Research Article

www.ijrap.net

(ISSN Online:2229–3566, ISSN Print:2277–4343)

DEVELOPMENT AND EVALUATION OF AN AYURVEDIC HERBAL FORMULATION (MUSA ASAVA) FOR ANTI-UROLITHIATIC ACTIVITY

Kumanan R *, Faseela Banu PK, Sudev K, Adarsh M, Santhosh C, Siniya TK

Department of Pharmacognosy, Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram District, Kerala, India

Received on: 08/12/22 Accepted on: 11/02/23

***Corresponding author**

E-mail: rkumanan@rediffmail.com

DOI: 10.7897/2277-4343.140247

ABSTRACT

The present study aims to develop and evaluate a herbal formulation with anti-urolithiatic activity. The study is planned to formulate an ayurvedic preparation (asava) as per official procedures using fresh stem extracts of *Musa paradisiaca*. The study also evaluates the impact of banana stem contents within the extract and prepared formulation (asava) on dissolving kidney stones for anti-urolithiatic activity. The objective of the current research work was to determine the anti-urolithiatic activity and its toxic element standardization analysis of the *Musa paradisiaca*. The banana stem asava was evaluated on different parameters like pH, viscosity phytochemical test, TLC, Heavy metal analysis, specific gravity, and organoleptic evaluation along with the fresh stem extract/juice. The phytochemical screening helps to find out the presence of alkaloids, flavonoids, carbohydrates, tannins, proteins, and cellulose. The ICP-MS heavy metal analysis showed the presence of arsenic and mercury. The anti-urolithiatic activity result indicated a 10% and 33% decrease in the stone weight in the presence of raw juice and Musa asava, respectively. Thus, it shows that regular Musa asava can reduce the chances of kidney stone formation.

Keywords: Anti-urolithiatic, Musa asava, ICP-MS analysis

INTRODUCTION

Medicinal plants continue to be used in traditional medicine to treat various ailments. The World Health Organization (WHO) estimates that 80% of the world's people depend on traditional medicine for their primary healthcare needs, and most of these therapies involve plant extracts or their active ingredients. It helps to explore different medicinal plants to determine the scientific basis for their traditional use. Ayurveda and other Indian texts mention the use of plants in the treatment of various human ailments. Due to the superiority and effectiveness of the activities provided by the botanical components of medicinal plants and the adverse effects of modern medicine, using medicinal plants to prevent and treat diseases is becoming increasingly important. Medicinal plants contain many medicinal properties; one such plant is *Musa paradisiaca.* It has been reported to have pharmacological activities such as anti-inflammatory, antioxidant, antibacterial, antidiabetic, antiulcer, antidiarrheal, cholesterol-lowering, hepatoprotective, antivenom, healing, hair growth promoting, antifungal, and antihemorrhagic activity[1].

Musa is a unique herbaceous plant of the Musaceae family, distributed in tropical and subtropical countries. Traditional medicine widely uses plant parts to treat various human ailments such as diabetes, diarrhoea, dysentery, hypertension, hysteria, epilepsy, leprosy, bleeding, kidney stones and stomach ulcers. This review presents information on plantain's morphology, traditional uses, and phytochemical and pharmacological activities. All parts of the banana tree have medicinal properties. The flowers are used to treat bronchitis, dysentery, menorrhagia and stomach ulcers. Boiled flowers are used to treat diabetes. Astringent plant juices are used for conditions such as hysteria, epilepsy, leprosy, fever, bleeding, dysentery, diarrhoea, haemorrhoids, insect bites, and others. Young leaves are used as a poultice for burns and other skin complaints. The ash from the bark and immature leaves is used against dysentery, diarrhoea, and malignant ulcers. The roots are used to treat digestive disorders, dysentery and other ailments. It also has insect-repellent properties. In India, mucilage from banana seeds treats catarrh and diarrhoea. The antifungal and antibiotic properties are found in the skin and pulp of fully ripe bananas. The plant is also used to treat inflammation, pain, and snakebites.[2]

A Banana is a herbaceous plant cultivated for its edible parts. Low in calories and high in fibre, banana stalks dissolve kidney stones, reduce weight and help cleanse the urinary tract. This study aimed to compare the chemical composition of the raw material and the effectiveness of asava against kidney stones. Fresh stem juice is extracted and labelled as raw juice; another is formulated as asava. Analyze biochemical parameters. Highest recorded protein concentration [0.435 g/100 g], iron [1.89 mg/100 g], phosphorus [3.16 mg/100 g] and calcium. The original juice is [35.05 mg/100 g], while the concentration of reducing sugars in plantain stem juice is the highest [2.45 g/100 g].[3]

