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Formulation And Evaluation Of Ayurvedic Shatmuli Asava For Treatment Of Menorrhagia

Mariyu Mma Kutty.V.T, Cheemadan Rasha, Asmina .Ck, Aiswarya .Av, Fathima Sinsiya.K, Riya Mohammedali Illikkal

[1]*Asst.professor,Department of Pharmacognosy ,KMCT College Of Pharmaceutical Sciences, KOZHIKKODE.*
[2;3;4;5;6]*Students, KMCT College Of Pharmaceutical Sciences, KOZHIKKODE.*

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ABSTRACT

OBJECTIVE: To prepare the formulation of Shatmuli Asava for menorrhagia activity and evaluate the Asava preparation according to AYUSH guidlines for internal use.

MATERIALS AND METHODS: The herbs collected, washed, dried and powdered. The suitable pot was prepared and sterilised. The herbs, excepients, fermenting agents trasfered into pot and kept for fermentation for one month . After that the Asava collected, filtered and go for evaluation procedure as per AYUSH guidelines. The alcohol content was determined by specific gravity method. Initially the distillation of Asava formulation was carried out and from the distillate the specific gravity calculated .

RESULT: The apparent specific gravity calculated was 0.97 kg/m2 which corresponded to 17ml of alcoholic content. Organoleptic evaluation and PH performed. The evaluation result of Shatmuli Asava formulation found to be ; Total solid content 94% w/v , Boiling point 85 ^{0}C, Reducing sugar present , Density 1.016 g/ml , Viscosity 1.2608 kg/ms, Extractive value 25.4%w/w, Total ash value 0.3%w/w, Water soluble ash value 3.3%w/w, Acid insoluble ash value 1% w/w .

DISCUSSION: The menorrhagia activity of Shatmuli Asava attributed to the presence of Ashoka, Shatavari, Muthanga, Bala, vibhitaki. Amla and Sati act as neutraceuticals which incease the RBC count and improving health. Evaluation results shows that the formulation comply for internal consumption. So Shatmuli Asava is a good formulation for curing and reduce the intensity of menorrhagia and act as health tonic for women.

KEYWORDS: Shatmuli Asava , Menorrhagia , Alcohol content , Extractive value , Ash value

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I. INTRODUCTION

Indian system of herbal medicine

It is a well-known fact that Traditional Systems of medicines always played important role in meeting the global health care needs. They are continuing to do so at present and shall play major role in future also. The system of medicines which are considered to be Indian in origin or the systems of medicine, which have come to India from outside and got assimilated in to Indian culture are known as Indian Systems of Medicine . India has the unique distinction of having six recognized systems of medicine in this category. They are- Ayurveda, Siddha, Unani and Yoga, Naturopathy and Homoeopathy[1]

Herbal medicines in Dietary Supplements

Dietary supplements and herbal remedies are popular complementary or alternative products for people. These are the supplements that are intended to supplement the diet and contain one or more dietary ingredients (including vitamins, minerals, herbs, or other botanicals, amino acids and other substances) or their constituents. These are intended to be taken by mouth as a pill, capsule, tablet, or liquids. Natural substances can be treated as either supplements to improve health or medicines for illness.[2]

Menorrhagia

Menorrhagia has been defined as blood loss of more than 80 ml in an otherwise normal menstrual cycle. A normal cycle is between 25 and 35 days in duration, with bleeding lasting from 5 to 7 days.[3] The pathophysiology of abnormal uterine bleeding (AUB) is as diverse as the classification of the disease. AUB can be caused by pelvic pathology like a distortion of the endometrial cavity due to fibroids, or endometrial protrusions into the cervix or vagina (polyps), or because of friable endometrial tissue. The friable endometrial tissue is likely caused by unopposed estrogen which causes the endometrium to become friable, vascular, and

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lacking sufficient stromal support which equates to heavy, continuous uterine bleeding. Systemic conditions are also responsible for AUB. Obesity is an epidemic whose consequences affect every aspect of life and every organ system. In women, obesity can lead to unopposed estrogen and can lead to the polycystic ovarian syndrome. Coagulopathies can also lead to AUB; 13% of women with AUB have a variant of Von Willebrand disease, and 20% have an underlying coagulopathy. With respect to treatment and management, the initial goal is to stabilize the patient's hemodynamics. If the patient is unstable, the patient should have two large-bore IVs, airway assessment, and have a type and cross in preparation for blood transfusion. For the unstable patient before surgical interventions, intrauterine tamponade should be performed. A Bakri balloon can be inserted into the uterus to tamponade uterine bleeding. If an intrauterine balloon is not available, gauze packing is an acceptable option. The physician should use a continuous piece to eliminate the risk of retained products.
If the bleeding is uncontrolled with tamponade, surgical intervention is necessary.[4]

Herbs used in menorrhagia condition.

Astringent herbs form a large category of tannin-containing plants that are used to reduce blood loss from the reproductive tract as well as the gastrointestinal tract, respiratory tract and skin. In the reproductive tract, the astringent herbs are used to correct uterine or cervical bleeding. Of these, shepherd's purse has a long history of use in the management of preventing or arresting gynecologic hemorrhage.[5] Saraca asoca dried bark has been used for menorrhagia in India . In India Sarac asoca dried bark as well as flower is givan as a tonic to ladies in case of Uterine disorders. Saraca asoca stem bark also used to treat all disorder associated with the menstrual cycle . Saraca asoca bark in Sri Lanka used for menstrual disorder and menorrhagia. Saraca asoca bark in India, used as a uterine sedative and hot water extracts administered to human adult female stimulates the uterus similar to ergot, but without producing tonic contraction.[6] The anti obesity potential of the aqueos tuber extract of Cyprus rotundus L. Atecr in high fat cafeteria diet fed obese rats. The result shows the significant weight reduction activity. The anti platelet effects of Cyperus Rotundus by examining their effects on platelet aggregations in rats.[7] The constituents of A.racemosus make it useful in menstural disorders such as dysmenorrhea, premenstural syndrome, irregular bleeding during perimenopausal period and also in situations after menopause. Asparagus racemosus contain saponins which hinder the oxytocic activity on uterine musculature, thereby maintain the spontaneous uterine motility, confirming its utility in dysmenorrhea which comprises of painful mensturation without significant pelvic pathology .[8]

