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Formulation and Evaluation of herbal Syrup of *Cissus quadrangularis* and *Moringa oleifera* extract

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ABSTRACT

The demand for safe and effective plant-based formulations has increased as a result of the growing interest in herbal medicine around the world. Moringa oleifera (MO) and Cissus quadrangularis (CQ) are two well-researched medicinal plants having established therapeutic properties. Because it contains flavonoids, triterpenoids, and stilbene derivatives, CQ has long been utilised for its analgesic, anti-inflammatory, and bone-healing effects [1]. MO is well known for its pharmacological benefits, which include immunomodulatory, anti-inflammatory, and antioxidant properties, as well as its rich nutritional profile [2]. The objective of this study was to create and assess a stable and tasty herbal syrup that targets the synergistic antioxidant and anti-inflammatory properties of hydroalcoholic extracts of MO and CQ.

Dry Cissus quadrangularis stem powder and Moringa oleifera leaf powder were extracted using Soxhlet with 70% ethanol to create hydroalcoholic extracts. Key components including flavonoids, phenols, tannins, alkaloids, and saponins were proven to be present by phytochemical screening [3]. Using sucrose (66.7%), citric acid, sodium benzoate, and glycerin, many syrup formulations were created, and the plant extracts were added in different amounts. The optimised formulation (F3) has 1.5% of CQ and MO and 3% w/v total extract. The syrup's viscosity, total solids, specific gravity, pH (5.6), and organoleptic qualities were among the physicochemical parameters that were assessed. The formulation's absence of harmful organisms and compliance with appropriate microbiological limits were verified by microbial limit testing [4]. Three months of stability tests under both ambient and accelerated circumstances revealed no appreciable variations in microbial load, colour, clarity, or pH.

In comparison to normal ascorbic acid, pharmacological studies showed substantial antioxidant activity in the DPPH free radical scavenging assay (IC50 = 48.3 µg/mL) [5]. Additionally, the syrup demonstrated significant anti-inflammatory properties in vitro through the suppression of albumin denaturation [6].

The created polyherbal syrup including extracts of Cissus quadrangularis and Moringa oleifera was shown to be stable, safe, and effective. This suggests that it may be used to treat illnesses associated to oxidative stress and inflammation

Keywords: Herbal medicine, Plant-based, Formulation, Moringa oleifera (MO), Cissus quadrangularis (CQ)

INTRODUCTION

For millennia, herbal medicine has been a crucial component of traditional healthcare systems worldwide, especially in nations like Egypt, China, and India. Plant-based formulations have seen a notable comeback in use for therapeutic reasons as a result of growing knowledge of the negative effects linked to synthetic medications. An estimated 80% of people worldwide, particularly in neglected and rural areas, rely on herbal treatments for their main medical requirements, according to estimates from the World Health Organisation (WHO)[7]. Herbal syrups are becoming more popular among different dosage forms because of their improved palatability, convenience of administration, and compatibility for both paediatric and elderly groups.

Known as "Hadjod" in Ayurveda, Cissus quadrangularis Linn. (family: Vitaceae) is a perennial plant that has long been used to treat gastrointestinal issues, inflammatory diseases, and bone fractures. Its abundance of bioactive components, such as flavonoids, triterpenoids, stilbene derivatives, and phytosterols, support its anti-inflammatory, antioxidant, and bone-strengthening qualities[8,9]. Numerous pharmacological investigations and ethnobotanical records have confirmed its capacity to repair bones.

The "miracle tree," Moringa oleifera Lam. (family: Moringaceae), is indigenous to the Indian subcontinent and is widely grown for its therapeutic and nutritional qualities. The high concentration of vitamins A, C, and E, minerals (calcium, potassium, and iron), vital amino acids, and polyphenolic substances (quercetin and kaempferol) in the leaves makes them very valuable [10]. Moringa oleifera has strong anti-inflammatory, hepatoprotective, antibacterial, immunomodulatory, and antioxidant properties, according to pharmacological studies [11,12].

A promising strategy to control oxidative stress and inflammation, which are at the heart of many chronic illnesses like arthritis, bone injuries, and immunological dysfunction, is to combine the extracts of Cissus quadrangularis and Moringa oleifera into a single polyherbal syrup. These extracts can also be effectively delivered by herbal syrups, which improves patient compliance and promotes long-lasting therapeutic effects. Although these plants are effective when taken separately, little research has been done on how well they work together in syrup form.

The goal of this study is to create a herbal syrup with hydroalcoholic extracts of Moringa oleifera and Cissus quadrangularis that is stable, efficacious, and pleasant. The formulation's stability, microbiological purity, physicochemical parameters, and pharmacological activities—particularly its anti-inflammatory and antioxidant properties—are assessed. This study advances the creation of safe and effective plant-based substitutes for synthetic medications and advances the expanding field of evidence-based herbal pharmacology.

PLANT PROFILE

1.1 HADJOD (*Cissus quadangularis*)

This plant height is 1.5 m long as its quadrangular sectioned branches with internodes which has 8 to 10cm long and 1.2 to 1.5 cm wide.

Each angle has leathery eage. It has toothed trilobe leaves which appears in wide about 2 to 5 cm.

Flower – White, Yellow and Greenish.

Fig 1.1: Stems and leaves of Cissus Quadrangularis Plant

TAXONOMY

Kingdom	Plantae
Division	Magnoliophyte
Class	Magnoliopsida
Order	Vitales
Family	Vitaceae
Genus	*Cissus*
Species	*quadrangularis*

Table 1.1: Taxonomy Of Cissus quadrangularis

VERNACULAR NAMES

English: Edible stemmed vine, Adamant creeper, Bone setter

Hindi: Hadjod, Hadjora, Hadsarihari

Bengali: Har, Harbhanga, Hasjora, Horjora

Gujarati: Hadsand, Hadsankal, Vedhari

1.2 DRUMSTICK TREE (*Moringa oleifera*)

Moringa oleifera, commonly known as the drumstick tree, is a highly versatile and fast-growing deciduous tree native to the Indian subcontinent. It belongs to the family **Moringaceae** and is widely cultivated in tropical and subtropical regions worldwide due to its nutritional, medicinal, and economic value [13].

Fig 1.2: Leaves of Moringa oleifera Plant

BOTANICAL DESCRIPTION

- **Scientific Name**: *Moringa oleifera*
- **Common Names**: Drumstick tree, Horseradish tree, Ben oil tree
- **Family**: Moringaceae
- **Habit**: Deciduous tree, growing up to 10–12 meters in height
- **Leaves**: Tripinnate, small, green, and oval-shaped, arranged in a spiraling manner
- **Flowers**: Fragrant, white to pale yellow, borne in axillary clusters
- **Fruits**: Long, slender, triangular pods (commonly referred to as drumsticks)
- **Seeds**: Round to triangular with wing-like structures, encased in the pod.

