear, uniform, and pharmaceutically stable herbal formulation.

POST EVALUATION TEST

i. Determination of pH:

The pH of the formulated polyherbal elixir was determined using a calibrated digital pH meter. Before measurement, the pH meter was standardized citric acid. The pH of the formulation was found to be within the acceptable range for oral liquid preparations, indicating suitability and stability of the elixir formulation.7

Fig 1. Digital pH meter

ii. **Physical stability**:

A UV spectrophotometer (Bioera) was used to assess the physical stability. Elixir's physical stability was assessed using a UV spectrophotometer.[7]

Fig 2. UV-Spectrophotometer

iii. **Density:**

A substance's density is its mass per unit volume. Elixir's density was measured using a specific gravity bottle. The specific gravity formula was used to get the density.[7]

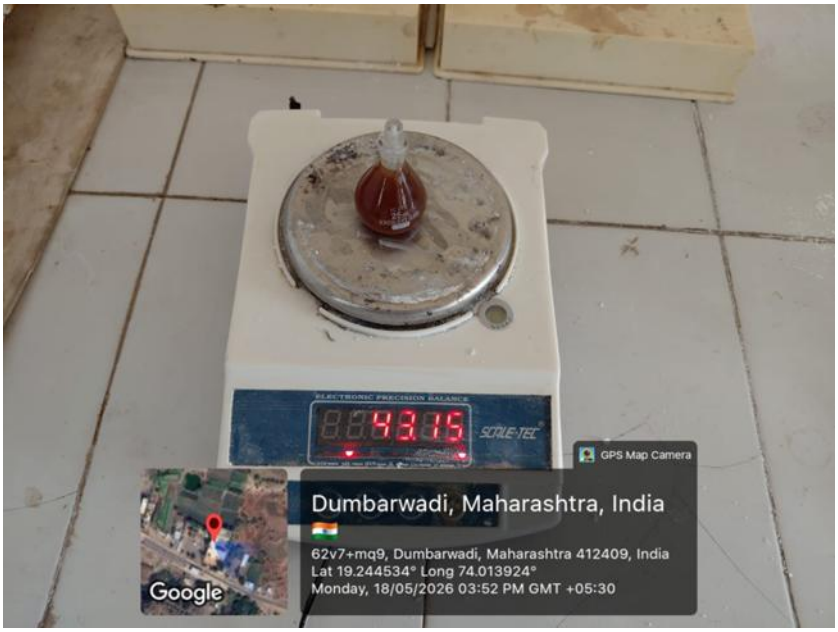


Fig 3. Specific Gravity Bottle

iv. Refractive index:

The value of the refractive index, also known as the index of refraction, is determined by dividing the speed of light in a vacuum by the speed of light in a second, denser medium. The Abbes refractometer was used to determine the elixir's refractive index. Three repetitions of the refractive index were made.[7]

v. **Measurement of viscosity:**

An Ostwald viscometer was used to measure the viscosity of the prepared batches. Three calculations were made using an Ostwald viscometer.[7]

RESULT AND DISCUSSION

Organoleptic properties: these studies are done for the tinospora cordifolia's organoleptic properties such as appearance, color, and odor. The results are similar to the literature studies.

Table 5: Organoleptic properties of Tinospora

Sr no.	Properties	Observed Results
1.	Appearance	Powder
2.	Color	Light brown powder
3.	Odor	Herbal odor
4.	Taste	Sweet with slight bitterness

Table 6: Evaluation properties of elixir

Sr no.	Formulation	pH	Viscosity	Density	Refractive Index

1	F1	6.3	0.054	0.031	1.3501
2	F2	6	0.0563	0.025	1.3308
3	F3	6.1	0.079	0.022	1.3415

Physical stability: UV spectrophotometer is used to determine physical stability
The peak shows similar wavelength and peak i.e. 277.5 nm which shows Elixir is stable.