Ayurvedic medicines

The Ayurvedic system of medicine has been prevalent in India since the Vedic period, and still remains the mainstay of medical relief to over 60 per cent of the population of the nation. In earlier times the practitioners of Ayurveda (Vaidya) were themselves collecting herbs and other ingredients and preparing medicines. For the purpose of acquiring raw materials Vaidyas now depend on commercial organizations trading in crude herbal drugs. Likewise, with passage of time a number of Ayurvedic Pharmaceutical units have come up for the manufacture of Ayurvedic drugs and formulations on commercial scale.[9] The word Ayurveda is composed of two components viz. 'Ayush' means life and 'Veda' means science hence Ayurveda is the 'Science Of Life'. The origin of this ancient science dates back to vedic period about 5000 years ago, Brahma, the creator, was the originator of this system who passed it on to the Ashwini kumars (Physician of God) who in turn imparted it to the Rishis from where it was promoted among the people through generation. The main objective of Ayurveda is maintenance and promotion notion of positive health and cure of disease through medicine, dietary restrictions and regulated life style The basic principles of Ayurveda involves two theories, one is Panchamahabhuta theory and the other is the Tridosha theory. According to Ayurvedic philosophy all the living and non living matters are made up of five basic elements in various proportions, they are Prithvi (Earth), Jala (Water), Teja (Fire), Vayu (Air) and Aakash (Ether). Even the human body is made up of these elements known collectively as the Panchamahabhutas. According to Ayurveda again all the physiological functions of the body are governed by three biological units viz. Vata, Pitta and Kafa each of which in turn is made up of the Mahabhutas. Physiologically these three doshas are responsible for various specific functions.

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Source of ayurvedic medicines :

A drug may be defined as an intended for use in diagnoses, cure, mitigation, prevention or treatment of disease in man or other animal, or indented to alter a body function or structure of man or other animals.

Classification of sources of Drugs :

1. Biological source
a) Higher plants
b) Microbes
c) Animals
2. Marine sources
3. Mineral source
4. Plant tissue culture

1. Biological Source:

Higher plants a source of drugs: Plants have been used in the treatment of various diseases from time immemorial. The traditional Indian systems of medicine. Ayurveda, Siddha, Unani systems are based on the use of plants & other natural substances. There are 200,000 to 250,000 species of flowering plants growing on earth, which belong to 10,500 general and about 300 families.[10]

Ayurvedic formulation

Ayurvedic formulations can be grouped into four types depending upon their physical nature. 1. Solid dosage forms, Eg: vatika, gutika, guggulu
2. Semisolid dosage forms, Eg: kalka, avaleha
3. Liquid dosage forms, Eg: asava, arista, swarasa, taila
4. Powder dosage form, Eg:churna

All the Ayurvedic preparations consist of two words. The first word may indicate either the disease for which the preparation is used or the property of the preparation or the drug contained or name of god or saint and second word indicates the type of preparation.

Asava and Arishtas

Ayurvedic classics treasures a rich repertory of medicinal plants used for the treatment, management and/or control of different types of diseases. Ayurveda comprises of various types of medicines including the fermented forms namely arishtas (fermented decoctions) and asavas (fermented infusions). These are regarded as valuable therapeutics due to their efficacy and desirable features.[11] The required quantity of water, to which jaggery or sugar as prescribed in the formula is added, is boiled and cooled. This is poured into the fermentation pot, vessel or barrel. Fine powders of the drugs mentioned in the formula are added. The container is covered with a lid and the edges are sealed with clay-smeared cloth wound in seven consecutive layers.

Asava and Arishta is a novel yet least continuous hydro alcoholic extraction method, being traditionally used in Ayurveda . This advanced dosage form probably results into transformation of several phytochemical compounds present in the herbs used to prepare it and there by either rendering them less toxic or more potent, besides helping in their faster absorption Asavas are medicinal preparations made by soaking the drugs, in powder form in a solution of sugar or jaggery, as the case may be for a specified period of time, during which it undergoes a process of fermentation generating alcohol, thus facilitating the extraction of the active principles contained in the drugs. The alcohol, so generated, also serves as a preservative. The characteristics include the filtered asava or arista should be clear without froth at the top. It should not become sour. The preparation has the characteristics of aromatic alcoholic odour.[12]

Powders

These are the preparation that comes as powdered herbal material meant for direct use or by incorporation into foods, beverage for drinking, insufflation, and wounds. They may finely sifted herbal materials from various parts of plants meant for a particular therapeutic effect.

Churna

It is a mixture of powdered herbs and or minerals. It is a fine powder of drug or drugs which are cleaned properly, dried thoroughly, crushed and then sieved. The churna is free flowing and should be preserved in airtight container. There are two type churna- simple churna and compound churna.It is mainly used in treatment of diabetics, indigestion, contipation etc.[13]

Taila

Tails are prepration where the taila is boiled with specified kasayas (decoction) and kalkas according to the recipe. This method guarantees that the active medicinal properties of the products are consumed.