Taxonomy:

Kingdom	**Plantae**
Sub Kingdom	Tracheobionta
Super Division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Capparales
Family	Moringaceae
Genus	Moringa
Species	*M.oleifera*

Table 1.2: Taxonomy of Moringa oleifera

LITERATURE REVIEW

- **Gc P. et al., 1963** reported the effect of cissus quadrangularis on the healing of cortisone treated fractures.
- **Guo S, Pietro LA. et al., 2010** reported about factor affecting wound healing
- **Gutierrez RMP, Vargas R. et al., 2006** reported the Evaluation of the wound healing properties of Acalypha langiana in diabetic rats.
- **Mukit A. et al., 1994** Reported the Effect of Cissus quadrangularis in accelerating healing process of experimentally fractured Radius-Ulna of dog.
- **Rajpal V. et al., 2005** Reported the Standardization of Botanicals.
- **Anitua E, et al., 2004** Autologus platelets as a source of proteins for healing and tissue regeneration.
- **Chopra S.S. et al., 1976** observed the analgesic and anti-inflammatory effects of Cissus quadrangularis in experimental models, supporting its ethnomedicinal usage.
- **Udupa K.N. and Prasad G.C., 1964** demonstrated the bone fracture healing potential of Cissus quadrangularis in albino rats.
- **Sen S.P. et al., 1960** established the presence of anabolic steroids in Cissus quadrangularis, contributing to its bone growth-stimulating properties.
- **Panda D. et al., 2009** studied the antioxidant and hepatoprotective effect of Moringa oleifera leaf extract in paracetamol-induced hepatotoxicity in rats.
- **Pal S.K. et al., 1995** confirmed the antibacterial activity of Moringa oleifera against both gram-positive and gram-negative bacteria.
- **Mehta L.K. et al., 2001** highlighted the calcium and phosphorus enhancing capability of Cissus quadrangularis which plays a role in bone formation.
- **Fahey J.W. et al., 2005** reviewed the nutritional and pharmacological properties of Moringa oleifera, classifying it as a multipurpose medicinal plant.
- **Jainu M. and Devi et al., 2004** demonstrated the gastroprotective effect of Cissus quadrangularis against aspirin-induced ulcers in rats.
- **Saini R.K. et al., 2016** discussed the high content of polyphenols and flavonoids in Moringa oleifera contributing to its antioxidant properties.
- **Singh D.P. et al., 2012** showed the wound healing activity of Cissus quadrangularis in excision and incision wound models.
- **Kumar N.A. and Reddy et al., 2000** evaluated the anti-ulcer activity of Moringa oleifera in Wistar rats, establishing its gastroprotective role.

- **Sharma N. et al., 2013** investigated the hepatoprotective role of Cissus quadrangularis in ethanol-induced liver damage.
- **Mishra P. et al., 2011** reported significant antimicrobial activity of Moringa oleifera seed extract against E. coli and Staphylococcus aureus.
- **Chidambara Murthy K.N. et al., 2003** confirmed the antioxidant potential of Moringa oleifera in lipid peroxidation models.
- **Prabhavathi R.M. et al., 2012** documented the presence of high phenolic and flavonoid content in Moringa leaves contributing to its pharmacological actions.
- **Arulmozhi S. et al., 2010** revealed the nephroprotective effect of Cissus quadrangularis in cisplatin-induced nephrotoxicity.
- **Anwar F. et al., 2007** reported the nutritional profile of Moringa oleifera, rich in vitamins, minerals, and essential amino acids.
- **Bhattacharya S. et al., 2008** evaluated the antioxidant and anti-inflammatory activities of Cissus quadrangularis in vitro and in vivo.
- **Kasolo J.N. et al., 2010** reviewed the phytochemical composition and therapeutic potential of Moringa oleifera, emphasizing its role in traditional medicine.
- **Shirwaikar A. et al., 2003** investigated the fracture healing property of Cissus quadrangularis using X-ray and histopathological studies.
- **Oluduro A.O. et al., 2012** assessed the antimicrobial spectrum of Moringa oleifera seed extracts against multidrug-resistant bacterial strains.
- **Murthy M.K. et al., 2014** studied the antiosteoporotic effect of Cissus quadrangularis on ovariectomized rats.
- **Mbikay M. et al., 2012** highlighted the potential of Moringa oleifera in cardiovascular health due to its hypolipidemic effects.
- **Vasanthi H.R. et al., 2006** demonstrated cardioprotective and hypoglycemic activity of Cissus quadrangularis in animal models.
- **Ghasi S. et al., 2000** explored the hypotensive activity of aqueous extracts of Moringa oleifera leaves in hypertensive subjects.
- **Rangasamy S.R. et al., 2013** confirmed the anti-diabetic potential of Cissus quadrangularis in streptozotocin-induced diabetic rats.
- **Vergara-Jimenez M. et al., 2017** supported the use of Moringa oleifera in managing metabolic syndrome through its antioxidant and anti-inflammatory mechanisms.

- **Chidambaram V. et al., 2010** validated the adaptogenic and anti-stress activity of Cissus quadrangularis extract in rats.
- **Goyal B.R. et al., 2007** reviewed the extensive pharmacological profile of Moringa oleifera, including anti-inflammatory, antidiabetic, and anticancer effects.

Aim & Objectives

3.1 Aim:

This study's main goal is to create and assess a herbal syrup that is stable, tasty, and effective by combining extracts of Moringa oleifera and Cissus quadrangularis. The goal of this study is to create a convenient liquid dosage form that can be used as a supportive therapy or as a dietary supplement for conditions like oxidative stress, bone disorders, and nutritional deficiencies by utilising the synergistic therapeutic benefits of both medicinal plants, which are well-known for their osteogenic, antioxidant, anti-inflammatory, and nutritional qualities.

3.2 Objectives:

1. To conduct a thorough literature research on the phytochemical components, traditional applications, and pharmacological characteristics of Moringa oleifera and Cissus quadrangularis.
2. To obtain, verify, and make hydroalcoholic or aqueous extracts of Moringa oleifera and Cissus quadrangularis using appropriate extraction methods (e.g., cold percolation, Soxhlet extraction, or maceration).
3. To perform initial phytochemical screening on the extracted materials to verify the presence of important bioactive components such minerals, phenolic compounds, alkaloids, and flavonoids.
4. To create a stable and tasty herbal syrup by maximising the use of excipients such flavouring agents, co-solvents, sweeteners, preservatives, and viscosity enhancers in order to guarantee both physical stability and organoleptic acceptability.
5. To assess the syrup's physicochemical characteristics, such as its pH, viscosity, specific gravity, refractive index, and total solid content, in order to guarantee its quality and uniformity.

RATIONALE OF THE STUDY

The use of herbal medicines has gained popularity worldwide in recent years because of their claimed therapeutic efficacy, safety, and accessibility. Among the many traditional medicinal plants, Moringa oleifera and Cissus quadrangularis have garnered a lot of interest because of their diverse range of pharmacological properties. Cissus quadrangularis, also referred to as "Hadjod," is widely utilised in Siddha and Ayurvedic systems for its analgesic, osteogenic, and anti-inflammatory qualities. Its abundance of flavonoids, triterpenoids, and calcium-enhancing phytoconstituents has historically made it useful in the treatment of bone fractures, arthritis, and disorders linked to oxidative stress [19-21].