Plant Profile

Botanical Name : *Musa paradisiaca*
Common Name : Banana
English : Plantain, French plantain
Malayalam : Vazha, Kadalivazha
Tamil : Ethakkai
Sanskrit : Kadali
Synonym : *Musa paradisiaca* var. subrubea Blanco, *Musa paradisiaca* var. ternatensis Blanco, *Musa paradisiaca* var. tetragona G. Forst, *Musa paradisiaca* var. tombak Blanco

Scientific Classification

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Division	: Magnoliophyta-Flowerings
Class	: Liliopsida-Monocots
Order	: Zingiberales
Family	: Musaceae
Genus	: *Musa*
Species	: *paradisiac* [4-6]

MATERIALS AND METHODS

Plant collection and authentication

Musa paradisiaca (L) is the accepted name for the hybrid between *Musa acuminata* and authenticated by Dr K. Kishore Kumar, Head, Department of Botany. Farook College. Calicut, Kerala and a voucher specimen were prepared. Herbarium (No.016) is documented in our department library.

Preparation of plant extract

The plant material was freed from foreign matter and washed with fresh water. It was cut into small pieces and made juice using a mixer grinder. Freshly obtained juice was filtered using a muslin cloth, and aqueous juice extract was preserved until its use in an air-tight and light-resistant container in a cold condition.[7, 8.]

Preliminary Phytochemical Investigation

The aqueous juice extract was subjected to qualitative chemical investigation. The following procedures were adopted to test for various phytochemical constituents in the extract as per C.K.Kokate, practical pharmacognosy[12]. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, phenols, carbohydrates, and glycosides. Phytochemicals are used as templates for lead optimization programs intended to make safe and effective drugs. The procedures for identifying Alkaloids, Carbohydrates, Tannins, Glycosides, Cellulose and Proteins were adapted to various chemical constituents in juice extract.[9-12]

Preparation of Musa Asava

Preparation of asava as per standard and existing procedure available. The jaggery is dissolved in the required quantity of water (previously boiled and cooled). This is poured into the fermentation vessel (mud pot used). Fine powder of the spices necessary is added to the mud pot, which is covered with a lid, and the edges are sealed with clay-smeared cloth in seven consecutive layers. Kept undisturbed in a dark room provided uniform temperature till the completion of the process. after the stipulated period, the lid was opened and checked for any froth on the top of the layer, then filtered and kept in a cleaned container. The same was taken for further studies. Cleanliness is ensured throughout the process to avoid contamination possibilities.

The materials and ingredients used to prepare Musa asava are an earthen pot with a lid, jaggery, pepper, ghee, fennel, honey, cinnamon, cloves, mixer grinder, muslin cloth, livestock feed (vaikol/dried rice paddy straw), cardboard box.

Standardisation of Musa Asava

Organoleptic Evaluation of Asava

Homogeneity and appearance: The formulation was tested for homogeneity by visual appearance.

Determination of pH

The pH of the formulation was determined by using a digital pH meter. About 10 ml of the asava was taken, a measure of the pH of the solution was done in triplicate, and average values were calculated.

Determination of viscosity

A Brookfield Dial Reading viscometer (RVT) was used to measure the viscosity (cps) of asava. The spindle (Helipath spindle set) was used for spindle number T-F. The spindle was rotated at 10 rpm. The determinations were carried out in triplicate, and the average of three readings was recorded.

Alcohol content determination

Steam distillation: The steam distillation method transfers 25 ml of the sample accurately measured at 25 ^{0}C to the distillation flask. Dilute with 150 ml of water and add a little pumice powder, attach the distillatory and condenser distilled and collect not less than 90 ml of distillate into 100 ml. Determine the specific gravity at 25 ^{0}C find the percentage w/v of ethyl alcohol.

Determination of specific gravity

Specific gravity is the ratio of the material's specific weight to the water's specific weight. A specific gravity bottle of 10 ml capacity was cleaned, dried and weighed. It is filled to the mark with water at the required temperature and weighed. The specific gravity bottle was next to fill up to the mark with the sample. The specific gravity was determined by dividing the weight of the sample expressed in grams by the weight of the water, expressed in grams.