Ghrita

Ghrita is a preparation in which Ghrita is boiled with recommended kasayas (decoction) and kalkas according to the recipe. This method guarantees that the active medicinal properties of

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the products are consumed.[14]

II. MATERIALS AND METHODS

Selection of medicinal plants:

Techniques and methods for selecting medicinal plants:

1. Randomized Approach
2. Chemosystematic Approach
3. Ecological Approach
4. Ethnoguided Approach

1. Randomized approach

The randomized investigations consist in random selection and collection of plant species for study, according to the plant availability. When carried out in regions with high diversity and endemism the probability of finding novel substances, bioactive or not, is certainly higher in this type of selection.

2. Chemosystematic approach

The chemosystematic approach, also called phylogenetic, consists of selecting a species from a family or genus, for which some phytochemical knowledge of at least one species of the group is known. The presence of different compounds, which can be used as biosynthetic markers, is used by botanists in taxonomy studies and the chemosystematic approach is used as a successful tool in the selection of families, subfamilies and genera to be investigated in terms of produced metabolites.

3. Ecological approach

The ecological approach, also known as field observations, consists in observations of interactions between organisms in their ecological environment, inducing to potential biological activity (antibacterial, antifungal, agrotoxic, pesticide).This approach searches for secondary metabolites and biological activities and it may be different species that are shadow resistant and not shadow resistant, among other characteristics, though little explored, it has achieved excellent results.

4. Ethnoguided approach

The ethnoguided approach consists of selecting plant species in accordance to the indication of specific population groups in certain contexts of use, emphasizing the search for the locally built knowledge regarding their natural resources and their application in their health systems. In this type of approach the ethnobotany, ethnopharmacology and ethnomedicine can be highlighted.

III. PLANT PROFILE:

SHATAVARI

Biological source: Asparagus racemosus is a woody climber growing to 1-2 m in height. The leaves are like pine needles, small and uniform and flowers are white and have small spikes. This plant belongs to the genus Asparagus in the family Liliaceae

Chemical constituents:

- Steroidal saponins, known as shatvarins. Shatvarin I to VI are present..
- Polycyclic alkaloid-Aspargamine A, a cage type pyrrolizidine alkaloid.
- Furan compound-Racemofuran.
- Carbohydrates-Polysacharides, mucilage.
- Flavanoids-Glycosides of quercitin, rutin and hyperoside are present in flower and fruits
- Kaepfrol-Kaepfrol along with Sarsapogenin from woody portions of tuberous roots could be isolated.

Uses:

- Diuretic activity
- Potential to prevent hepatocarcinogenesis.
- A versatile female tonic
- Cytotoxicity, analgesic and antidiarrhoeal activities
- Anti-inflammatory effects
- Antioxidant effects
- Reproductive tonic
- Lactogenic[40]

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Shatavari

ASHOKA

Biological source: Saraca indica (Ashoka) Linn is a rainforest tree belongs to the family of Caesalpiniaceae.

Chemical constituents:

The bark of plant contain epicatechin, procyanidin p2, catechin, 22-dien-33-ol, leucopelargonidin-3-O-p-Dglucoside, leucopelargonidin and leucocyanidin. The flower part of plant contain Oleic, linoleic, palmitic and stearic acids, P-sitosterol, quercetin, kaempferol 3-0-P-D-glucoside.It mainly contains glycoside, flavanoids, tannins, saponins, esters and primary alcohols.

Uses:

- Saraca indica is used in many pharmacological activities like,
- anti menorrhagic
- anti-cancer
- anti oxytoxic and anti-inflammatory
- antiulcer
- Anti progestational activity
- Anti estrogenic activity
- Anti implantation
- anti-microbial activity and have extend uses in indigenous system of medicine. It mainly contains glycoside, flavanoids, tannins, saponins, esters and primary alcohols.[41]

Ashoka

MUTHANGA

Biological source: Cyperus rotundus L. is a perennial, monocotyledonous and herbaceous plant of the Cyperaceae family.

Chemical constituents:

C. rotundus contained many secondary metabolites such as sesquiterpenes, quinones, flavonoids (visnagin, khellin, isorhamnetin, and tricin), saponins, alkaloids, phenolic acids (salicylic acid, protocatechuic acid, caffeic acid and pcoumaric acid), coumarins and steroids,β dgalactopyranoside

Uses:

It has numerous range of pharmacological properties like ,

- antibacterialand microbial activity.
- antiplatelet activity.
- gastrointestinal activity.
- anti-inflammatory, anti-laceration, wound healing activity.
- anti-rumotoid, anti-cancer, antioxidant.
- Catamenia, Stomachache[42]

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Cyperus rotundus

CITRAKA

Biological sources: Plumbago zeylanica is also known as citraka, doctor bush, or leadwort. It is the most popular herbal plant that belongs to the family Plumbaginaceae or leadwort.

Chemical constituents:

P. zeylanica contains secondary metabolites which include flavonoids, alkaloids, saponins, glycosides, tannins, steroids, triterpenoids, carbohydrates, coumarins, fixed oil, phenolic compounds, fats, naphthoquinones and proteins. The screening of different parts of P.zeylanica plant also revealed the presence of linoleic acid, nonylnonanoate, palmitic acid, stigmasterol acetate, lupeol acetate, lupeol, friedelinol, stigmasterol.

Uses:

Traditionally it is used to treat variety of diseases such as;

- dysmenorrhea,
- leprosy, anaemia, rheumatic pain and cold
- anti-malarial, anti-obese and anti-diabetic.
- anti-microbial, anti-ulcer, anti-inflammatory.
- anti-oxidant and anti-cancer.[43]

Citraka

KURUNTHOTTI

Biological sources: Sida cordifolia is a perennial subshrub of the mallow family Malvaceae native to India.

Chemical constituents:

The following alkaloids were reported from S. Cordifolia,β-phenethylamine, ephedrine, pseudoephedrine, hypaphorine, vasicinone, vasicinol, choline, and betaine. The roots and stems contain the alkaloid ephedrine. Recent analyses have revealed that ephedrine and pseudoephedrine constitute the major alkaloids from the aerial parts of the plant, which also show traces of sitosterol and palmitic, stearic and hexacosanoic acids.