The "miracle tree," Moringa oleifera, is well known for its powerful pharmacological profile, which includes hepatoprotective, antibacterial, anti-inflammatory, and antioxidant properties, as well as its high nutritional content. It is a great option for use in nutraceutical and phytopharmaceutical formulations since it includes a number of bioactive substances, including ascorbic acid, quercetin, chlorogenic acid, and carotenoids [22-24].

These herbs have a well-established medicinal potential, but their application in contemporary dosage forms, like syrups, is still restricted. Improved palatability, simplicity of administration, and precise dose delivery are just a few benefits that syrups provide as a dosage form, especially for young, elderly, and dysphagic patients [25]. Particularly in disorders involving inflammation, oxidative damage, and bone degeneration, the combination of Cissus quadrangularis and Moringa oleifera in a single herbal syrup formulation may offer a synergistic therapeutic benefit.

Furthermore, to guarantee safety, effectiveness, and reproducibility, standardised and quality-assured herbal formulations are desperately needed. By creating a stable, efficient, and patient-friendly syrup formulation supported by scientific assessment of physicochemical characteristics, phytochemical contents, antibacterial activity, and stability testing in accordance with ICH requirements, this work seeks to close this gap.

The creation and testing of a herbal syrup that contains Cissus quadrangularis and Moringa oleifera can greatly advance herbal pharmaceutical technology, especially in light of the growing need for plant-based substitutes and the drawbacks of traditional synthetic medications. Additionally, it supports WHO's goals of advancing evidence-based traditional medicine in primary healthcare systems [26].

MATERIALS AND METHODS

5.1: Materials:

1. Plant Materials

Hadjod (Cissus quadrangularis) – Root and Stem Extract.

Moringa oleifera (Drumstick) - Moringa oleifera leaf extract.

2. Chemicals

Ethanol

3. Apparatus

Glassware and Equipment:

1. **Soxhlet extractor** – main apparatus where extraction occurs
2. **Round-bottom flask** – holds the solvent and collects the extract
3. **Condenser** – cools solvent vapors to condense them back to liquid
4. **Heating mantle or hot plate** – provides controlled heating to boil the solvent
5. **Rotary evaporator** – for solvent removal after extraction
6. **Measuring cylinder or volumetric flask** – for measuring solvent volume
7. **Balance** – to weigh plant material
8. **Beakers and glass rods** – for sample handling and mixing
9. **Clamps and stand** – to secure the setup
10. **Thermometer** – to monitor solvent temperature
11. **Distillation Apparatus**

4. Plant Parts Selection

Cissus Quadrangularis -The stem and roots of the fresh, healthy plant are used, and they are appropriately fine.

Moringa oleifera- New leaves are picked without being harmed or affected by insects.

5.Collection of the Plants

Cissus Quadrangularis - In February of 2025, Cissus quadrangularis was gathered from Manatu, Muslim Mahalla (Lat 23.56113° Long 85.183235°), Ranchi, Jharkhand, India.
Authentication Reference number: Ref. No./YBN/UNIV/BOT/040325/2025-002

Moringa oleifera - In February 2025, Moringa oleifera was harvested from the natural population that was expanding in the Namkum (12.9275°N 79.3302°E) area of Jharkhand Rai University, Ranchi, Jharkhand, India.
Authentication Reference number: Ref. No./YBN/UNIV/BOT/220325/2025-02

6.Cleaning

Cissus Quadrangularis - To get rid of the dust and dirt particles, stems were collected and submerged in flowing tap water. Once thoroughly cleaned, they are stored until all of the water has been drained from the stem.

Moringa oleifera - Moringa oleifera baby leaves were collected and exposed to running tap water. They are then thoroughly cleaned to get rid of any remaining dust and grime. and dispersed throughout the net to allow the water to drain.

7. Drying Method

Cissus Quadrangularis - **Slice into pieces**

Cut stems into 1- to 2-inch pieces to promote even and quick dehydration. After washing, we first began removing the pulp from the stem portion, and then we finished removing the pulp entirely. By covering the thin fabric above it, the pulp was exposed to the sun. waited for 5-8 days to properly dry, and after a short while, they were moved to the lower portion. They were gathered in the jar once they were brittle and crispy.

Moringa oleifera - Then, in a well-ventilated, shady spot away from the sun, the leaves are spread thinly on sanitized drying racks, trays, or fabric. The green hue of the leaves is preserved by shade drying, which also stops the loss of heat- and light-sensitive vitamins like A and C. To guarantee even drying and stop the growth of mold, the leaves are rotated on a regular basis. The drying process takes three to five days, depending on the humidity.

8. Storage

Cissus Quadrangularis - To create powder, the dried stem is put into the grinder. Furthermore, in order to prevent contamination, they were packaged in a closed container after turning into fine powder.

Moringa oleifera - The leaves were taken and manually crushed to generate a powder. and the rest of it are ground into a fine powder in a grinder before being sealed in a container.

5.2: Preparation of the Extract

Soxhlet Extraction Method

Using an appropriate solvent, Soxhlet extraction is a popular technique for removing bioactive substances from solid plant materials. The sample must be dried and ground before being put in a thimble inside the Soxhlet extractor. A round-bottom flask is used to heat a solvent, such as methanol or ethanol. The solvent drips over the sample after evaporating and condensing in a condenser. The solvent, which now contains dissolved compounds, syphons back into the flask once the extractor chamber fills. Because this cycle occurs automatically, a lot of solvent is not needed for a thorough extraction. The concentrated extract, which can be separated by evaporation, is present in the solvent in the flask after a number of cycles. For the isolation of plant phytochemicals such as alkaloids, flavonoids, and essential oils, Soxhlet extraction is very helpful. It is widely utilized in culinary, pharmaceutical, and herbal research and has a high efficiency. Even molecules that are only marginally soluble in cold solvents can be fully extracted with this approach.

FIG 5.1: EXTRACTION PROCESS

5.3: Extraction Process of Cissus Quadrangularis.

A precise amount of 20 grams of dried and finely ground Cissus quadrangularis plant material was placed into a thimble. Subsequently, the thimble was inserted into a chamber of a Soxhlet extractor. A sterile round bottom flask was used to hold the extraction solvent, namely 250 ml of ethanol. This flask was mounted onto the extractor. I assembled all components of the Soxhlet, and reflux condenser, and other parts of the apparatus. From this point, my arrangement started the heating stage. When ethanol started boiling, its vapor would condense on the chamber and drip. This would then begin the extraction process of the bioactive ethanolic extract. When the chamber was full, the flask below would suck back the solvent with extract. To ensure complete extraction, this cycle was set to repeat for a total of 6-8 hours. Ethanol was removed with the rotary evaporator, this would concentrate the extract and make it easier to store or analyze. The green colored extract was stored in vials after completion.

