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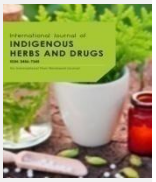

ROOT POWDER EXTRACT, PHYTOCHEMICAL STUDIES & PREPARATION OF HERBAL FORMULATION TINOSPORA CORDIFOLIA ASAVA

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Abstract

Asava, a traditional formulation in Ayurveda, is renowned for its superior preservation and enhanced therapeutic efficacy. This phenomenon is largely attributed to the intricate process of fermentation facilitated by a consortium of microbes. Microbial activity not only extends the shelf-life of Asava but also transforms its constituent ingredients into potent therapeutics through biotransformation. The alcohol-aqueous milieu generated during fermentation not only preserves the formulation but also facilitates improved drug delivery within the body. This milieu, predominantly produced by microbial action, acts as a medium for the potentization of drugs, enhancing their bioavailability and efficacy. Moreover, the biotransformation mediated by native microbes leads to the synthesis of novel compounds with enhanced therapeutic properties, thereby elevating the overall efficacy of Asava. The preservative properties inherent in these formulations, coupled with microbial-mediated potentization, underscore the significance of microbial fermentation in augmenting the therapeutic potential of Asava in Ayurveda.

Keywords: Asava, Ayurveda, fermentation, microbial activity, therapeutic properties, preservation.

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Introduction

Since ancient times, humanity has relied upon the plant kingdom for essential elements of survival, including food, clothing, shelter, and medicines. This enduring relationship between humans and plants has shaped the course of human culture and development, underscoring the profound interdependence between the two [1,2,3].

The relentless pursuit of happiness and well-being propels humans along diverse paths, known and unknown. Disease, an omnipresent aspect of human existence, has spurred the quest for remedies since time immemorial. Early humans, grappling with afflictions, turned to the abundant flora around them, recognizing the healing potential of plants as a primary source of relief [4,5,6].

Today, plants stand as the silent giants of drug development, serving as the primary reservoir of medicinal compounds worldwide [7]. The historical evolution of pharmacology has been intricately intertwined with plant-derived remedies, which continue to hold a significant place in ethnomedicine and traditional healing practices [8,9].

In recent decades, the spotlight on medicinal plants has intensified, driven by the realization of their untapped therapeutic potential. This burgeoning interest has prompted multinational pharmaceutical companies and research institutions to delve into the vast wealth of the plant kingdom in search of novel drug candidates and lead molecules [10].

Central to this exploration is the investigation of traditional remedies, predominantly derived from botanical sources. These remedies, upon which a majority of the global population still relies for healthcare, represent a rich repository of therapeutic compounds waiting to be discovered and harnessed for the benefit of humanity [11,12].

This paper explores the enduring alliance between humans and plants, illuminating the pivotal role of the plant kingdom

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in human survival and the ongoing quest for health and well-being [13].

Medicinalplants have been used as natural medicines, since prehistoric times [14-16]

(i) Direct Utilization of Plant Extracts in Medicine:

The utilization of crude plant extracts directly in medicinal formulations is a common practice, owing to the presence of naturally occurring chemical constituents such as berberine, morphine, psilocin, and vincristine. These compounds, inherent to various plant species, possess therapeutic properties that make them valuable assets in pharmaceutical preparations.

(ii) Phyto Compounds in Drug Synthesis [17]:

Phyto compounds found in plants are often utilized in the synthesis of drugs for therapeutic purposes. Examples include tubocurarine, colchicine, nicotine, and quinine, which have been synthesized from natural sources by folk practitioners. Tinospora cordifolia, a widely used plant in traditional Ayurvedic medicine, exemplifies this trend. Its diverse array of constituents, including morphine alkaloids, clerodane diterpenes, berberine, and palmatine, among others, have been isolated from different parts of the plant. These compounds exhibit a range of biological activities, including hypoglycemic, antipyretic, anti-allergic, anti-neoplastic, anti-inflammatory, antioxidant, and immuno modulatory properties.

The extensive usage of Tinospora cordifolia in traditional and modern medicine is attributed to its effectiveness in treating various ailments such as fever, vomiting, diabetes, jaundice, anaemia, polyuria, and skin diseases. Its inclusion as a key ingredient in numerous natural remedies underscores its significance in healthcare practices, both traditional and contemporary.