Uses:

- It is a plant used for the treatment of inflammation of the oral mucosa, blennorrhea, asthmatic bronchitis and nasal congestion, stomatitis, asthma and nasal congestion.
- It has been investigated as an anti-inflammatory, for preventing cell proliferation and for encouraging liver re-growth, Analgesic and anti inflammatory.[44]

Kurunthotti

HARITAKI

Biological sources: Terminalia chebula Retzius (T.chebula Retz) is a medium to large-sized tree that belongs to the family Combretaceae and is widely distributed throughout Asia.

Chemical constituents:

Contained a maximum value for total phenols, total flavonoids, gallic acid, catechin, chlorogenic acid, and coumaric acid. It is likewise probably the most abundant source of ascorbic acid. The most abundant tannins in the fruit include gallic acid, ellagic acid, chebulic acid, chebulinic acid,

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punicalagin, terflavin A, corilagin, galloyl glucose, tannic acid. At the same time, available flavonoids like quercetin, catechin, and kaempferol are found.

Uses:

- Antioxidative
- Antiproliferative
- Anti-microbial
- Proapoptotic, hepatoprotective
- Anti-diabetic
- Anti-ageing
- Anti-inflammatory
- Antiepileptic
- It is also beneficial in glucose and lipid metabolism and prevents atherogenesis and endothelial dysfunction.[45]

Haritaki

VIBHITAKI:

Biological source: Terminalia bellirica Roxb., known as Bahera or bastard myrobalan, belonging to the family Combretaceae.

Chemical constituents:

It contain hexahydroxydiphenic acid, methyl ester, β- sitosterol, gallic acid, ellagic acid, ethyl gallate, galloyl glucose, chebulagic acid, mannitol, glucose, galactose, and rhamnose.

Uses:

- Antisecretory
- Analgesic
- Antihypertensive
- Antidiarrhoeal activity
- Antimicrobial activity
- Antidiabetic
- Antioxidant
- Antiulcer
- Antipyretic
- Hepatoprotective
- Anticancer
- Angiogenesis
- Antidepressant-like and anti-urolithiatic activity.[46]

Vibhitaki

AMLA

Biological sources: Phyllanthus emblica L. (popular known as amla or Indian gooseberry) is an ephemeral tree belonging to the Euphorbiaceae family.

Chemical constituents:

Polyphenols comprise the main group of secondary metabolites wherein several compounds belonging to phenolic acids, flavonoids, tannins, other phenolics and derivatives. Another class of compounds reported in the amla plant is flavonoids. Flavonols,Kampferol their derivatives and quercetin and its derivatives are found.

Uses:

- Anti-hyperglycemic,
- Hypoglycemic,
- Anti-inflammatory, anti-hyperlipidemic, and antioxidant activities.
- Help in RBC formation.[47]

Amla

BHUNIMBA

Biological sources: Andrographis paniculata (Burm. f.) Wall. ex Nees (AP) is an important medicinal plant and widely used around the world.

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It belongs to the family Acanthaceae.

Chemical constituents:

All parts of this plant are used to extract the active phytochemicals, but the compositions are widely differ from one part to another and with place, season, and time of harvest. Our extensive data mining of the phytoconstituents revealed more than 55 ent-labdane diterpenoids, 30 flavonoids, 8 quinic acids, 4 xanthones, and 5 rare nor iridoids.

Uses:

This is traditionally used for the treatment of common cold, diarrhoea, fever due to several infective cause, jaundice, as a health tonic for the liver and cardiovascular health, and as an antioxidant.
It is also used to improve sexual dysfunctions and serve as a contraceptive.

Bhunimba

KACHOLAM

Biological sources: Kaempferia galanga L.is an endangered medicinal plant with potent medicinal activities belongs to the family Zingiberaceae

Chemical constituents:

Its rhizome contains a volatile oil ,several alkaloids, starch, protein, aminoacids, minerals and fatty matter. The essential oil is reported to contain over 54 components of which the major constituents are ethyl–trans p–methoxy cinnamate, entadecane. In addition, it contains camphene, kaempferol, kaempferide, cinnamaldehyde and ethyl cinnamate.

Uses:

- Antimicrobial
- Antioxidant
- Analgesic
- Amebicidal
- Anti-dengue
- Anti-inflammatory
- Anti-tuberculosis
- Anti-thrombotic
- Anti-angiogenic
- Anticancer
- Hypotriglyceridemic, hypopigmentary and osteolysis.[49]

Kacholam

HONEY

Biological sources: Honey is a natural product formed from nectar of flowers by honeybees (Apis mellifera) belongs to the Family: Apidae.

Chemical constituents:

The main composition of honey is carbohydrates that contribute 95–97% of its dry weight.Honey includes main compounds, such as proteins, vitamins, amino acids, minerals, and organic acids. Pure honey also consists of flavonoids, polyphenols, reducing compounds, alkaloids, glycosides, cardiac glycosides, anthraquinone, and volatile compounds.

Uses:

- Antioxidant
- Anti-inflammatory
- Antibacterial
- Antidiabetic
- Respiratory
- Gastrointestinal, cardiovascular, and nervous system protective effects. [50]

Honey

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PREPARATION OF ASAVA

Preparation of Raw materials and Earthen pot:

Preparation of Raw Materials:

Series of steps were followed for the Preparation of raw materials which are described below:

Collection –

Whole plants were collected and are properly washed with clean water to remove the dirt and soil aadhere to it. Kurunthotti, thannikka, mallika was collected from kuttyadi,kozhikode. Ashoka, Shatavari ,Muthanga, Koduveli, Kaduka,Kacholam was collected from Ayurvedhic shop mukkam, kozhikode in the month of february 2024.