Quantitative determination of heavy metals

Quantitative determination of major heavy metals and minerals of asava were determined by (ICP-MS), Instrument: ICP- MS-Agilent Technologies 7700 series ICP MS. (Source care Keralam, Thrissur)

Reagents: Water (SIEMENS), Supra pure nitric acid or sub-boiled nitric acid, NIST traceable calibration standards.

Apparatus: Polypropylene bottles for storing standards, polypropylene volumetric flasks for preparing standards, polypropylene pipettes, inductively coupled plasma mass spectrometer.

Procedure: Standard preparation: Preparation of 1ppm stock solution; 1ppm stock solution is prepared by 1 ml of 1000 ppm standard solution to a 100 ml standard flask and made up to the mark using ultra-pure water.

Preparation of working standard solution: 0.5 ppb, 5 ppb, 50 ppb, 100 ppb, 200 ppb, 250 ppb, and make up to the mark using HPLC water. 1 ml of 20% extra pure concentrated HNO3 was added to the standard flask prior to making it up to the mark.

Sample preparation: 0.25 g to 0.5 g sample is weighed accurately into an MDS digestion tube. Add 5.0 ml conc. HNO3 (extra pure), 0.5 ml HCL (extra pure) and 1.0 ml water (extra pure) and allow 15 min. self-digestion. Tighten the cap and keep it for digestion in MDS. After digestion, quantitatively transfer the contents into a 50 ml tube and makeup to 50 ml using extra pure water.[13]

Anti-urolithiatic property of *Musa paradisica*

To investigate the effect of asava prepared from banana stem juice

on kidney stones, stones were collected from Dr Sunil G, Department of Nephrology, PVS Hospital, Kozhikode, Kerala. The collected kidney stones were weight accurately and inoculated into a sterile conical flask containing 25 ml of extracted raw banana stem juice and prepared Musa asava for 5 days to obtain results.[14]

Determination of Thin Layer Chromatography

Principle of separation by thin layer chromatography
The program is based on a family member's affinity for a compound for mobile and stationary phases. The process is activated by displacing the mobile phase on the surface of the stationary phase. During this movement, compounds of higher affinity are acquired more slowly than those of lower affinity, leading to their separation from the components involved in the TLC procedure, as follows. TLC Plates: These are used to apply thin films on stationary phases. They are either inert or stable. The stationary phase layer remains in these plates for better analysis. Often, those who perform experiments prefer ready-to-use plates.

Mobile Phase: This comprises a solvent/solvent mixture. The taken solvent needs to be chemically inert, of the highest purity and particulate-free. Only then can the TLC spots develop on the stationary phase.

TLC Chamber: TLC procedure takes place here, and it keeps the dust particles away from the process and ensures not allowing the solvent to evaporate. That develops the spots appropriately, and a uniform atmosphere is maintained inside this chamber.

Filter Paper: This gets placed inside the chamber after being prepared in the required ratio with the mobile phase. It confirms that the mobile phase is saturated thoroughly, and no vapours exist in the air inside the mobile phase-containing chamber.

After collecting all these components, the steps followed: The process starts by making a thin mark on the TLC plate's bottom. It assists in the application of sample spots. These spots are kept at equal distances. The sample is then applied to these spots made on the line. Then the TLC chamber is filled with the mobile phase of its bottom up to the required volume. Finally, the prepared stationary phase plate is kept inside the chamber. The chamber is then closed after placing the plate into it. Let the process end, and the plate is removed and allowed to dry. The sample spots get analysed through a suitable method for the sample, such as UV light, KMnO4 stain, and iodine staining. After analysing the compound, it gets described in its relative mobility terms, i.e., its Rf value is calculated. This value changes for each compound, even under the same circumstances. These aspects include adsorbent, temperature, thickness, spotted material amount, and solvent system.