Introduction to Pharmacognosy [18-19]

Pharmacognosy is the study and science of medicine from natural sources. Although usually associated with herbal medicine, this field includes animals, fungi, microbes, and minerals, as well as parts of/or entire organisms such as flowers, leaves, roots, etc. Natural medicines have been used for many thousands of years to enhance human health and treat diseases, and modern pharmaceutical medicine is largely dependent on drugs originally discovered in and isolated from natural source. In fact, natural sources are the basis for approximately 50% of all prescription medicines currently marketed.

Important examples include morphine, ephedrine, atropine, salicylic acid, colchicine, and tamoxifen. Alternative medicinal sources increasingly investigated for therapeutic use include animals such as frogs, snakes (venom), and worms and marine species. Parasites (e.g., helminths) and fungi (e.g., psilocybin) are also included in the scope of pharmacognosy.

The roots of pharmacognosy are embedded in traditional medicine practices globally, and are recorded through traditional knowledge systems, folklore, incantations, Materia Medica, and pharmacopoeias.Pharmacopeia reflects investigation and standards for identity, purity, quality, and clinical efficacy of drugs, whereas material medica reflects traditional indications and applications. Currently, the principles have been entrenched into pharmacological sciences through systematic evidence-based investigations related to purity, potency, extraction methodology, isolation of active constituents, consistency, efficacy, and safety [20-25].

Pharmacognosy remains a central feature in traditional medicine and pharmacology, with the former remaining the primary source of medicine in developing countries and emerging economies. However, modern pharmacognosy includes increased scientific focus, particularly through the introduction of molecular, genomic, and metabolomic techniques, increasingly taken up in a variety of fields including molecular biology, biotechnology, proteomics, and bioinformatics with the emergence of increasing global demand for more holistic, safe, and effective medicinal approaches, there is a significant resurgence in the field of pharmacognosy in recent years. This chapter aims to provide an overview of herbal pharmacognosy, with a focus on secondary metabolite classes relevant to human biology, pathology, and clinical practice [26].

Pharmacognosy as a multiplex of overlapping and integrated sciences is at the forefront of numerous technological advances, both in the laboratory and the field. Thus, Pharmacognosists are involved in a number of selected activities and these include:

Identification of Natural Drug Sources [27-30]:

- **Determination of Morphological Characters:** This involves examining the physical attributes of plants to identify and classify them.
- **Planning for the Cultivation of Medicinal Plants:** Developing strategies for growing medicinal plants to ensure a consistent supply.
- **Protocol Development and Implementation for Collection, Drying, and Preservation:** Establishing and following specific procedures to collect, dry, and preserve plant materials to maintain their medicinal properties.
- **Evaluation of Crude Natural Drugs:** Assessing the quality of raw plant materials through microscopic, macroscopic, genetic, chemical, and biological analyses.
- **Pharmacological Evaluation of Crude Extracts and Active Constituents:** Investigating the therapeutic effects of plant extracts and their active compounds.
- **Isolation and Characterization of Active Metabolites:** Extracting and identifying the key active ingredients from natural sources.
- **Interdisciplinary Relationships:** Collaborating with fields like ethnobotany, ethnopharmacology, botany, chemistry, enzymology, genetics, pharmacology, horticulture, quality control, and biotechnology.
- **Legal and Regulatory Issues:** Addressing the legal and regulatory aspects of pharmacognosy.

The Role of Pharmacognosy in Society [30-35]

There are several challenges in the global healthcare system, including:

1. Differences in health care spending per capita among countries.
2. The availability of trained physicians per thousand people in various regions.

3. Worldwide access to medications, including treatments for rare diseases.
4. The depletion of natural resources necessary for medicinal use.

Research Methodology in Ayurveda [35-36]:

Ayurvedic research varies depending on the project but generally falls into five main categories: literary, fundamental, drug, pharmaceutical, and clinical research. While there is no single approach to conducting research, employing certain methods and skills can enhance efficiency and effectiveness.

The Gunas or Qualities [37]:

In Sanskrit, "Guna" describes the qualities or attributes inherent in every substance or being. These are categorized into three main types:

- **Rajas:** Associated with activity, passion, and preservation. Individuals with rajasic qualities are typically self-centered, egoistic, and dynamic.
- **Tamas:** Linked to imbalance, disorder, chaos, and ignorance. Those with tamasic qualities tend to be destructive, vindictive, and prone to negative emotions.
- **Sattvas:** Represent balance, goodness, purity, and positivity. People with sattvic qualities are generally good-hearted, creative, peaceful, and virtuous.