Grapes, honey, nellika and jaggery were collected from kattangal.

Drying –

- Drying is one of the most important step. Drying is the easiest method of preserving herbs.
- Simply expose the leaves, flowers, or seeds to warm, dry air.
- Leave the herbs in well ventilated area until the moisture evaporates.
- Common drying techniques are: Sun drying

Shade drying

Drying by artificial heat

- Nellika and shatavari were dried using hot air oven.

Powdering

Preheat oven to 100 ^{0} C , Spread out herbs on a wire rack set inside a rimmed baking sheet and bake until dry and brittle, 1-2 hours. Grind herbs in a spice mill to a fine powder Seperately.

Preparation of Earthen pot:

Earthen pot for the Formulation of asava was prepared as follows:

- Earthen pot of 2 litre capacity was bought for the preparation of Asava.
- Pot was properly washed with clean water to remove the dirt, and was kept for drying under sun.
- 1 Tablespoon of ghee (around 15g) was taken and smeared inside the pot and was kept under the sun to dry, as ghee will be absorbed by the pot.
- This process was continued continuously for 5 times.

Ghee smeared pot

FORMULATION OF ASAVA

Asava is a special Ayurvedic medicine made by soaking the herbs in the form of dry powder in a solution of jaggery or sugar. It is kept such for a specialized period of time so that it undergoes a process called Sandhanakriya (Fermentation). This fermentation generates alcohol which facilitates the extraction of active principles contained in the herbs or drugs.The alcohol generated act as self preservative.

INGREDIENTS FOR 1000ML

Table No :1

Sl No.	Ingredients	Quantity
1	Ashoka	132g
2	Shatavari	33g
3	Muthanga	99g

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4	Citraka	66g
5	Bala	99g
6	Haritaki	33g
7	Vibhitaki	33g
8	Amla	66g
9	Bhunimba	19.8g
10	Sati	1.65g
11	Honey	15ml
12	Sugar	250g
13	Grapes	500g
14	Ghee	1 Tablespoon -15g

Ingredients for the formulation of asava

Nilavepu

Haritaki

Koduveli

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Thannikka

Kacholam

Ashoka

Kurunthotti

Steps involved in preparation of shatmuli asava

- The required quantity of water, to which jaggery or sugar as prescribed in the formula is added, boiled and cooled.
- This is poured into the fermentation pot, vessel or barrel. Fine powders of the drugs mentioned in the formula are added.
- Smear the pot with ghee before the liquids poured into it (in large scale manufacture, wooden-vats, porcelain-jars or metal vessels are used in place of earthen vessels.).
- The container is covered with a lid and the edges are sealed with clay-smeared cloth wound in seven consecutive layers. The container is kept either in a special room (Alternatively, in an underground cellar or in a heap of paddy, so as to ensure that for the duration of fermentation, as far as possible, a constant temperatures may impede or accelerate the fermentation).
- After the specified period, the lid is removed, and the contents examined to ascertain whether the process of fermentation has been completed.
- The fluid is first decanted and then strained after two or three days. When the fine suspended particles settle down, it is strained again.
- If the fermentation is to be carried in an earthen vessel, it should not be new. Water should be boiled first in the vessel. Absolute cleanliness is required during the process.
- Smear the pot with ghee before the liquids poured into it (in large scale manufacture, wooden-vats, porcelain-jars or metal vessels are used in place of earthen vessels.).

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- The filtered asava should be clear without froth at the top. It should not become sour(Cukra). The preparation has the characteristics of aromatic alcoholic odour.
- Asava can be kept indefinitely. They should be kept in well-stoppered bottles or jars. Honey, where mentioned, should be added as such without being dissolved or boiled.

Tied pot

Pot kept inside the cardboard

Standardisation of asava :

It is the establishment of standards for the quality and purity of raw materials,quality control throughout the drug manufacturing method, development of high-quality finished product, storage and distribution to preserve the quality of the finished product.

Generally it involves the following parameters;

1. Organoleptic characters
   - Colour of sample
   - Odour of sample
   - Taste of sample

2. Identification
   - Thin layer chromatography
   - High performance thin layer chromatography

3. Physico-chemical analysis
4. Ash value
5. Extractive value
6. Heavy metals
7. Total solid content
8. pH
9. Boiling point
10. Viscosity
11. Specific gravity
12. Alcohol content
13. Reducing sugar

1. DESCRIPTION

Colour : Clear dark brown without frothing.
Odour : Aromatic odour
Taste : Acrid taste

2. IDENTIFICATION

Thin Layer Chromatography:

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic, metal sheet or plate. Pre-coated plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent. Preparation of plates: Prepare a suspension of the coating substance in accordance with the instructions of the supplier and, using the spreading device designed for the purpose, spread a uniform layer of the suspension, 0.20 to 0.30 mm thick, on a flat glass plate 20 cm long. Allow the coated plates to dry in air, heat at 1000 to 1050 for at least 1 hour (except in the case of plates prepared with cellulose

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when heating for 10 minutes is normally sufficient) and allow to cool, protected from moisture. Store the plates protected from moisture and use within 3 days of preparation. At the time of use, dry the plates again, if necessary, as prescribed in the monographs.

3. PHYSICO-CHEMICAL ANALYSIS:
DETERMINATION OF ASH VALUES:
Determination of Total Ash:

Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450 0 until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450^0. Calculate the percentage of ash with reference to the air-dried drug.

Determination of Acid-Insoluble Ash:

To the crucible containing total ash, add 25 ml of dilute hydrochloric acid. Collect the insoluble matter on an ashless filter paper (Whatman 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes and weigh without delay. Calculate the content of acid insoluble ash with reference to the air-dried drug.

