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RESEARCH ARTICLE

Preparation and Standardization of Asava from *Emblica officinalis* Gertin by using flowers of *woodfordia fruticosa* as a fermenter

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Abstract:
Asava and Arishta are the very popular dosage form of Ayurveda, used since ancient time as a medicine for the treatment of various diseases.The Asava formulation was prepared by using flowers of *woodfordia fruticosa* as a fermenter with addition of jaggery. Heavy metal analysis was carried out using AAS and active chemical constituent was identified using RP-HPLC methods. The UV spectrophotometric method for formulation was developed and validated as per ICH Q2B guidelines.The self generated alcohol (SGA) content as ethanol was observed to be 8.69%, also the higher alcohol like n-butanol, n-propanol, iso-butanol were also found in trace amount as a byproduct of alcoholic fermentation. From the present study it can be said that the Ascorbic acid is found in Asava formulation also the properties of Amla and flowers of plant *Woodfordia fruticosa*, will helps to improve the health benefits of Asava formulation.

Keywords: Asava, Arishta, Ascorbic acid,Fermentation, RP-HPLC, UV

Introduction

The *Emblica officinalis* Gertinis a popular medicine in Ayurveda having many health benefits due to presence of phytoconstituents in it[1]. Ascorbic acid (AA) and Gallic acid (GA) is the active chemical constituent found abundant in Amla and having neuroprotective and antioxidant properties[2].Ascorbic acid is the water soluble antioxidants naturally found in plant-based foods like fruits and vegetables including peppers, citrus fruits, tropical fruits, spinach and cabbage[3].

Asava (fermented infusion) and Arishta (fermented decoction) are the form of Ayurvedic system of medicine. The concept of ayurvedic medicine is to promote the health rather than to fight disease. In the present study flowers of plant*Woodfordia fruticosa* kurz.(family- Lytheraceae) is used as a fermenter without any source of nutrients (Fig. 1). From these flowers various types of phytoconstituents were isolated and identified them as woodfordins A, B, C…I, oenothein A and B, citric acid, punicaline, estrone, insulin, mannitol, protein, lawsone, carbohydrates, aspartic acid, quercetin, glycosides, pentose, malvidin[4] etc.Flowers are use as source of yeast, from these six-types of yeast cultures are isolated and identified them as a *S. cerevisiae* and *Rhodutorula mucilaginosa*[5].Flowers are commonly use for the treatment of rheumatism, leucorrhea, menorrhagia, asthma, liver disorder and also posses significant antibacterial, antifirtility, antiulcer[6]antihyperglycemic, anti-inflammatory, cardioprotective, antitumor, immunomodulatory[7], anti-nociceptive[8]activity.

Fig 1: Flowers of Woodfordia fruticosa Kurz.

Materials and Methods

Collection and Authentification of Amla fruits and fermenter

Fresh Amla fruits were collected from the local market of Kopargaon, Dist-Ahmadnagar, India and the flowers of plant *Woodfordia fruticosa* Kurz. (Dhataki) were collected from Mahatma Phule Krushi Vidyapeth, Rahuri in June 2013, Dist-Ahmadnagar, India. Its authentication was carried out at department of botany, S. S. G. M. College Kopargaon, Dist-Ahmadnagar, India.

Physicochemical Examination of Amla fruits

The physicochemical examination of Amla fruit powder was carried out as per WHO guidelines[9].

Asava formulation

Formulation was prepared by using flowers of plant*Woodfordia fruticosa* as a fermenter for 3 months fermentation periods at 30-35^{0}C. The flow chart for making Asava formulation was given in Fig 2.

Heavy Metal Analysis (HMA)

Heavy metals were analyzed by using Atomic Absorption Spectrophotometer (AAS) coupled with inductive coupled plasma. Objective of this study is determined the concentration of heavy metals present in formulation that are consumed by community.