Ayurvedic Medicine Formulations [38]:

Method of Preparation:

1. **Asava and Arista:**

These are prepared by soaking herbs in either powdered form or as a decoction (kasaya) in a solution of sugar or jaggery. During fermentation, alcohol is produced, which helps in extracting the active ingredients from the herbs.

Standardization of Asava:

Standardization is divided into three main approaches:

1. **Raw Material and Equipment:**

- Ensuring the quality and proper storage of raw materials and selecting appropriate equipment for fermentation and storage.
- Testing for heavy metals, microbial contamination, and residual pesticides, as these can affect the fermentation process and the quality of the final product.
- **Manufacturing Processes**
- **Temperature:** Temperature impacts fermentation; high temperatures can destroy yeast cells, while low temperatures may inhibit fermentation.
- **Fermentation Time:** Over time, specific gravity, total solid content, and sugar levels decrease while alcohol content increases, stabilizing after about six months.
- **Vessels and Preparation Conditions:** Different materials like glass, aluminum, tinned-copper, stainless steel, porcelain jars, and earthen pots can be used, each affecting the fermentation process differently.

2. **Properties and Quality of the End Product [39]:**

- **Organoleptic Evaluation:** Assessing the color, odor, and taste. Asava should be a clear liquid with a pleasant aroma and slightly sweet taste, without sourness.
- **Physical and Chemical Parameters:** Evaluating properties like specific gravity, pH, density, extractive value, and the presence of phytochemicals such as tannins and alkaloids.
- **Analytical Studies:** Using techniques like Thin Layer Chromatography (TLC) for testing and additional analyses for nitrogen content, proteins, and lipids.

Plant Profile [40]

Biological source:

It consists of dried, matured root and pieces of stem *Tinospora cordifolia Miers*.

Family: Menispermaceae

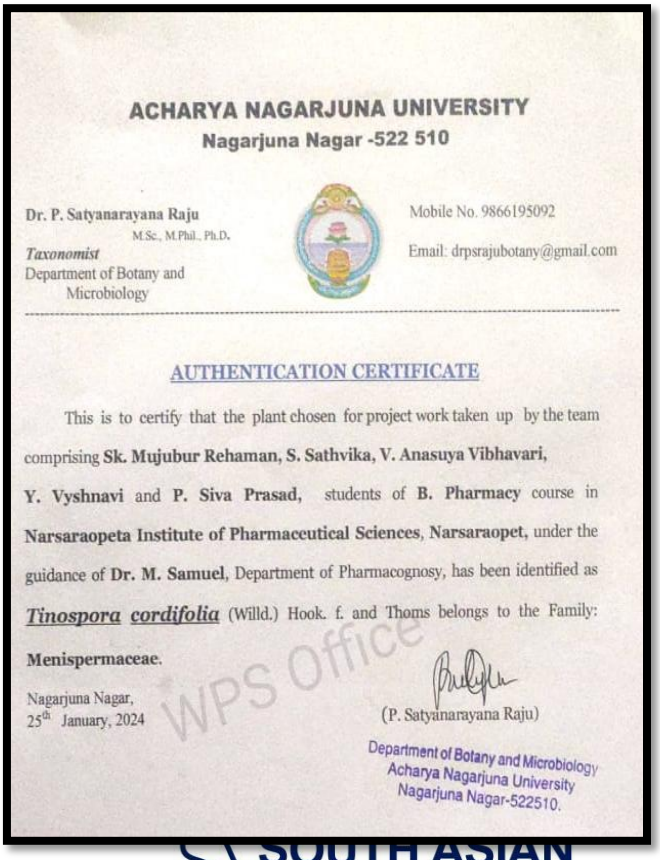
Scientific classification:

Kingdom - Plantae
Subkingdom - Angiosperms
Super division - Eudicots
Orders - Ranunculates
Family -Menispermaceae
Genus -*Tinospora*
Species - *cordifolia*

Vernacular names: *Tinosporacordifolia*is commonly called Heart-leaved moon seed, Gulvel, Guduchi, Gaduchi, and Giloe.

Telugu -Tippateega.
Sanskrit - Amrita, Guduchi
Hindi - Giloy, Guruc, Gurcha.