Table 7: Stability of formulation

Sr no.	Formulation	Wavelength	Absorbance
1.	F1	277.5	-0.069
2.	F2	277.5	-0.021
3.	F3	277.5	-0.069

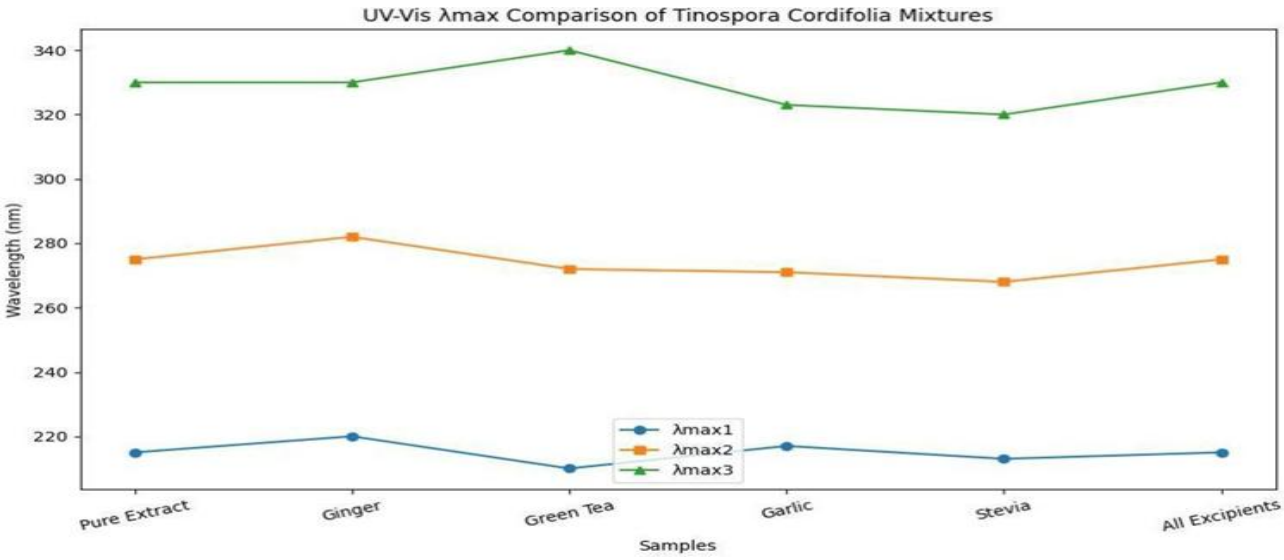
Organoleptic properties of prepared elixir were determined on the basis of appearance, taste, odor.

Table 8. Organoleptic properties of formulation

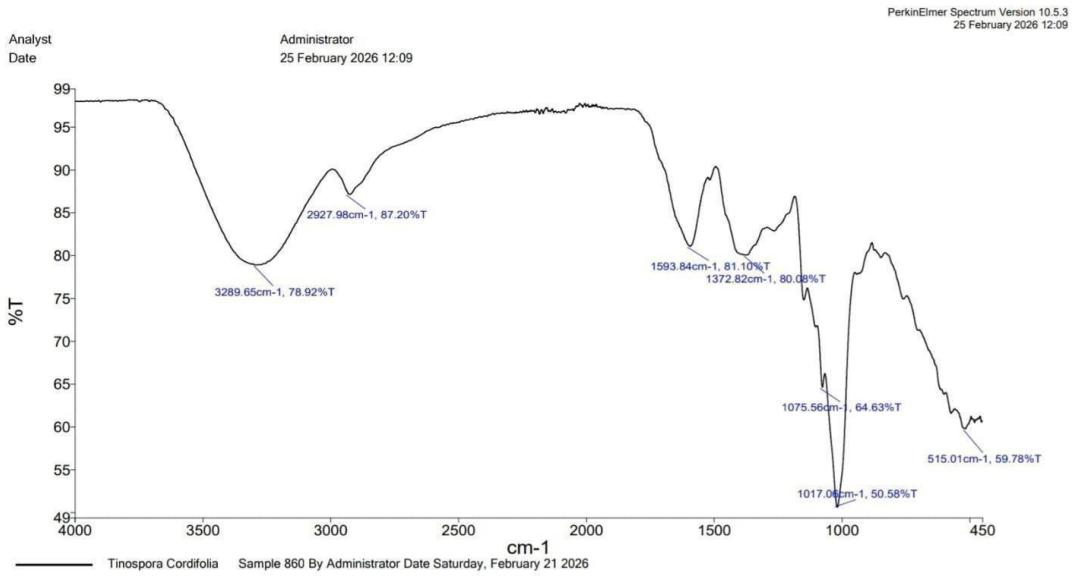
Sr no.	F1	F2	F3
Color	Greenish brown	Greenish brown	Greenish brown
Taste	Lingering sweet aftertaste with mild bitterness	Lingering sweet after taste with mild bitterness	Lingering sweet aftertaste with mild bitterness
Odor	Herbal odor	Herbal odor	Herbal odor