RP-HPLC analysis

Reverse phase High Performance Liquid Chromatography(RP-HPLC) describes methods that utilize a polar mobile phase in combination with non-polar stationary phase[10, 11]Chromatographic analysis was performed using a Shimadzu HPLC (LC-20AD, isocratic) Japan, was equipped with the UV-Vis detector (SPD-20A) and LUNA C18 reversed phase column (dimension ID 4.6mm × length 150mm, particle size 5µm)with flow rate of 1.0ml/min at wavelength 260nm.The mercury (Hg) lamp was used for inspection of the wavelength accuracy for the UV-Vis detector. The HPLC graded Acetonitrile: water (50:50v/v) was the selected mobile phase for HPLC analysis. The selected mobile phase was filtered through 0.45µ membrane filter and degassed by sonication before used. The room temperature was controlled at 16^{0}C ± 1.Standard solution was prepared by dissolving 10mg of AA in Acetonitrile: water (50:50v/v) solvent system. Formulation was concentrated tilled to form a hard mass was then triturated to formed a powder. Test solution was prepared by dissolving 10mg of triturated powder in 100ml solvent system.

UV method development

Method validation was performed by using Ultraviolet-vis-spectrophotometer (model no.1650 PC), Shimadzu. Validation is assuring that a developed method shows a valid measurement. It is also use to estimate the quality, reliability and consistency of analytical results. Stock solution was prepared by dissolving 10mg of powder sample in to 100ml methanol. Method was developed and validated as per ICH Q2B guidelines includes, linearity, range, precision, recovery, robustness, ruggedness, LOD and LOQ.

Results and Discussion

Amla juice was prepared by crushing and pressing the Amla fruits which contains the total soluble solids 3.9^{0}Brix with near about 4 pH.The physicochemical examination of Amla fruits was carried out and calculatedas total ash content 4%, acid insoluble ash 0.5%, alcohol soluble extractive values 48%, water soluble extractive values 52%, moisture content 3.33%, and crude fiber content 20% w/w. The potassium or Sodium salt of metabisulphite will helpful to inhibit the growth of undesirable microorganisms. The Amla fruits are sour, astringent, bitter, acrid, sweet, cooling, anodyne, ophthalmic, carminative, digestive, stomachic, laxative, alterant, aphrodisiac, rejuvenative, diuretic, antipyretic and tonic. They are useful in various conditions of tridosha, diabetes, cough, asthma, cephalalgia, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, skin diseases, leprosy, haematogenesis, inflammations[12]anemia, emaciation, hepatopathy, jaundice, strangury, diarrhoea, dysentery, hemorrhages, leucorrhoea, menorrhagia, cardiac disorders, intermittent fevers and greyness of hair[13]. Amla is having the greater antioxidant, antibacterial, antimutagenic, anti-ulcer, antitumour, anti-aging, hepatoprotective, spasmolytic activity and also use in cough, bronchitis haemoptysis, tuberculosis and scurvy[14, 15, 16]. Because of these properties of Amla and flowers of plant *Woodfordia fruticosa*, will helps to improve the health benefits of Asava formulation.

During fermentation the yeast *S. cerevisiae* contains the enzymes invertase which was carried out the hydrolysis of sucrose to glucose and fructose. Enzyme zymase further converts glucose to alcohol and carbon dioxide[17, 18]. Jaggery is the good medium for the growth of *S. cerevisiae* and other organisms. Anaerobic fermentation was preferred because during aerobic fermentation the growth of other microorganism (such as acetic acid bacteria, yeast or moulds) will occurred which affect on