Fig: 1 Guduchi Plant



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AUTHENTICATION CERTIFICATE

This is to certify that the plant chosen for project work taken up by the team comprising **Sk. Mujubur Rehaman, S. Sathvika, V. Anasuya Vibhavari, Y. Vyshnavi** and **P. Siva Prasad,** students of **B. Pharmacy** course in **Narsaraopeta Institute of Pharmaceutical Sciences, Narsaraopet,** under the guidance of **Dr. M. Samuel**, Department of Pharmacognosy, has been identified as ***Tinospora cordifolia*** (Willd.) Hook. f. and Thoms belongs to the Family: **Menispermaceae.**

Nagarjuna Nagar,
25th January, 2024

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Plant Authentication

Collection and Preparation of Plant Extract:

Freshly collected roots of Tinospora cordifolia were dried in the shade and then ground into a powder. This powdered material was sifted to separate the coarse particles from the fine powder. The coarse powder underwent extraction with ethanol using a continuous Soxhlet apparatus. The resultant extract was concentrated to a smaller volume under a vacuum at 50℃, followed by drying in a vacuum desiccator.

For the fine powder, 2 liters of ethanol were added in a glass jar and left for cold maceration over seven days. The resulting liquid extract was then concentrated and evaporated under reduced pressure, maintaining a temperature below 50℃ until a soft mass was obtained. The dried ethanolic extract was subsequently analyzed qualitatively for its phytochemical constituents.

Fig: 3 Collection of Roots

Fig: 4 Roots Powder

Soxhlation Process

Soxhlet Apparatus:

The Soxhlet apparatus is a specialized glass unit primarily used for the extraction of compounds using organic solvents. This method is widely recognized for its efficiency, often outperforming other conventional extraction techniques except in specific applications involving thermolabile compounds. The powdered solid material is placed in a filter paper thimble, which is then positioned inside the Soxhlet apparatus. The setup includes a round bottom flask (RBF) containing the solvent, connected to a reflux condenser.

During the extraction process, the solvent in the RBF is gently heated, causing the vapor to ascend through the side tube. The vapor is then condensed by the condenser, allowing it to drip into the thimble containing the material. The Soxhlet gradually fills with solvent, and when it reaches the top of the siphon tube, the solvent flows back into the flask, carrying the extracted compounds with it. This cycle repeats until the extraction is complete.

Advantages of Soxhlet Extraction:

1. Enables the extraction of large quantities of plant material at once.
2. Allows for the reuse of the solvent multiple times.
3. Eliminates the need for filtration post-extraction.
4. Independent of the type of matrix used.
5. It is a straightforward technique.

Disadvantages of Soxhlet Extraction:

1. High temperatures over extended periods can degrade heat-sensitive compounds.
2. The extraction process is time-consuming and labor-intensive.
3. Limited ability to manipulate variables, coupled with the need for large solvent volumes, leads to criticism of this technique.

Distillation Process:

Distillation involves converting a liquid mixture into vapor, transferring the vapor to another location, and then recovering the liquid through condensation. The principle behind distillation is that a liquid boils when its vapor pressure matches atmospheric pressure. Simple distillation occurs at the boiling point of the liquid, and the efficiency of separation improves with higher relative volatility.

Construction: A typical distillation setup includes a distillation flask with a side arm, a condenser (usually a water condenser) connected to the side arm via a cork, and an adapter linking the condenser to a receiver flask.

Procedure: The liquid to be distilled is placed in the flask. A thermometer is inserted into the flask, and water is circulated through the condenser jacket. Upon heating, the liquid boils, and the vapor rises, traveling through the side arm into the condenser. The temperature stabilizes at the boiling point of the liquid. The vapor condenses back into liquid form and is collected in the receiver flask.

Applications:

1. Producing distilled water and water for injection.
2. Preparing volatile and aromatic waters.
3. Separating non-volatile solids from volatile liquids.

Qualitative Phytochemical Screening:

Plants synthesize a variety of natural compounds, such as alkaloids, glycosides, tannins, essential oils, and other secondary metabolites with physiological activities, in addition to carbohydrates, proteins, and lipids used as food by humans. A comprehensive study of crude drugs involves examining both primary and secondary metabolites produced by plant metabolism. Various qualitative chemical tests are conducted to profile the chemical composition of a given extract or fraction.

The following tests were carried out on the extract to detect various phytoconstituents present in them.