Determination of Water Soluble Ash:

Boil the ash for 5 minutes with 25 ml of water; collect insoluble matter in a Gooch crucible or on an ashless filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450^0. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water
soluble ash with reference to the air-dried drug.

DETERMINATION OF EXTRACTIVE VALUES:
Determination of Alcohol Soluble Extractive:

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of alcohol the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallowdish, and dry at 1050, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Determination of Water Soluble Extractive:
Proceed as directed for the determination of alcohol-soluble extractive, using chloroform water instead of ethanol.

3. DETERMINATION OF HEAVY METALS:

The test for heavy metals is designed to determine the content of metallic impurities that are coloured by sulphide ion, under specified conditions. The limit for heavy metals is indicated in the individual monographs in terms of the parts of lead per million parts of the substance (by weight), as determined by visual comparison of the colour produced by the substance with that of a control prepared from a standard lead solution.

Method :
Standard solution: Into a 50 ml Nessler cylinder, pipette 2 ml of standard lead solution and dilute with water to 25 ml. Adiust with dilute acetic acid Sp. or dilute ammonia solution Sp to a pH between 3.0 and 4.0, dilute with water to about 35 ml, and mix.
Test solution: In to a 50 ml Nessler cylinder, place 25 ml of the solution prepared for the test as directed in the individual monograph, or using the stated volume of acid when specified in the individual monograph, dissolve and dilute with water to 25 ml the specified quantity of the substance being tested. Adjust with dilute acetic acid Sp. or dilute ammonia solution Sp. to a pH between 3.0 and 4.0, dilute with water to about 35 ml and mix.
Procedure: To each of the cylinders containing the standard solution and test solution, respectively, add 10 ml of freshly prepared hydrogen sulphide solution, mix, dilute with water to 50 ml, allow to stand for five minutes, and view downwards over a white surface; the colour produced in the test solution is not darker than that produced in the standard solution.

4. TOTAL SOLID CONTENT:

Transfer accurately 50 ml of the clear Asava/ Aristha to an evaporable dish,which has been dried to a constant weight and evaporate to dryness on a water bath, then dry at 1050 for 3

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hours. After cooling the dish containing the residue in a desiccator for 30min, weigh it immediately. The weight of residue should comply with the requirements stated under the individual monograph.

5. DETERMINATION OF pH VALUES:

The pH value of an aqueous liquid may be defined as the common logarithum of the reciprocal of the hydrogen ion concentration expressed in g per litre. The pH value of a liquid can be determined potentiometrically by means of the glass electrode, a reference electrode and a pH meter either of the digital or analogue type.

6. VISCOSITY:

The liquid under test is filled in a U tube viscometer in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer when the capillary is vertical and the specified temperature is attained by the test liquid. The liquid is sucked or blown to the specified weight of the viscometer and the time taken for the meniscus to pass the two specified marks is measured. The kinematic viscosity in centi stokes is calculated from the following equation:

Kinematic viscosity = kt

Where,

k = the constant of the viscometer tube

t = time in seconds for meniscus to pass through the two specified marks.

7. BOILING POINT:

100 ml of the liquid to be examined is placed in the distillation flask, and a few glass beads or other suitable substance is added. The bulb of the flask is placed centrally over a circular hole varying from 3 to 5 cm in diameter (according to the boiling range of the substance under examination), in a suitable asbestos board. The thermometer is held concentrically in the neck of the flask by means of a well fitting cork in such a manner that the bulb of the thermometer remains just below the level of the opening of the side-tube. Heat the flask slowly in the beginning and When distillation starts. Adjust heating in such a manner that the liquid distils at a constant rate of 4 to > ml per minute. 'The temperature is read when the first drop runs from the condenser. and again when the last quantity of liquid in the flask is evaporated.

8. SPECIFIC GRAVITY:

The specific gravity of a liquid is the weight of a given volume of the liquid at 25^0(unless otherwise specified) compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air.

9. ALCOHOL CONTENT:

The ethanol content of a liquid is expressed as the number of volumes of ethanol contained in 100 volumes of the liquid, the volumes being measured at 24.90 to 25.10. This is known as the "percentage of ethanol by volume". Transfer 25 ml of the preparation being examined, accurately measured at 24.90 to 25.10, to the distillation flask. Dilute with 150 ml of water and add porcelin peice. Attach the distillation head and condenser. Distil and collect not less than 90 ml of the distillate into a 100-ml volumetric flask. Adjust the temperature to $24.9^0$to 25.1 ^{0}and dilute to volume with distilled water at $24.9^0$to 25.1^0. Determine the relative density at 24.9^0 to 25.1^0. The values indicated in column 2 of Table are multiplied by 4 in order to obtain the percentage of ethanol by volume contained in the preparation. If the specific gravity is found to be between two values, the percentage of ethanol should be obtained by interpolation. After calculation of the ethanol content, report the result to one decimal place.

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Table no : 2

Specific gravity at 25^0	Ethanol content
1.0000	0
0.9985	1
0.9970	2
0.9956	3
0.9941	4
0.9927	5
0.9914	6
0.9901	7
0.9888	8
0.9875	9
0.9862	10
0.9850	11
0.9838	12
0.9826	13
0.9814	14
0.9802	15
0.9790	16
0.9778	17
0.9767	18
0.9756	19
0.9744	20
0.9733	21
0.9721	22
0.9710	23
0.9698	24
0.9685	25

10. TEST FOR REDUCING AND NON REDUCING SUGAR :

Table no: 3

Procedure	**Observation**	**Inference**
Molisch's test: 3ml of sample solution+1-2 drops of molisch reagent + 3ml of concentrated sulphuric acid along sides of test tube	Reddish – violet ring at the junction of 2 liquids	Presence of Carbohydrates
Benedict's test: 5ml of Benedicts reagent +8 drops of solution, mix and boil for 2 minutes	Appearance of red yellow green colour Absence of red yellow green colour	Presence of reducing sugar Absence of reducing sugar
Fehling's test: To 1 ml of test solution add Fehlings solution A and B and boil for 2 minutes.	Appearance Brick red precipitate No red precipitate	Presence of reducing sugar Absence of reducing sugar

11. ELEMENTALANALYSIS:

Determination of Iron (Fe):

Preparation of sample solution: Ignite a suitable quantity of the sample (in the presence of organic matter) in a crucible in amuffle furnace at 500-550 0 until the residue is free from organic

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matter. Moisten with 5- 10ml of hydrochloric acid, boil for two min, add 30 ml of water, heat on the water bath for few min, filter and wash thoroughly the residue with water and make up to volume in a volumentric flask.