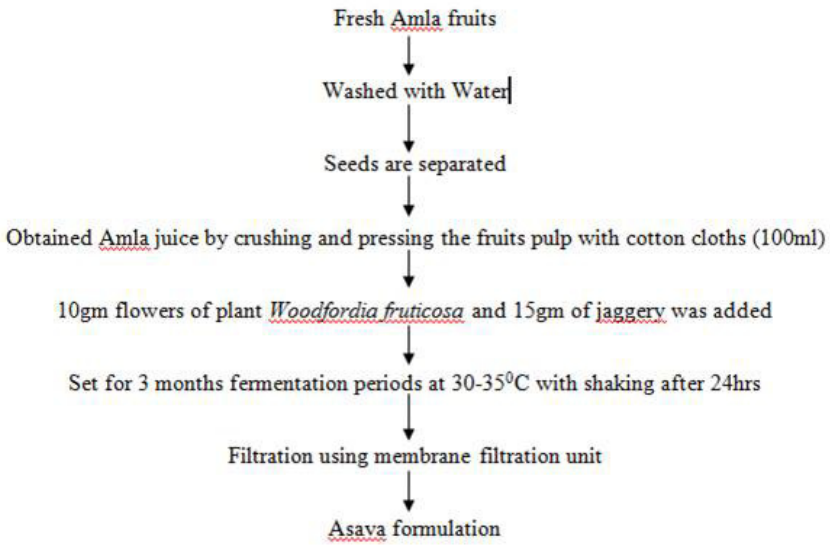

Fig 2: Flow chart for making Asava formulation

Table 1: Observation table for HMA
ND means less than 0.01ppm

Sample	Pb	Cd	As	Hg
	ppm	ppm	ppm	ppm
Sample	ND	ND	ND	ND

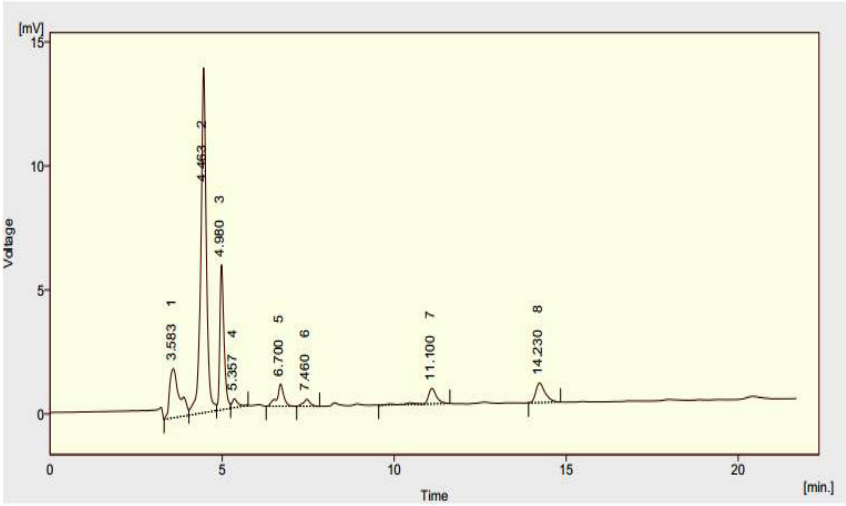

Fig 3: RP-HPLC spectrum of Asava formulation

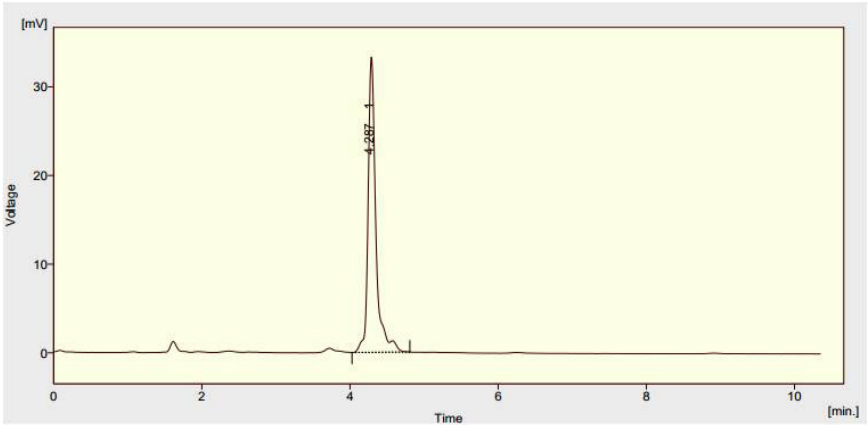

Fig 4: RP-HPLC spectrum of Ascorbic acid

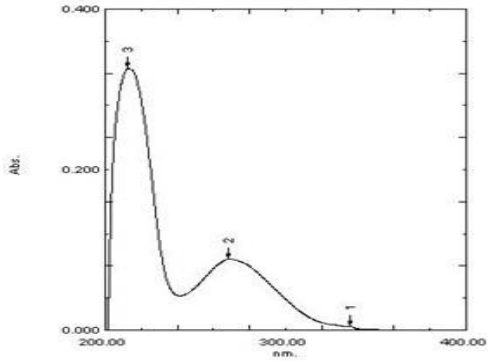

Fig 5: UV spectrum of Asava formulation

Table 2: Method validation parameters for Asava formulation

Sr. no.	**Parameters**	**Asava Formulation**	
1.	λ_{max}	258nm	
2.	Linearity a)Correlation coefficient b)Slope c)Intercept d)Range	 0.99 0.60 0.005 0.4-1.8µg/ml	
		SD	%RSD
3.	Precision a)Intraday precision b)Interday precision c)Intermediate precision	 0.0018 0.00086 0.0044	 0.38 0.17 0.97
4.	Ruggedness Analyst I Analyst II	 0.0029 0.00054	 0.29 0.054
5.	Robustness (Scanning speed-fast)	0.0044	0.44
6.	LOD	0.024µg/ml	
7.	LOQ	0.072µg/ml	
8.	% Recovery	50% - 91% 100% - 92.8% 150% - 94.9%	

ethanol fermentation. The presence of (%) SGA as ethanol in Amla wine was determined by using Gas Chromatography and it was found to be 8.69%.

The results of HMA suggest that not any heavy metal will detected in formulation beyond limit (Table 1), means the Amla fruits are used for preparation of formulationshould produces pollutant free formulation.

RP-HPLC data

RP-HPLC is the very suitable techniques for identification of AA in various types of plant and plant products. The standard AA showed peak at R_t 4.2 (Fig 4) at 259nm wavelength, while on the other hand the Asava formulation showed eight peaks (Fig 3). It was clearly observed that the R_t of standard AA was coinciding with the R_t of second peak (R_t-4.4) in Asava formulation.