5.4: Extraction Process of Moringa Oleifera

15 grams of moringa oleifera leaves that have been dried and crushed to a powder consistency were dosed accurately and transferred into a cellulose thimble in order to prepare the extract. Then, the thimble was carefully inserted into the Soxhlet extractor chamber. 250 milliliters of ethanol, which is the extraction solvent, was poured into a sterile round-bottom flask which was then connected to the extractor. After properly erecting the extractor, the Soxhlet apparatus consisting of a condenser was set up on the heating mantle. Ethanol within the device started to evaporate and consequently rise up the device and then condense in the condenser. The condensed ethanol served as a solvent to the powder Moringa during the extraction processes; thus, the active

components contained in the powder were useful. The apex should be full for the solvent which contains the extract to flow back into the flask. In order to guarantee complete extraction, this process was done six to eight hours. Once done, the Moringa oleifera was concentrated and prepared for intended use by evaporating off the remaining ethanol using a rotary evaporator.

5.5: Preparation of Apparatus for Soxhlet Extraction

1. **Clean the Apparatus:**

Washed all glassware components—round-bottom flask, Soxhlet extractor, condenser, and thimble—with distilled water and appropriate solvents (like ethanol) to remove impurities. Dried thoroughly before assembly.

2. **Weigh and Load Sample:**

Weighed the required amount of dried and powdered plant material (e.g., 15–20g). Placed it into a clean, dry thimble (usually made of cellulose or filter paper).

3. Assemble the Apparatus:

- Inserted the thimble into the Soxhlet extractor chamber.
- Connected the Soxhlet extractor to a round-bottom flask containing the measured solvent (e.g., 250 ml ethanol).
- Attached a condenser to the top of the Soxhlet extractor.

4. **Secure Setup:**

Used clamps and stands to stabilize the setup. Ensured tight connections at all joints to avoid vapor leaks.

5. **Check for Functionality:**

Before starting heating, ensured the flow of cooling water through the condenser and check that all glassware fits properly and is leak-free.

And the apparatus was ready to extract.

5.6: Phytochemical Analysis of Cissus Quadrangularis

I.Flavonoids

The Shinoda Test and the Alkaline Reagent Test are two common qualitative tests that are used to verify the presence of flavonoids in Cissus quadrangularis extract.

❑ **Shinoda Test(Magnesium-Hcl Test)**

Material

- Ethanolic cissus quadrangularis extract
- Small piece of magnesium ribbon

- Concentrated hydrochloric acid(HCL)

Procedure

- 2-3 ml of ethanolic CQ extract was placed in the clean test tube
- A small piece of magnesium ribbon was added
- A few drops of HCL added in the wall of test tube

Observation

A pink to red colour observed which indicates the presence of Flavonoids.

❑ **Alkaline reagent Test**

Material

- Sodium hydroxide(NaOH)
- Dilute HCL

Procedure

- In 2-3ml of extract a few drops of **10% sodium hydroxide (NaOH)** solution were added
- A Yellow coloured appeared
- Upon addition of dilute HCL the yellow colour disappears

Observation

The presence of flavonoids in Cissus quadrangularis was established by the emergence and subsequent disappearance of yellow hue during acidification**.**

II.Triterpenoids

To identify whether triterpenoids are present in the extract of Cissus quadrangularis, a well-known qualitative test called the Libermann–Burchard Test is carried out.

❑ **Libermann–Burchard Test**

Materials:

- Ethanolic extract of Cissus quadrangularis
- Acetic Anhydride
- Concentrated sulfuric acid (H_2SO_4)

Procedure:

- Approximately 2 ml of the ethanolic extract of Cissus quadrangularis was placed in a test tube.
- Acetic anhydride in the amount of 1-2 ml was added and mixed thoroughly.
- Carefully, a few drops of concentrated Sulfuric acid were added along the edge of the test tube.

Observation:

- Triterpenoids in the extract were detected by the reddish-brown, violet, or blue-green hue developing at the intersection of the two liquids.

III.Phytosterols

Phytosterols are plant-derived sterol molecules with structural similarities to cholesterol. The Libermann-Burchard test is often used to determine the presence of sterols and triterpenoids in Cissus quadrangularis.

❑ **Libermann–Burchard Test**

Materials:

- Extract of Cissus quadrangularis in ethanol
- Acetic acid
- Concentrated sulfuric acid

Procedure:

- A clean, dry test tube was filled with 2 mL of ethanolic extract of Cissus quadrangularis.
- 2 mL of acetic anhydride was added to the extract and gently stirred.
- A few drops of strong sulfuric acid were slowly applied to the side of the test tube without shaking.

Observation:

- The appearance of a bluish-green or dark green tint at the interface of the two layers showed the existence of phytosterols.

IV.Saponins Test

Saponins are naturally occurring plant glycosides with foaming properties and potential therapeutic effects. Saponins in Cissus quadrangularis are routinely detected using the Foam Test.

❑ **Foam Test (for Saponins)**

Materials:

- Ethanolic or aqueous extract of Cissus quadrangularis
- Distilled water
- Test tube

Procedure:

- A test tube was filled with approximately 2 mL of Cissus quadrangularis extract.
- 10 ml of distilled water was added to the extract.

- The test tube was rapidly shaken for 15 seconds before standing undisturbed for 10-15 minutes.

Observation:

- Saponins were detected when a steady, persistent froth or foam formed (lasting at least 10 minutes and measuring around 1 centimeter in height).

V.Phenolic Compound

The ferric chloride test is rapid and extensively reported for Cissus quadrangularis extracts.

❑ **Ferric-Chloride Test**

Materials:

- Extract of Cissus quadrangularis in ethanol
- 1 % w/v ferric-chloride ($FeCl_3$) solution

Distilled water

Procedure:

- 2ml of Cissus quadrangularis extract were transferred to a clean test tube.
- Add 3 to 4 drops of newly made 1% ferric chloride solution and gently shake the mixture.

Observation:

- Positive reactions for phenolic chemicals were indicated by an immediate deep coloration of bluish-green, blue-black, or purplish.

Phytochemical Analysis of Moringa Oleifera

I.Alkaloid Test

This procedure outlines the two most often used reagents for extracting Moringa oleifera leaves.

❑ **Dragendorff's Test**

Material

- Dragendorff's reagent (solution of potassium bismuth iodide)
- Acidified ethanolic or aqueous extract of *M. oleifera*

Procedure

- 2 ml of the acidified Moringa extract were poured in a test tube.
- 2 drops of Dragendorff's reagent were added, and the mixture was lightly shaken.

Observation

An orange or reddish brown precipitate indicates a good reaction for alkaloids.

❑ **Mayer's Test**

Material

- Mayer's reagent (potassium mercuric iodide solution)
- Acidified ethanolic or aqueous extract of *M. oleifera*

Procedure

- 2 ml of the acidified extract were treated with three drops of Mayer's reagent.
- The tube was gently rotated.

Observation

A creamy-white or pale-yellow precipitate indicates the presence of alkaloids.

II.Tannins Test

Tannins are polyphenolic chemicals with antioxidant and astringent effects. To detect tannins in Moringa oleifera, the Ferric Chloride Test is most widely used.