The formula used for Rf value calculation is:

$$Rf(retention\ factor) = \frac{distance\ traveled\ by\ the\ compound}{distance\ traveled\ by\ the\ solvent\ front}$$

Quantitative determination of heavy metals

RESULT AND DISCUSSION

Preliminary analysis of phytochemicals

Plant constituents	Test	Observation
Carbohydrates	Molisch's	+
	Fehling's	+
	Legal's	+
	Benedict's	+
Protein	Protein test	+
Tannins and phenols	Gelatine	+
	Vanillin	-
	Catechin	+
Flavonoids	Lead acetate	+
	Zinc HCl	+
	Shinoda's	+
Alkaloids	Mayers test	+
	Dragendorff's	+
	Wagner's test	+
	Hager's test	+
	Van-urks	-
	Vitali Morin's	-
	Thalleoquine	-
Cellulose	Cellulose test	+
Glycosides	Raymond's test	+
	Kedde's test	+
	Baljet test	+
	Tollen's test	+
	Legal's test	+

Note: + Present - Absent

Organoleptic evaluation of asava
Organoleptic evaluation can be done employing the organ of sense. This refers to assessing asava by colour, odour, taste, special features, etc. Colour: Greyish brown, Odour: Aromatic, Taste: Astringent sour

Measurement of viscosity
The viscosity of the formulation was determined by Brookfield Viscometer spindle type s-24 (LVDVE) at 10 rpm. The viscosity of the formulation was found to be 421.7 cp, which indicates that the formulation is less viscous.

Measurement of pH
The pH of the asava formulation was determined using a pH meter and was around 4.5.

Determination of specific gravity
The specific gravity was determined by dividing the weight of the sample expressed in grams by the weight of the water. The result of the specific gravity of the formulation was found to be 1.1242.

Determination of alcohol content
The alcohol content of the asava formulation was determined using a simple distillation method. The alcohol content (% v/v) was found to be 13.5%v/v.
The formula used [Alcohol by Volume = SG X 131.25].

Quantitative determination of major heavy metals and minerals of asava were determined by (ICP-MS).

Parameters	Unit	Result	Specification	Detection Limit	Asava Test Method
Arsenic	mg/kg	0.18	NMT 3.0	0.05	CKL/ANL/AY-008*
Cadmium	mg/kg	Not Detected	NMT 0.3	0.05	
Lead	mg/kg	BDL	NMT 10.0	0.05	
Mercury	mg/kg	1.11	NMT 1.0	0.05	

The Sample complies as per API, with respect to the tested parameters only. *Ref code of Care Keralam. Note: NMT- Not More Than, BDL-Below Detection Limit, Total Number of Determination: 4 only

TLC: Mobile Phase: Toluene: ethyl acetate: formic acid (5:1:0.5), Rf Value: 0.4

Determination of the anti-urolithiatic activity of Musa asava

To investigate the effect of banana stem juice on kidney stones were collected from PVS Hospital, Calicut. Kidney stones were weighed accurately and taken into a sterile conical flask containing 25 ml of extracted raw banana stem juice and asava for seven days. The result indicated a 10% and 33% decrease in the stone weight in the presence of raw juice and Musa asava, respectively. As banana stem juice and Musa asava has anti-urolithiatic property, regular consumption of a moderate dose of banana stem juice can reduce the chances of kidney stone formation.

Figure 1: Development of TLC

Figure 2: Measurement of Viscosity

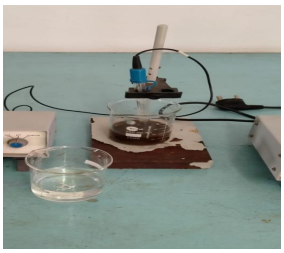

Figure 3: Measurement of pH

Figure 4: Determination of Alcohol content

Figure 5, 6, 7: Determination of Anti-Urolithiatic Activity of *Musa* Asava with fresh juice and control

Figure 8: *Musa* asava Ingredients

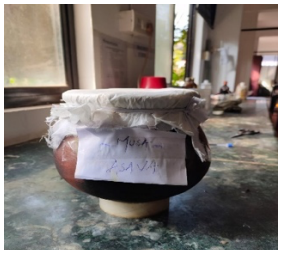

Figure 9: Asava preparation step 1

Figure 10: Asava Preparation step 2

Figure 11: *Musa paradisiaca* plant

Figure 12: Musa plant Stem

CONCLUSION

We studied the anti-urolithiatic activity of our novel herbal formulation (Musa asava), followed by preliminary analysis of phytochemicals, organoleptic evaluations, quantitative determination of heavy metals and specific gravity determination. Carbohydrates, protein, tannins, phenols, flavonoids, alkaloids, cellulose, and glycosides are the phytochemicals present in our formulation. The formulation is less viscous by measurement on viscometer (421.7cp) pH (4.5) and alcohol content 13.5% v/v. The weight of kidney stones was much less after the experimentation. This indicates that Musa asava gives a significant response to anti-urolithiatic activity.