Procedure:

Take /withdraw a suitable aliquot from the stock solution in 250 ml in duplicate. Dilute to about 100 ml with distilled water. Add 1-2 drops of methyl red indicator. Add 1-2 g ammonium chloride. Add dil. Ammonium solution till brown precipitate appears. Boil the solution with ppt. for 4-5 minutes. Cool the content and filter through Whatman 41 no. filter paper. Wash the residue with hot water 4-6 times. Dissolve the residue in dil. HCl in 250 ml beaker. Wash with hot water and make the volume to 100 ml approx. Boil the solution on burner. Reduce the Fe 3+ to Fe 2+ by adding stannous chloride solution drop wise till solution becomes colourless. Add 1-2 drops of stannous chloride solution in excess. Cool the content in water. Add 10-15 ml 10per cent solution of mercuric chloride. Add 25 ml acid mixture. Add 2-3 drops of diphenylamine barium sulphonate indicator. Add distilled water, if required. Titrate against standard potassium dichromate solution. Appearance of violet colour show end point.

Determination of Sulphur:

Procedure:

Take 0.5 – 1 g powdered sample in 250 ml beaker. Add 10 ml carbon tetrachloride saturated with bromine. Keep in cold condition in fume chamber over night. Add 10 – 15 ml concentrated Nitric acid. Digest on water bath. Add 10 ml concentrated hydrochloric acid, digest it to expel nitrate fumes till syrupy mass. Cool and extract with hydrochloric acid, make volume to 100 ml. Boil and filter through Whatman No 40. filter paper. Wash the residue with hot water. Filter through Whatman 41 No. paper to 600 ml beaker. Acidify the filtrate with hydrochloric acid. Add 20 ml of 10 per cent Barium chloride solution. Stir the solution and digest on burner. Allow to settle $BaSO_4$ precipitate over night. Filter the precipitate through Whatman No. 42 filter paper. Wash the precipitate with water. Ignite the precipitate in muffle furnace in pre weighed platinum crucible up to 850^0. Allow to cool and weigh.

Chlorides:

Chlorides, heated with manganese dioxide and sulphuric acid, yield chlorine, recognisable by its odour and by giving a blue colour with potassium iodide and solution of starch.

Calcium:

Solutions of calcium salts yield, with solution of ammonium carbonate, a white precipitate which after boiling and cooling the mixture, is insoluble in solution of ammonium chloride.[51]

IV. RESULT AND DISCUSSION

Selection of medicinal plants

Thorough study was performed and the following plants were found to have menorrhagia activity.

- Ashoka
- Shatavari
- Muthanga
- Bala
- Vibhitaki
- Amla
- Sati

The following parameters were performed for the standardization of Asava ;

- Description
- Identification
- Total Solids
- Boiling point
- Test for reducing and non reducing sugars
- Alcohol content
- Viscosity
- Ash value
- Extractive value

INGREDIENTS:

Table no : 4

Sl no.	Ingredients	Official Formula	Working Formula
1	Ashoka	132g	26.4g

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2	Shatavari	33g	6.6g
3	Muthanga	99g	19.8g
4	Citraka	66g	13.2g
5	Bala	99g	19.8g
6	Haritaki	33g	6.6g
7	Vibhitaki	33g	6.6g
8	Amla	66g	13.2g
9	Bhunimba	19.8g	3.96g
10	Sati	1.65g	0.33g
11	Honey	15ml	3 ml
12	Sugar	250g	50g
13	Grapes	500g	100g
14	Ghee	1 Tablespoon -15g	3g

Description:

Table no : 5

Description	Observation
Colour	Clear light brown without frothing and significant sedimentation
Odour	Aromatic odour
Taste	Acrid

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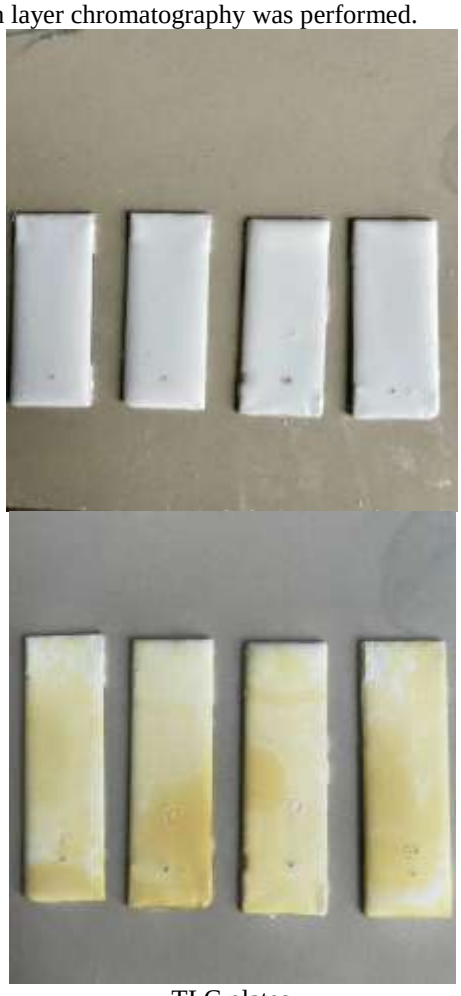
Filled bottles

Identification:

Thin layer chromatography was performed.