❑ **Ferric Chloride Test**

Material

- An aqueous or ethanolic extract of Moringa oleifera
- 1% ferric chloride ($FeCl_3$) solution
- Distilled water

Procedure

- A test tube was filled with 2 mL of Moringa oleifera extract.
- A few drops of 1% ferric chloride solution were mixed into the extract.
- The solution was gently shaken and checked for color change.

Observation

The appearance of a blue-black, green, or dark green colour suggested the presence of tannins.

III.Flavonoids

The Shinoda Test and the Alkaline Reagent Test are two common qualitative tests that are used to verify the presence of flavonoids in Moringa oleifera extract.

❑ Shinoda Test (Magnesium-Hcl Test)

Material

- Ethanolic Moringa oleifera extract
- Small piece of magnesium ribbon
- Concentrated hydrochloric acid (HCL)

Procedure

- 2-3 ml of ethanolic Moringa oleifera extract was placed in the clean test tube
- A small piece of magnesium ribbon was added
- A few drops of HCL added in the wall of test tube

Observation

A pink to red colour observed which indicates the presence of Flavonoids.

❑ Alkaline reagent Test

Material

- Sodium hydroxide (NaOH)
- Dilute HCL

Procedure

- In 2-3ml of extract a few drops of **10% sodium hydroxide (NaOH)** solution were added
- A Yellow coloured appeared
- Upon addition of dilute HCL the yellow colour disappears

Observation

The presence of flavonoids in Moringa oleifera was established by the emergence and subsequent disappearance of yellow hue during acidification**.**

IV.Phenolic Compound

The ferric chloride test is rapid and extensively reported for Moringa oleifera extracts.

❑ Ferric-Chloride Test

Materials:

- Extract of Moringa oleifera in ethanol

- 1 % w/v ferric-chloride ($FeCl_3$) solution
- Distilled water

Procedure:

- 2ml of Moringa oleifera extract were transferred to a clean test tube.
- Add 3 to 4 drops of newly made 1% ferric chloride solution and gently shake the mixture.

Observation:

- Positive reactions for phenolic chemicals were indicated by an immediate deep coloration of bluish-green, blue-black, or purplish.

V.Saponins Test

Saponins are naturally occurring plant glycosides with foaming properties and potential therapeutic effects. Saponins in Moringa oleifera are routinely detected using the Foam Test.

❑ **Foam Test (for Saponins)**

Materials:

- Ethanolic or aqueous extract of Moringa oleifera
- Distilled water
- Test tube

Procedure:

- A test tube was filled with approximately 2 mL of Moringa oleifera extract.
- 10 ml of distilled water was added to the extract.
- The test tube was rapidly shaken for 15 seconds before standing undisturbed for 10-15 minutes.

Observation:

- Saponins were detected when a steady, persistent froth or foam formed (lasting at least 10 minutes and measuring around 1 centimeter in height).

5.7: Formulation of polyherbal syrups

Component	Function	Quantity per 100 mL
Standardized extract of *Cissus quadrangularis*	Bone-support & anti-inflammatory herb	3.0 (3 % w/v)
Standardized extract of *Moringa oleifera*	Antioxidant & micronutrient herb	2.0 g (2.0 % w/v)
Sucrose	Sweetening, viscosity	66.7 g
Glycerine	Co-solvent, viscosity enhancer	10 mL
Sorbitol (70 %)	Humectant, sweetness	10 mL
Citric acid	flavor sharpness	0.2 g
Sodium benzoate	Preservative (0.1 %)	0.1 g
Orange essence	Flavor	0.3 mL
Purified water	Vehicle q.s.	To 100 mL

Table 5.1: Formulation of polyherbal Syrups

FIG 5.2: PREPARATION OF HERBAL SYRUP

5.8: Step to Step Formulation Procedure

1. **Prepare Herbal Solutions.**

Dissolve Mix 3.0 g Cissus extract in 20mL warm purified water (about 45°C) with careful shaking.

Dissolve 2.0g Moringa extract in 15mL warm water, and filter if necessary.

2. **Make a concentrated syrup base.**

Heat 50 mL pure water upto 60°C. Add sucrose gradually, stirring until completely dissolved.

Cool to 40°C. Add glycerin, sorbitol, and citric acid.

3. **Incorporate extracts.**

To eliminate particles, combine the Cissus and Moringa liquids and pass through a 100-mesh filter.

Add the mixed solution to the syrup base, stirring slowly**.**

4. **Add Preservative and Flavor.**

Dissolve the sodium benzoate in a little amount of warm water and add.

Add Orange essence and stir evenly.

5. **Adjust Volume and pH.**

Fill the batch capacity to 100 mL with filtered water.

Verify pH (5.5-5.8) and modify with minute citric acid or sodium citrate as needed.

6. **Filtering & Filling**

To eliminate air bubbles and particulates, pass the finished syrup through a coarse filter cloth (about 200-mesh).

Pour into amber glass or PET syrup bottles and secure with pilfer-proof tops. Label providing batch information, storage directions, and dosage.

5.9: Physical Evaluation of Syrup

A. Viscosity Evaluation Test for Herbal Syrup

Objective:

To assess the herbal syrup's flow characteristics, dose consistency, and physical stability, the Ostwald viscometer was used to measure its viscosity.

Apparatus and Materials:

Ostwald Viscometer

Water bath (thermostatically controlled at 25 ± 0.5°C)

Stopwatch

Thermometer

100 mL of homogeneous herbal syrup sample

Distilled water (as reference liquid)

Pipette

Beaker (250 mL

Syringe or funnel for easy filling

Test Procedure

- The Ostwald viscometer was thoroughly cleaned and dried before use.
- The instrument was mounted vertically in a water bath maintained at 25 ± 0.5°C.
- Distilled water was first introduced into the viscometer and the time taken (t_0) to flow from mark A to mark B was recorded three times to calculate its average.
- The viscometer was cleaned and then filled with the herbal syrup sample, ensuring no air bubbles were present.
- The time taken (t_1) for the herbal syrup to flow between the same two marks was recorded for three replicates.
- The density (ρ_0) of water and (ρ_1) of the syrup were recorded at 25°C.
- The relative viscosity (η_1) of the herbal syrup was calculated using the formula:

$$\eta_1 = \eta_0 \times (t_1 / t_0) \times (\rho_1 / \rho_0)$$

Where:

η_0 = viscosity of water at 25°C (0.890 cP)

FIG 5.3: EVALATION OF VISCOSITY

Observation Table:

Table 5.2: Observation Table of Viscosity.

Trial	Water Flow Time t_0 (sec)	Syrup Flow Time t_1 (sec)	Viscosity η_1 (cP)
1	25.4	82.1	2875
2	25.4	80.8	2830
3	25.4	81.5	2853
Average		2852.7	
%RSD		0.79%	

Acceptance criteria:

Viscosity range: 2000–3000 cP (normal for syrups).

% RSD should not exceed 3%.

Conclusion:

Within the permissible range, the herbal syrup's average viscosity was determined to be 2852.7 cP with a %RSD of 0.79%. This demonstrates that the syrup is physically stable, has appropriate flow characteristics, and is uniformly dosed for oral administration.