UV Spectrometer

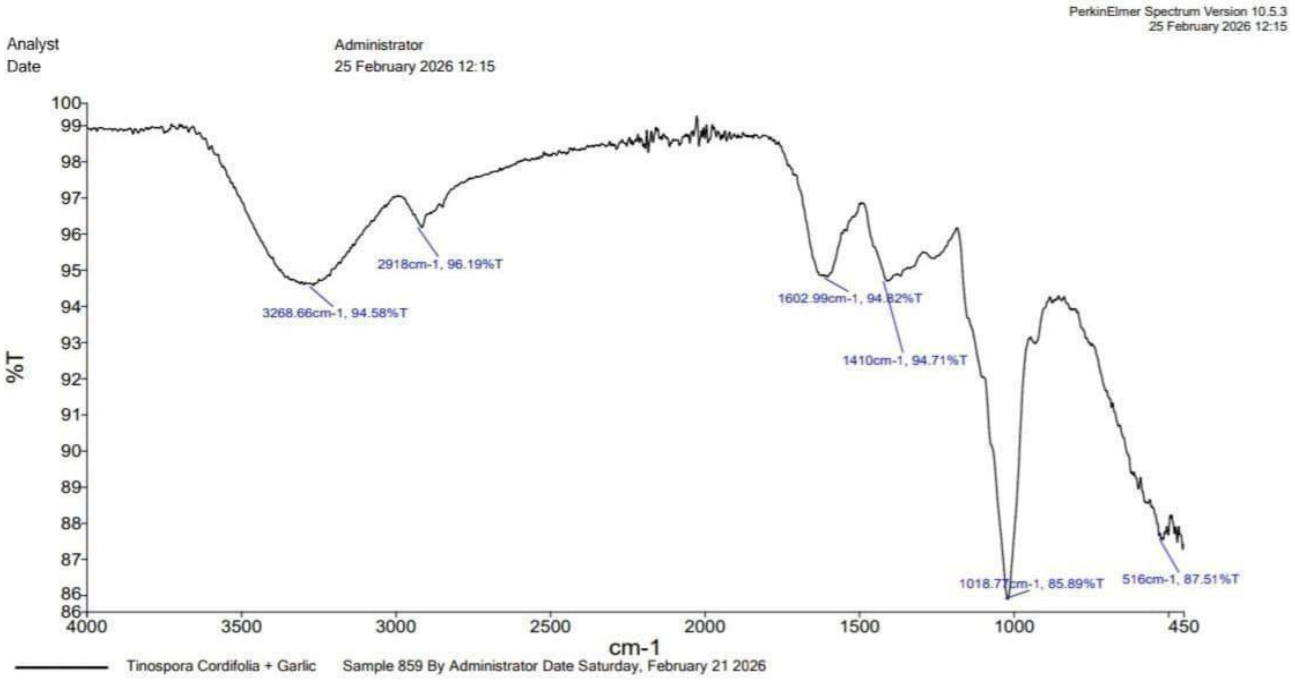
A UV-visible spectrophotometer operating in the 200–400 nm wavelength range was used to analyses the herbal elixir. Maximum absorbance was demonstrated by the optimized formulation at roughly: λ max = 277.5 nm. This wavelength verifies the formulation's stability and the existence of active phytoconstituents. The user-uploaded reference study showed similar UV absorption.