UV data

For proper selection of wavelength diluted sample was scanned at 200-400nm on spectrum mode and method was developed in photometric mode. The λ_{max} of formulation was observed to be at 258nm (Fig 5), which was nearer to the wavelength of standard ascorbic acid i.e. 254nm, another peak is also observed at 222nm.The calibration curve was plotted using concentration (ppm) Vs absorbance; curve obtained was linear within the concentration range from 0.4-1.8 ppm. Precision was determined by studying the repeatability and intermediate precision. The method was found to be precise as the RSD values for repeatability and intermediate precision were found to be less than 2.0%. LOD and LOQ were confirmed to be 0.024, 0.072µg/ml. Accuracy of the method was performed by recovery studies. The percentage recovery was calculated as 91.5%, 92.8%and 94.9%for 50%, 100%, and 150% of test concentration (Table 2). Robustness study of the developed method shows that, by varying the condition such as changing in scanning speed of instrument the results of test solutions was not affected the analytical method were concluded as robust.

Conclusion

In present study, we have formulated the Asava formulation by using the flowers of *Woodfordia fruticosa*as a fermenter, which was free from heavy metal (Pb, Cd, As, Hg). The RP-HPLC analysis strongly suggested the presence of medicinally important compound (AA) inAsava formulation; its presence in formulation plays the important role in the treatment and prevention of infectious diseases and protection of body from free radicals damage.The proposed analytical UV-method was developed, validated and was found to be linear, accurate, precise and robust.