Our study revealed the anti-urolithiatic activity of Musa asava, and its standardization shows a 10% and 33% decrease in kidney stones, which represents regular consumption of Musa stem juice and Musa asava, respectively can be very effective against kidney stone formation.

REFERENCES

1. Lavanya K, Abi Beaulah G, and Vani G. Phytochemistry and pharmacology of *Musa paradisiaca*, World journal of pharmaceutical and medicinal research. 2016; 2 (6): 163-17.
2. Varsha J, Galani A. Comprehensive Review on *Musa paradisiaca* Linn. Scholars International Journal of Traditional and Complementary Medicine, Sch Int J Trad Complement Med. June 2019; 2 (4): 45-56.
3. Vanitha S, Sree Vyshnavi P. Determination of Chemical Composition in Cooked and Raw Banana Stem (*Musa accuminata*) and Its Efficacy on Kidney Stones *in vitro* Study, Journal of Scientific Research Institute of Science Banaras Hindu University Varanasi. 2020; 64 (3): 66-74.
4. Ridwan Adesola. The pharmacological potentials of *Musa paradisiaca* Linn. Researchgate. Plant Science Today, 2021; 8(4): 1-8.
5. Ananta Swargiary, Mritunjoy Kumar Roy, Muhammed Akram. Phytochemistry and pharmacological property of *Musa balbisiana* Colla, Research gate Pharmacognosy Reviews. 2021; 15 (29): 16-20.
6. Kamoldeen Abiodun Ajijolakewu, Abiodun Saheed Ayoola, Tariq Oluwakunmi Agbabiaka, *et.al*. The ethnomedical antimicrobial and phytochemical properties of *Musa paradisiaca*, Springeropen. Bulletin of the National Research Centre volume. 2021; 45: 86.
7. Subrata Das Arunava Das, L Arunthathi, R Chandhini, R Madhumitha. Evaluation of Antimicrobial Activity of *Musa paradisiaca* Pseudo Stem Powder on Sustainable Textiles, Journal of Technical Education and Training. 2018; 4: 1-7.
8. Ajay Singh Bisht, Divya Juyal, Monika, Sangeeta Verma, Sweeta Joshi. Study on *in-vitro* antidiabetic potential of stem of *Musa paradisiaca* Linn, International Journal of Comprehensive and Advanced Pharmacology. 2017; 2 (1): 1-5.
9. Gowri Shankar, Jeevitha P, Shadeesh. Nutritional Analysis of *Musa acuminata* Research and Reviews, Journal of Food and Dairy Technology. 2017; 5 (4).
10. Ememobong Gideon Asuquo, Chinweizu Ejikeme Udobi. Antibacterial and toxicity studies of the ethanol extract of *Musa paradisiaca* leaf, Cogent Biology. 2016;2: 1-10.
11. U S Mahadeva Rao. Phytochemical screening total flavonoid and phenolic content assays of various solvent extracts of tepal of *Musa paradisiaca*, Malaysian Journal of Analytical Sciences; 20 (5); 2016: 1181-1190.
12. C K Kokate, A P Purohit, S B Gokhale. Study on preliminary phytochemical investigation, Textbook of Practical pharmacognosy 5th Edition Vallabh Prakashan New Delhi. 2005: p 107-111.
13. Deshraj S Chumbhale, S R Chaudhari, C D Upasani. Standardization of marketed Kumari asava - A polyherbal Ayurvedic *Pleurotus ostreatus* formulation, International Journal of Pharmaceutical Chemical and Biological Science. 2014; 4 (3): 681-685.
14. S Vanitha, P Sree Vyshnavi. Determination of chemical composition in cooked and raw banana stem (*Musa accuminata*) and Its Efficacy on Kidney Stones *in vitro* Study, Journal of Scientific Research. 2020; 64 (3): 66-74.

Cite this article as:

Kumanan R, Faseela Banu PK, Sudev K, Adarsh M, Santhosh C and Siniya TK. Development and evaluation of an Ayurvedic herbal formulation (Musa asava) for anti-urolithiatic activity. Int. J. Res. Ayurveda Pharm. 2023;14(2):86-90
DOI: http://dx.doi.org/10.7897/2277-4343.140247

Source of support: Nil, Conflict of interest: None Declared