TLC plates

Total solids :

As per the equation;

Total solid % (W/V) = (W1-W)/v×100

W1 = 65.86

W = 63.04

V = 3ml

= 65.86-63.04/3×100

= 94 %W/V

Total solid was found to be 94 %w/v.

Boiling points :

Boiling point of the sample was identified using a thermometer and the temperature was found to be 85°**C.**

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Test for reducing and non reducing sugars:
Molisch's test – Reddish violet ring at the junction of 2 liquids, which indicates the presence of carbohydrates.
Benedicts test – Appearance of yellow/red/green colour indicates the presence of reducing sugar.
Fehling's test – Appearance of Brick red precipitate indicates presence of reducing sugars.

Alcohol content :
Weight of empty specific gravity bottle = 19.03g
Weight of bottle + alcohol = 40.07g
Volume of alcohol = 21.5ml
Density of water = 0.997 g/ml
Mass = 40.07-19.03
= 21.04g
Density= Mass/volume
= 21.04/21.5
= 0.97g/ml
Specific gravity = 0.97/0.99
= 0.97kg/m^3
Alcohol content in the given sample was found to be 17ml.

Viscocity :
Weight of empty bottle w_1 = 18.01g
Weight of bottle + water w_2 = 42.57g
Weight of bottle + sample w_3 = 43.25g
Density = $w_3 - w_1 / w_2 - w_1$
= 1.027g/ml
34
FORMULATION AND STANDARDIZATION OF AYURVEDIC SHATMULI ASAVA FOR TREATMENT OF MENORRHAGIA
Density of given liquid = 1.02 × 0.997
= 1.016g/ml
Density of sample ρ_1 = 1.016g/ml
Density of water ρ_2 = 0.997g/ml
Time taken by sample t_1 = 103 seconds
Time taken by water t_2 = 74 seconds
Viscosity of given liquid $\eta = t_1 \rho_1 / t_2 \rho_2 \times \eta_w$
η = (103 × 1.016 / 74 × 0.997) × 0.8904
= 1.2608 kg/ms
Viscometer was used for finding the viscocity and the result was found to be 1.2608 kg/ms.

PH :
Digital pH meter was used to check the pH of formulation, before the experiment the machine was calibrated by using standard buffer solution of pH . pH of the sample was found to be 7

Specific gravity :
Weight of empty specific gravity bottle = 18.01g
Weight of bottle + weight of water = 42.57g
Weight of bottle + weight of sample = 43.25g
Specific gravity= w_1-w/w_2-w
= 43.25-18.01/42.57-18.01
= 1.027kg/m^3.
Specific gravity of the sample was found out using

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Specific gravity bottle and the value was found to be 1.027 kg/m^3.

Extractive value

Extractive value = Weight of residue/initial weight of drug ×100
= 1.27/5 × 100
= 25.4 %w/w.
Extractive value of the given sample was found to be 25.4%w/w.

Ash value

Total ash value

Weight of crucible W_1 = 26.98g
Weight of crucible+ash W_2 = 26.99g
%Total ash = W_2-W_1/3 × 100
= 26.99-26.98/3×100
= 0.3%w/w

Water soluble ash

Weight of crucible W_1 = 68.00g
Weight of powder W_2 = 3ml

Weight of water soluble ash + crucible W_3= 68.02g
Weight of insoluble ash W_4 = W_3-W_1 = 68.04-68.00
= 0.04g
Weight of total ash W_5 = 0.3g
Weight of water soluble ash W_6 = W_5-W_4 = 0.1g
%Water soluble ash value = W_6/W_2 × 100 = 3.3%w/w

Acid insoluble ash value

Weight of crucible W_1 = 64g
Weight of crucible + ash = 64.03g
Weight of sample = 3 ml
Weight of acid insoluble ash = 0.03g
% Acid insoluble ash = 0.03/3 × 100 = 1%w/w

Labelling of Asava

SHATMULI ASAVA 200ml
Rx Ashoka - 52.8g Shatmuli - 13.2g Muthanga - 39.6g Citraka - 26.4g Bala - 39.6g Haritaki - 13.2g Vibhitaki - 13.2g Amla - 26.4g Bhunimba - 7.92g Sati - 0.66g Honey - 6ml Sugar - 100g Grapes - 200g Ghee - 6g
Storage : Store in a cool place in tightly closed amber coloured bottle, protected from light and moisture. Dose : 15- 30ml orally with equal amount of water after meals twice a day.

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Mfg date: 04/03/2024 Exp date: 04/03/2025 MRP : 130/-
Manufactured and packed by: KMCT College of Pharmaceutical Science, Kallanthode, NITC, Calicut, 673601

V. SUMMARY AND CONCLUSION

The medicinal plants named saraca indica, asparagus racemosus, cyperus rotundus Sida cordata, Terminalia Belerica and Kaempferia Galanga was taken as the ingredients for the formulation of shatmuli asava for the treatment of menorrhagia. From the literature review above plants were found to have activity in menorrhagia condition. These plants reduces the severity,pain. Inflammation and flow volume in menorrhagia.Also shatmuli formulation contain Phyllanthus emblica , these herbs increases the RBC ,vitamins and mineral contents during the disease condition.

The formulation of Shatmuli Asava was subjected to various standardization parameters as per AYUSH such as TLC, alcohol content, total solid contents , PH, viscosity, boiling point, extractive value, ash value, total solid content and reducing sugers to ensure the quality , safety and efficacy of the product.

Thus formulation and evaluation of Shatmuli Asava was performed and it was found to be effective for the treatment of menorrhagia.

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