Table: 1PHYTOCHEMICAL SCREENING

S. No	Name of the test	Ethanolic extract	Aqueous Ethanolic extract
1.	Shinoda test (for flavonoids)	Positive	Positive
2.	Neutral Fecl3 test (for Phenolic and Tanins)	Positive	Positive
3.	Benedict's test (for carbohydrates)	Negative	Negative
4.	Liberman& Buchard test (for Steroids&triterpenes)	Positive	Positive
5.	Mayer's test (for alkaloids)	Negative	Negative
6.	Foam test (for Saponin glycosides)	Positive	Positive

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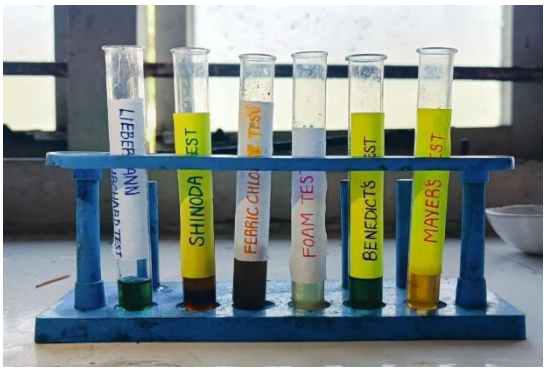

Fig 5:Phytochemical Screening

Process Involved in Asava:

Asava refers to medicinal preparations made by soaking drugs, either in powdered form or as a decoction (known as kasaya in Ayurveda), in a solution of sugar and jaggery (Gur) for a designated period. During this soaking process, fermentation occurs, generating alcohol which helps extract the active constituents from the drugs. The alcohol produced also acts as a preservative for the product.

Examples of Asava:

- Kumariasava
- Madhukasava
- Punarnavasava
- Arvindaasava
- Chandanaasava

- Kanakasava
- Lohasava

Properties of Asava:

1. **Stability:** These formulations are highly stable, with the bioactive products of fermentation being continuously exposed to low concentrations of alcohol. The medicinal properties of Asava are believed to enhance over time.
2. **Alcohol Content:** The presence of alcohol in these formulations can detoxify certain phytochemicals and increase the potency of others.
3. **Therapeutic Efficiency:** These fermented extracts provide rapid therapeutic effects at low doses. The aqueous nature of the extract promotes better uptake of the drug by target organs, improves the efficiency of extracting drug molecules from the herbs, and enhances drug delivery to the body.
4. **Alcohol Content and Taste:** Asava formulations are moderately alcoholic (up to 12% by volume) and generally sweetish with a slight acidic taste and an agreeable aroma.

Requirements:

The basic equipment needed includes a sufficiently large and strong earthen pot with a glazed exterior or a glazed porcelain jar of suitable size, a properly fitting lid, a cloth to seal the vessel, a paddle-like stirrer, a clean cloth of fine and strong texture for filtering, and vessels for keeping the juices or boiling the drugs.

The main components are categorized based on their specific roles in the process:

1. **Main Herbs:** These herbs provide the primary extract or decoction.
2. **Naming Herbs:** The medicine's name is derived from these herbs, indicating their importance.
3. **Flavoring Agents:** These herbs contribute to the medicine's flavor and have their own pharmacological actions.
4. **Fermentation Initiator:** Woodfordiafructicosa acts as the inoculum to start fermentation, with jaggery (the sugar medium) required for the fermentation process.

Method of Preparation of Asava:

1. Dissolve jaggery or sugar in the required quantity of water, boil, and cool the solution.
2. Pour the cooled solution into the fermentation vessel.
3. Add finely powdered drugs to the container, cover it with a lid, and seal the edges with a cloth smeared with clay wound in seven consecutive layers.
4. Maintain a constant temperature for fermentation by placing the container in a special room, an underground cellar, or a heap of paddy.
5. After a specified period, remove the lid and examine the contents to determine if fermentation is complete.
6. Decant the fluid and strain it after two or three days.

Fig: 6 Preparation Of Guduchi Asava

Precautions:

i. The filtered Asava should be clear and free from froth on the surface. ii. It should not develop a sour taste. iii. The preparation should have a characteristic aromatic alcoholic odor.

Storage:

Asava should be stored in well-sealed bottles or jars.

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Conclusion

Asava is regarded as one of the best formulations in Ayurveda due to its superior preservation qualities, attributed to the fermentation process. The microbes involved in fermentation enhance the therapeutic properties by transforming the initial ingredients into more effective end products. The alcohol-aqueous environment created by these microbes also improves drug delivery within the body. Additionally, these products possess preservative properties and benefit from the biotransformation of drugs mediated by native microbes, resulting in increased potency.

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All authors are contributed equally.

Conflict of Interest

No Conflict of interest

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Ethical statement and inform consent

Not Applicable

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