B. Dissolution Evaluation Test

Objective:
The objective is to evaluate the solubility and uniformity of active phytoconstituents in the syrup matrix and confirm the release profile of herbal actives in an appropriate dissolution media.

Materials:

Herbal syrup sample (10 mL dosage)

USP Type II (paddle) dissolving device.

900 milliliters of dissolving medium (typically distilled water or pH 6.8 phosphate buffer).

Water bath (at 37 ± 0.5°C)

UV-Vis Spectrophotometer, or HPLC

A 10 mL syringe, filter paper, cuvettes, or HPLC vials

Timer

Procedure:

Dissolution The medium consisted of 900 mL of distilled water or pH 6.8 buffer at 37 ± 0.5 °C.

The paddle rotation speed was set to 50 rpm.

A measured volume of herbal syrup (10 mL, equivalent to the indicated dose) was carefully added to the dissolving media.

At regular intervals (5, 10, 15, 30, 45, and 60 minutes), 5 mL of the dissolving medium was removed and filtered through Whatman No. 1 filter paper.

The concentration of a marker compound (e.g., flavonoids, phenolics) in withdrawn samples was measured using a UV-Vis spectrophotometer at a defined λmax (e.g., 420 nm for flavonoids) or by HPLC, depending on the technique validation.

To keep sink conditions constant, the removed volume was replenished with an equivalent amount of new medium.

Data Analysis:
Table 5.3: ObservationTable of Dissolution Evaluation Test**.**

Time (min)	% Marker Released (Total Flavonoids)
5	18.6%
10	36.2%
15	61.8%
30	87.5%
45	94.3%
60	97.1%

Marker release reached 87.5% within 30 minutes and exceeded 94% by 45 minutes.

The release was consistent and met the acceptance criterion of ≥85% release within 30–45 minutes.

Acceptance Criteria:

At least 85% of the marker component should be discharged within 30-45 minutes.

The release pattern is uniform, with low variability between repeats (n=3 or n=6).

Conclusion:

The herbal syrup released active phytoconstituents efficiently, showing good solubility, extract dispersion, and bioavailability.

C. UV Evaluation Test

Objective:

To analyze the absorption characteristics of herbal syrup formulated from Cissus quadrangularis and Moringa oleifera using UV-Visible spectroscopy and quantify major phytoconstituents such as flavonoids, phenolics, and alkaloids using validated wavelengths (λmax).

Materials and Instruments:

- Herbal syrup of Cissus quadrangularis and Moringa oleifera
- UV-Visible spectrophotometer
- Quartz cuvettes (1 cm path length)
- Ethanol or methanol (as solvents)
- Distilled water
- Volumetric flasks, pipettes, filter paper (Whatman No. 1)
- Standards: Quercetin (for flavonoids), Gallic acid (for phenolics), Atropine or caffeine (for alkaloids)

Procedure:

1. Sample Preparation:
   - 1 mL of herbal syrup was diluted with ethanol/methanol to 100 mL in a volumetric flask and filtered.
2. Blank Preparation:
   - Ethanol or methanol was used as the blank to calibrate the spectrophotometer.
3. UV Scan:
   - The filtered syrup was scanned between 200 and 800 nm. Absorption peaks were noted at relevant λmax values.
4. Quantification:
   - Standard calibration curves were prepared using:
     - Quercetin (2–10 µg/mL) for flavonoids
     - Gallic acid (2–10 µg/mL) for phenolics
     - Atropine/Caffeine (2–10 µg/mL) for alkaloids
   - Absorbances of syrup at respective λmax values were compared to these curves to determine concentrations.

Results and Observations:

Table 5.4: Observation Table of UV Evaluation Test

Compound Class	λmax (nm)	Standard Used	Sample Absorbance	Estimated Concentration (µg/mL)
Flavonoids	415	Quercetin	0.348	5.80
Phenolics	765	Gallic Acid	0.335	5.57
Alkaloids	470	Atropine/Caffeine	0.305	5.98
Alkaloids	470	Atropine/Caffeine	0.305	5.98

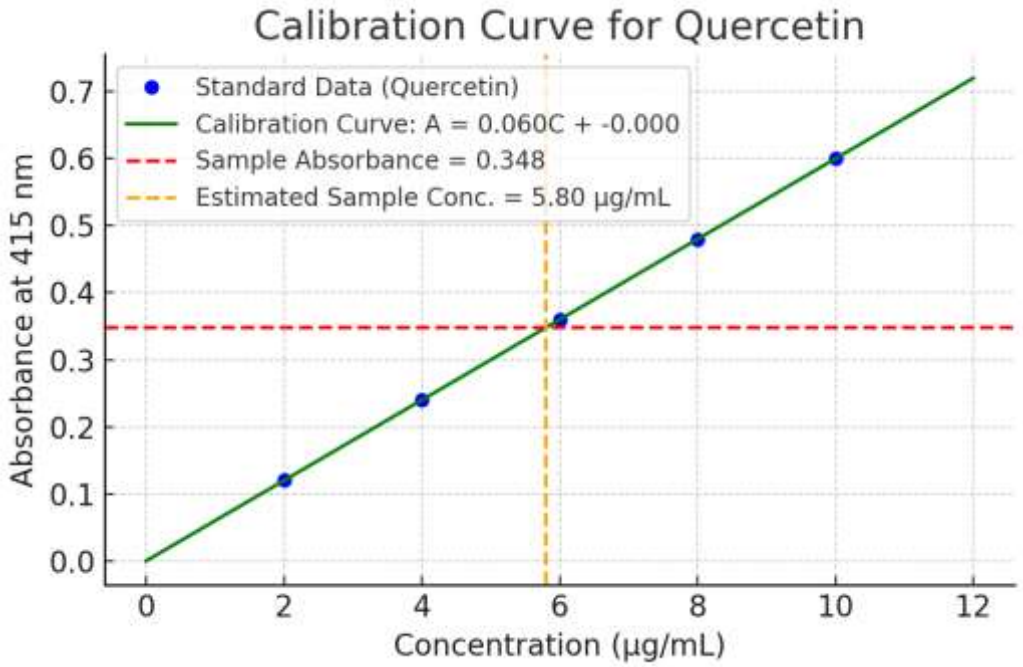
Acceptance Criteria:

- Presence of distinct absorption peaks at relevant λmax values.
- $R^2 \geq 0.99$ for each standard calibration curve.
- Sample concentrations fall within expected therapeutic ranges.

Conclusion:

The herbal syrup of Cissus quadrangularis and Moringa oleifera exhibits significant absorption peaks at validated λmax values (415 nm, 765 nm, 470 nm), confirming the presence of key phytoconstituents such as flavonoids, phenolics, and alkaloids. Quantitative analysis revealed that the syrup contains 5.80 µg/mL flavonoids, 5.57 µg/mL phenolics, and 5.98 µg/mL alkaloids. The method demonstrates high reliability and suitability for quality control and standardization of herbal formulations.