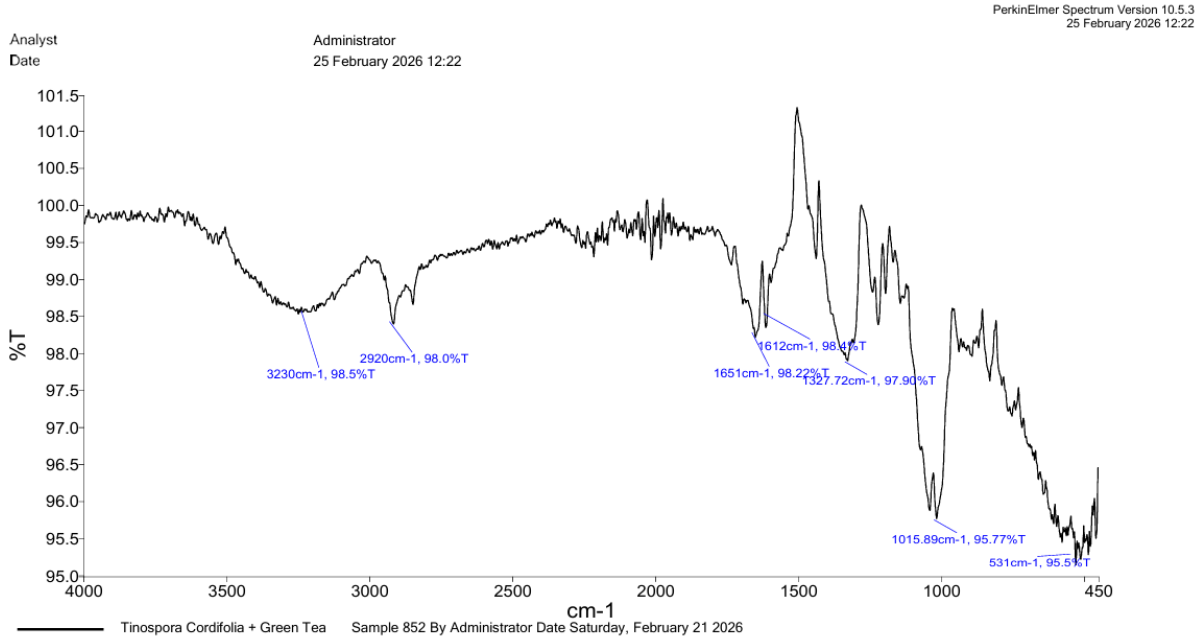
Graph 1. Formulation Stability



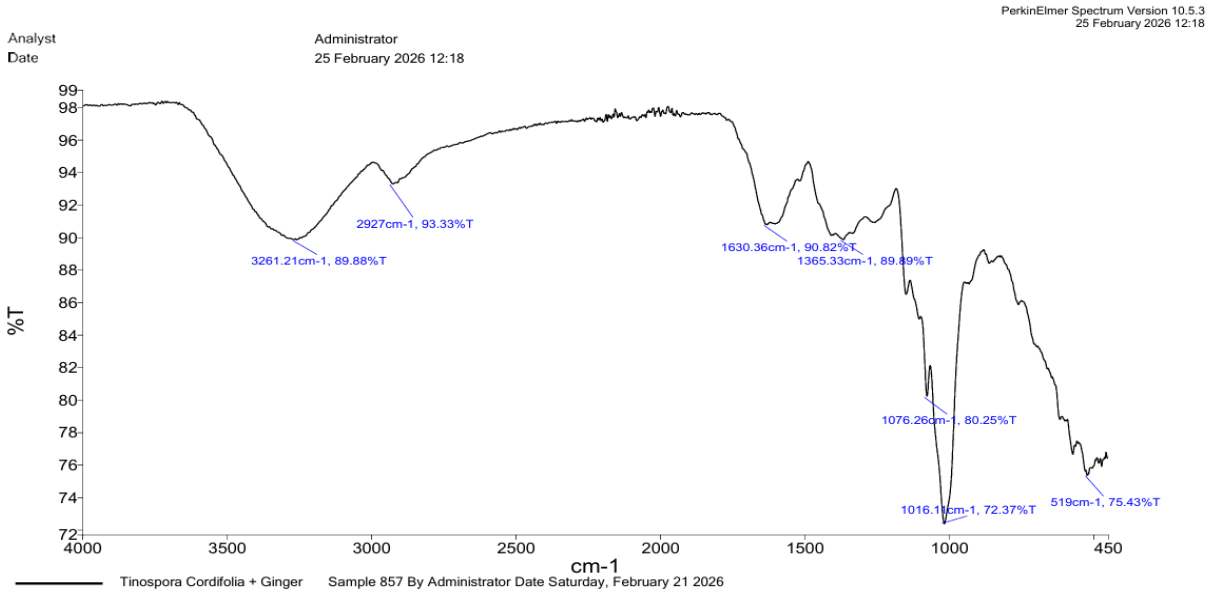
TINOSPORA CORDIFOLIA



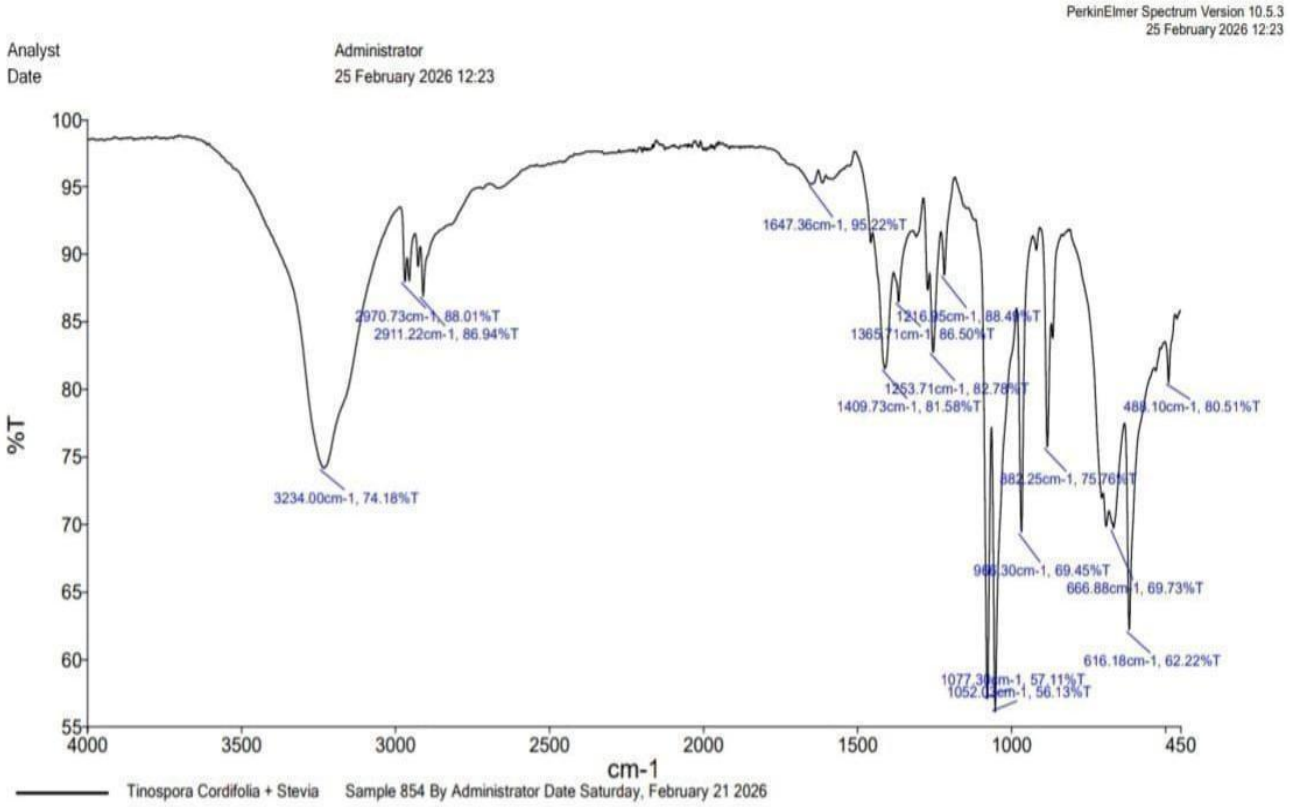
TINOSPORA CORDIFOLIA + GARLIC



TINOSPORA CORDIFOLIA + GREEN TEA



TINOSPORA + GINGER



TINOSPORA CORDIFOLIA + STEVIA

DISCUSSION

Because F2's ratio of herbal extracts, solvent system, and excipients was tuned, it demonstrated superior physicochemical stability and general acceptability across all formulations. The solubility and even dispersion of phytoconstituents in the elixir were enhanced by the balanced concentrations of ethanol and propylene glycol. The pH of F2 showed high stability and administration appropriateness, falling within the permitted range for oral liquid formulations. Additionally, the formulation demonstrated suitable density, viscosity, and refractive index, indicating improved pourability and homogeneity. Stable absorbance was revealed by UV spectrophotometric measurement at: λ max = 277.5 nm

Results showed that the active phytoconstituents were stable and did not significantly degrade. The antioxidant phytoconstituents included in green tea, ginger, garlic, and Tinospora cordifolia may enhance the formulation's stability and therapeutic efficacy.

. CONCLUSION

For the treatment of obesity and diabetes mellitus, the current study effectively concentrated on the creation and assessment of a multipurpose polyherbal elixir using Tinospora cordifolia as the main ingredient, along with ginger, garlic, green tea, and stevia. These herbal components were chosen because of their documented anti-diabetic, antioxidant, hypolipidemic, thermogenic, and immunomodulatory properties. (pmc.ncbi.nlm.nih.gov)