References

1. Ali Ghasemzadeh and Neda Ghasemzadeh Flavonoids and phenolic acids: Role and biochemical activity in plant and human. J. Med. Plants Res.2011; 5: 6697-6703.
2. Lu Z, Nie G, Belton PS, Tang H and Zhao B. Structure activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. Neurochem Int. 2006; 48: 263-74.
3. Kamal Kardani, Nilesh Gurav, Bhavna Solanki, Prateek patel and Bhavna Patel. RP-HPLC method development and validation of Gallic acid in Polyherbal tablet formulation. Journal of Applied Pharmaceutical Sciences 2013; 3: 37-42.
4. A. Finose and K. Devaki. Phytochemical and Chromatographic studies in the flowers of Woodfordia fruticosa (L) Kurz. Asian J. Plant Sci. Res2011; 1:81-85.
5. Prashant Bhondave, Raghunath Burase, Abhay Harsulkar, Shrikant Takale, Anant Paradkar and Sanjay Patil. Yeast consortium isolated from *Woodfordia fruticosa* flowers proved to be instrumental for traditional Ayurvedic fermentation.Int. J Pharm BiomedRes 2013; 4: 37-45.
6. V. Mihira, K V. Ramana, S. Ramakrishna and P. Pambabu. Evaluation of Anti-ulcer activity of *Woodfordia fruticosa* roots. An International Journal of Advances in Pharmaceutical Sciences2011;2: 158-160.
7. Hemraj, Neeraj Upmanyu, Avneet Gupta, Anil Jindal and Sunny Jalhan. Pharmacological activities of Stephania Glabra, Woodfordia fruticosa and Cissempelos Pareira- A review. Int J Pharm Pharm Sci 2012; 4: 16-23.
8. Neeraj Verma, G. Amresh, P. K. Sahu, Neelam Mishra and Ch. V. Rao. Anti-inflammatory and antinociceptive activity of hydroethanolic extracts of *Woodfordia fruticosa* kurz flowers. Der pharmacia Sinica 2012; 3: 289-294.
9. Patel Parthik, Patel N. M and Patel P. M. WHO guidelines on quality control of herbal medicines. IJRAP2011; 2: 1148-1154.
10. John Kenkel. Analytical Chemistry for Technicians, third edition. CRC press LLC,2003; 367-389.
11. S. Gorinstein, R. Moshe, J. Deutsch, F. H. Wolfe, K. Tilis, A. Stiller, I. Flam and YA.Gat. Determination of basic components in white wines by HPLC, FT-IR Spectroscopy and Electrophoretic techniques. J Food Compost Anal 1992; 5: 236-245.
12. Kroes BH, Van Den Berg JJ, Quarles van Ufford HC, Van Dijk H and Labadie RP. Anti-inflammatory activity of Gallic acid. Planta Med1992; 58: 499-504.
13. Indian Medicinal Plants a compendium of 500 species. Part 3 by Orient Longman Publications, 1997; 256-263.

14. Indian Herbal Pharmacopoeia. Revised new edition. Indian drug manufacturers association, Mumbai, 2002; 214-221.
15. Shah and Quadry's. Pharmacognosy. Twelfth revised edition. B.S. Shah prakashan, 2004; 303-304.
16. P. K. Mukhergee. Quality control Herbal drugs, an approach to evaluation of Botanicals. 1st edition. Business Horizons, New Delhi,2002; 1-515.
17. Jagdish Manwar, Kakasaheb Mahadik and Anant Paradkar. Plackett-Burman Design: A statistical method for the optimization of flowers of *Woodfordia fruticosa*. Ferment Technol,2013; 2-6.
18. Lin Y, Tanaka S. Ethanol fermentation from biomass resources: Current state and prospects. Appl Microbiol Biotechnol2006; 69: 627-642.