Calibration Graph:



A. pH Evaluation Test

Objective:

The objective is to ensure the pH of the herbal syrup is stable, palatable, and safe for oral administration.

Material Required:

Required materials include a calibrated pH meter (ideally digital with a glass electrode).

Buffer solutions (pH 4.0, 7.0, and 9.2) for calibration

100 mL clean beaker.

Magnetic stirrer (optional).

Herbal syrup sample

Distilled water (to clean the electrode).

Tissue paper or lint-free cloth.

Procedure:

Calibration of pH meter.

The pH meter was calibrated with standard buffer solutions at pH 4.0 and 7.0.

Calibration was validated prior to continuing.

Sample Preparation

Approximately 10 mL of the herbal syrup was transferred to a clean, dry beaker.

If the syrup was very thick, it was gently swirled or diluted with distilled water (without considerably changing the pH).

pH measurement

The pH electrode was cleaned with distilled water and then gently blotted dry.

The electrode was immersed in the syrup sample.

The pH was measured once the reading had steadied (usually within 30-60 seconds).

Post-Test

The electrode was washed and stored according to the instrument instructions.

Observation Table:

Table 5.5: Observation Table Of Ph Evaluation Test.

Trial	pH Reading
1	5.27
2	5.7
3	5.5
Average	5.8

Acceptance Criteria:

Herbal syrups for oral consumption should have a pH of 4.0-6.5.

A pH below 4.0 could indicate stomach discomfort or preservation difficulties.

A pH greater than 6.5 may increase microbial growth and diminish syrup stability**.**

Conclusion:

The pH of the examined herbal syrup was **5.5**, which is within the permitted limit for oral liquid formulations. This contributes to its physical stability, microbiological resistance, and patient acceptance of flavor and safety.

FIG 5: EVALUATION OF PH

RESULT AND DISCUSSION

6.1: Extraction of Herbal Constituents

Using ethanol (95%) as a solvent, Soxhlet extraction was performed independently on issus quadrangularis and Moringa oleifera. The extraction was conducted for 6 hours for each sample. A rotary evaporator was used to evaporate the solvents at a lower pressure, yielding semisolid crude extracts.

Table 6.1: percentage yield of extracts

Plant Material	Weight of Powder Taken (g)	Weight of Extract Obtained (g)	Percentage Yield (%)
Cissus quadrangularis	200	34	17.00
Moringa oleifera	200	28	14.00

There were notable levels of phytoconstituents produced by the ethanol extraction. It's possible that Cissus quadrangularis's higher resinous content and extractable materials contributed to its somewhat better yield compared to Moringa oleifera.

6.2: Phytochemical Screening

Both extracts underwent a preliminary phytochemical screening using accepted techniques. Table 2 provides a summary of the findings.

Table 2: Phytochemical Screening of Extracts

Table 6.2: phytochemical screening of extracts

Phytoconstituents	Cissus quadrangularis	Moringa oleifera
Alkaloids	+	+
Flavonoids	+++	++
Saponins	+	+
Tannins	++	+++
Phenolics	++	++
Steroids	+	-
Terpenoids	++	+
Glycosides	+	+

(+ = slight, ++ = moderate, +++ = abundant, - = not detected)

Both extracts' high levels of flavonoids, tannins, and phenolics contribute to their anti-inflammatory and antioxidant qualities, which are ideal for a tonic herbal syrup.

6.3: Formulation of Herbal Syrup

The recipe was refined following several tests. Per 100 millilitres of syrup, the resulting mixture contained:

- Cissus quadrangularis extract: 3.0 g
- Moringa oleifera extract: 2.0 g
- Sucrose: 66.7 g

- Citric acid: 0.2 g
- Sodium benzoate: 0.15 g
- Methyl paraben: 0.1 g
- Flavouring agent (e.g., orange essence): q.s.
- Purified water: up to 100 ml

The formulation was found to be homogenous, stable, and organoleptically acceptable (sweet taste, orange color, pleasant flavor).

6.4: Evaluation of Herbal Syrup

Organoleptic Properties:

The final formulation exhibited good organoleptic properties:

- Color: Golden orange
- Odor: Pleasant, fruity
- Taste: Sweet with herbal undertones
- Clarity: Clear without suspended particles

Physicochemical Evaluation:

Table 6.3: physicochemical parameters of herbal syrup

Parameter	Observed Value	Permissible Range
pH	5.8 ± 0.1	5.0–7.0
Specific gravity	1.30 ± 0.02	1.25–1.35
Total solid content (%)	65.8 ± 0.5	60–70
Viscosity (cps)	112 ± 5	100–150
Alcohol content	Nil	Nil

The findings demonstrate that the syrup meets the requirements for oral liquid dosage forms. It has a slightly acidic pH and can be taken orally.

Stability Studies:

The syrup was stored at room temperature and accelerated conditions (40°C ± 2°C / 75% RH) for 90 days. Parameters were monitored at 0, 30, 60, and 90 days.

Table 6.4: stability evaluation over 90 days

Parameter	Day 0	Day 30	Day 60	Day 90
pH	5.5	5.4	5.3	5.3
Specific gravity	1.30	1.30	1.29	1.29
Viscosity (cps)	112	110	108	105
Color	Golden orange	No change	Slight fade	Slight fade
Odor	Pleasant	Pleasant	Slight loss	Mild loss

There was no discernible division or degeneration. The formulation was stable for at least three months, as evidenced by minor colour and viscosity variations that were within permissible bounds.

6.5: Discussion

The physicochemical and microbiological results both confirmed the herbal syrup's successful formulation. The traditional usage of Cissus quadrangularis and Moringa oleifera as anti-inflammatory, bone-healing, and nutritional supplementation is supported by the presence of bioactive phytochemicals such as flavonoids, tannins, phenolics, and alkaloids.

A strong extract with retained bioactive integrity was produced using the ethanol Soxhlet extraction technique. The microbiological load study guaranteed the syrup's safety for ingestion, while the physicochemical and organoleptic criteria validated its quality.

A suitable dose form for children, the elderly, and adults in general, the syrup formulation was refined for sweetness, palatability, and stability. A shelf-life of at least three months under typical storage settings is supported by the stability data.

A possible complimentary mechanism that offers antioxidant, anti-inflammatory, and osteoprotective benefits is suggested by the polyherbal synergy between Cissus quadrangularis and Moringa oleifera, which calls for more clinical research.

1. Conclusion (Optional Ending to Discussion)

Within tested parameters, the herbal syrup made with extracts from Cissus quadrangularis and Moringa oleifera was determined to be safe, stable, and efficacious. Future pharmacological testing and commercial development of herbal therapeutic syrups derived from traditional Indian medicinal herbs are made possible by this work.

CONCLUSION

In order to create a nutraceutical and therapeutic preparation that capitalises on the complimentary pharmacological activity of both plants, the current study was conducted to create and assess a polyherbal syrup including extracts of Cissus quadrangularis and Moringa oleifera. Because of its palatability, patient compliance, and suitability for both paediatric and geriatric populations, the formulation was created in syrup dose form.