Important bioactive components like alkaloids, flavonoids, tannins, glycosides, phenols, and saponins were found in the extracts, according to preliminary phytochemical screening. It is well recognized that these phytoconstituents are important in lowering oxidative stress, boosting therapeutic efficacy in metabolic diseases, regulating lipid metabolism, and improving insulin sensitivity.

The produced polyherbal elixir exhibited acceptable color, odor, taste, pH, viscosity, density, and refractive index, among other organoleptic and physicochemical characteristics. Herbal extracts were compatible with the chosen hydroalcoholic vehicle system, as evidenced by the formulation's physical stability and lack of precipitation or phase separation.

Because of the optimum concentration of ethanol, excipients, and herbal extracts, which enhanced the solubility and uniform distribution of phytoconstituents, F2 showed the best stability and general acceptance of all the formulations. The formulation's pH was within the permitted range for oral liquid dosage forms, suggesting that it was suitable for administration and that there was less likelihood of irritation. UV-visible spectrophotometric analysis demonstrated stable absorbance at:

λ max = 277.5 nm which confirmed the stability and uniformity of the active phytoconstituents present in the formulation.

In terms of science, Tinospora cordifolia has been well documented for its anti-diabetic, antioxidant, and immunomodulatory qualities,

while green tea and ginger promote fat oxidation and metabolic activity to provide thermogenic and anti-obesity benefits. Because of its hypoglycemic and antihyperlipidemic properties, garlic may help lower blood cholesterol and enhance cardiovascular health. Stevia enhanced the formulation's palatability without raising the glucose load and functioned as a natural, non-caloric sweetener appropriate for diabetic patients. Pubmed.ncbi.nlm.nih.gov (www.ncbi.nlm.nih.gov)

The creation of a single multifunctional herbal elixir that combines anti-diabetic, antioxidant, hypolipidemic, and anti-obesity herbs into a stable oral liquid dosage form with enhanced patient compliance is what makes the study novel. In comparison to individual herbal therapy, the synergistic combination of specific herbal medications may offer greater therapeutic efficacy. (Springer.com link)

Thus, compared to traditional synthetic medications, the new polyherbal elixir may be a viable natural therapeutic formulation for the treatment of obesity, diabetes mellitus, and related metabolic problems with maybe fewer adverse effects. To determine the formulation's long-term safety, effectiveness, and commercial application, more pharmacological research, toxicity research, stability research, and clinical trials are required. (www.mdpi.com)

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