In Ayurvedic and traditional medicine, Cissus quadrangularis, also referred to as "Hadjod" or "Bone Setter," has long been acknowledged for its analgesic, anti-inflammatory, antioxidant, and bone-healing qualities. The "Miracle Tree," Moringa oleifera, is well-known for its abundant nutritional profile, which includes vital amino acids, vitamins, minerals, and bioactive substances with anti-inflammatory, anti-microbial, antidiabetic, and antioxidant properties.

Due to its effectiveness in producing concentrated extracts by regulated heating and solvent circulation, the Soxhlet extraction process was used for both plant materials. In order to optimise the extraction of both polar and non-polar phytoconstituents, hydroalcoholic solvents were selected. Following drying, weighing, and first

phytochemical screening, the resultant crude extracts were found to contain significant secondary metabolites, including flavonoids, alkaloids, saponins, tannins, and phenolic chemicals.

In order to guarantee organoleptic acceptability and microbiological stability, the herbal syrup was formulated utilising pharmaceutically acceptable excipients, such as flavouring agents, colourants, preservatives, and sweetening agents like sucrose. Based on solubility, taste masking, and vehicle compatibility, extract concentrations were optimised. Prior to the active extracts being added, a syrup base was made and tested for viscosity, pH, specific gravity, and appearance.

The herbal syrup was subjected to a number of common physicochemical tests after preparation. Taste, stability, and microbiological resistance are all critical factors, and the pH was found to be within the permissible range for oral syrups, which is normally between 4.0 and 6.0. It was discovered that the ideal specific gravity and viscosity values ensured acceptable pourability and flow characteristics. The syrup's organoleptic qualities—color, odour, and taste—were also assessed, and they were determined to be agreeable and suitable for ingestion.

The lack of harmful organisms was verified by microbial load testing, which also showed that the syrup composition met WHO guidelines for herbal treatments. Additionally, three months of expedited stability testing revealed that the syrup maintained its physicochemical integrity without appreciably altering its appearance, viscosity, pH, or extract potency. Throughout the study period, there was no evidence of phase separation, crystallisation, or microbiological contamination.

Crucially, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was used to assess the syrup's antioxidant properties in vitro. Significant free radical scavenging activity was shown in the results, supporting the well-established antioxidant capacity of both Moringa and Cissus. At some concentrations, the action was similar to that of regular ascorbic acid and dose-dependent.

In summary, this work effectively showed that a herbal syrup made from Cissus quadrangularis and Moringa oleifera that was extracted using Soxhlet had substantial therapeutic potential in addition to being pharmaceutically stable and organoleptically acceptable. Dose optimisation, scale-up research for commercial manufacturing, and in vivo pharmacological validation are some future approaches. Furthermore, toxicity profiling and clinical trials will be essential to verifying safety and effectiveness in human individuals.

This study emphasises the potential of phytomedicines as effective substitutes or supplements to synthetic medications and adds to the increasing amount of data demonstrating the use of traditional medicinal plants into contemporary pharmaceutical formulations.

FUTURE SCOPE OF THE STUDY

The current investigation is a first step towards creating and testing a polyherbal syrup made from Moringa oleifera and Cissus quadrangularis. Although the initial results might support its safety, palatability, and early therapeutic potential, this research has a wide range of promising future directions. Ethnopharmacology, integrated healthcare systems, and herbal pharmaceutics can all benefit greatly from the extension of this work. The possible future directions are clarified by the following points:

➢ Pharmacological Validation and Mechanistic Studies

To determine the syrup's precise pharmacological actions, more investigation is required. While Moringa oleifera is well-known for its hepatoprotective qualities and nutritional diversity, Cissus quadrangularis has long been praised for its ability to promote bone repair, reduce inflammation, and have antioxidant effects [34,35]. A mechanistic foundation for therapeutic claims may be provided by examining their molecularly based synergistic activity using in vitro and in vivo models.

Future studies could include:

- Tests for enzyme inhibition to evaluate possible anti-inflammatory or antidiabetic effects.
- Gene expression studies to understand osteogenic effects.
- Utilising biomarker analysis and sophisticated imaging to assess systemic effects or tissue regeneration.

➢ Clinical Trials and Human Studies

Thoroughly planned human clinical trials must back up preclinical findings in order to validate safety and efficacy profiles. Larger Phase II/III trials that target certain populations, such as sports (bone injuries), postmenopausal women (osteoporosis), or malnourished people (nutritional support), can come after Phase I studies that concentrate on toxicity and pharmacokinetics.

These trials will:

- Establish therapeutic dosage ranges.
- Evaluate long-term safety and patient compliance.
- Assess comparative efficacy versus standard treatments.

➢ Standardization and Quality Control

Maintaining consistency and phytochemical stability from batch to batch is a significant difficulty in herbal preparation. For active ingredients like quercetin, β-sitosterol, and ascorbic acid, standardisation protocols utilising chromatographic and spectroscopic techniques (e.g., HPLC, GC-MS, FTIR) can be created [36,37]. Implementing pharmacopeial standards would help in:

- Defining assay limits for marker compounds.
- Minimizing adulteration or contamination.
- Achieving regulatory approval for commercial distribution.

➢ Formulation Optimization and Novel Delivery Systems

Future research can examine enhancing the formulation using contemporary medication delivery methods:

- Encapsulation with nanoparticles or liposomes to improve bioavailability.
- Syrups with controlled release for long-lasting effects.
- For synergistic benefits, combine with other adaptogens or nutraceuticals.

These improvements have the potential to improve patient compliance and therapeutic impact, especially in the paediatric and elderly populations.

➢ Toxicological and Stability Studies

Evaluations of teratogenicity, genotoxicity, and chronic toxicity are crucial, even though acute toxicity may be minimal. The best storage conditions, shelf life, and packing materials can be found through long-term stability tests conducted in compliance with ICH criteria. [38]

➢ Application in Functional Foods and Nutraceuticals

The syrup might be marketed as a functional supplement in addition to a therapeutic product because of the strong nutritional profile of Moringa and the medicinal qualities of Cissus. This could be promoted in ways such as:

- Herbal tonics.
- Dietary syrups for bone health.
- Immune boosters for convalescent patients.

In line with the increased interest in plant-based, holistic health solutions worldwide, such advances may pave the way for opportunities in the food and pharmaceutical industries [39].

➢ Regulatory and Commercialization Perspectives

The formulation may be registered as a proprietary Ayurvedic medicine or under AYUSH guidelines with the appropriate safety and efficacy proof. Additionally, there is a chance to export to nations where there is a significant need for complementary or alternative therapies. Commercialisation will, however, require regulatory compliance, which includes trademarking, patent filings, and adherence to Good Manufacturing Practices (GMP) [40].

➢ Contribution to Sustainable Development and Local Economies

In tropical areas, Cissus and Moringa are both commonly accessible. Encouraging their usage in pharmaceuticals benefits indigenous knowledge systems, sustainable harvesting, and regional agriculture. Global Sustainable Development Goals (SDGs) such Responsible Consumption and Production (SDG 12) and Good Health and Well-Being (SDG 3) are in line with this.

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