Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/18779
DOI URL: http://dx.doi.org/10.21474/IJAR01/18779

RESEARCH ARTICLE

PRELIMINARY PHYSICO CHEMICAL AND CHROMATOGRAPHICAL ANALYSIS OF VASA ARKA (LEAF OF ADATHODA VASICA NEES) FOR DEVELOPMENT OF DOSAGE FORM FOR NEBULISATION

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Manuscript Info

Manuscript History
Received: 21 March 2024
Final Accepted: 28 April 2024
Published: May 2024

Key words:-
Arka, Adhatoda vasica, Swarasa, Chromatography Profile, Nebulization

Abstract

In the present scenario where the number of people being affected with respiratory ailments is at high, a proper management technique with best effect and least side effects has become a necessity. A nebulizing agent is far more potent than an oral medicine due to its increased absorption as well as its assimilation rate. Vasa (Adathoda vasica Nees) is one of the best medicines used for respiratory illness in the science of Ayurveda. According to literature review it possesses swasa kasahara action. Researches have been conducted on this herbal medicine over respiratory ailments and showed positive results. But no information is obtained on the nebulization effect of Vasa. The present study aims to evaluate the physicochemical parameters of Vasa arka & Vasa swarasa and to analyse the chromatography profile of Vasa arka and Vasa swarasa. Through this research, an attempt is done to expand the potential possibilities of Vasa as a nebulizing agent. The Pharmaceutical study includes collection of genuine Vasa leaves followed by its pre- processing and finally conversion to the Vasa swarasa and Vasa arka. Physico-chemical analysis was carried out as per parameters mentioned in Ayurvedic Pharmacopoeia of India.8. Preliminary phytochemical investigations were carried out by following standard procedure.However In the present study, the preliminary phytochemical analysis of the swarasa and arka of vasa revealed the presence of important antioxidant phytochemicals such as phenolic compounds. The study revealed that vasa is inherently having bronchodilatory action. Preparing arka of the same retained the volatile oil and active principles that were responsible for the bronchodilating action. The present study concluded that vasa arka are effective in the management of respiratory illness in the form of nebulizing agents.

Introduction:-

As an ancient science of life and health,Ayurveda has evolved with the objective mainly keeping the body fit and prevention and cure of diseases.In Ayurveda, Kalpana's denote different dosage forms of drugs. So our Acharyas have elaborately described about Panchavidha kashaya Kalpanas namely Swarasa,Kalka,Srta,Seetha and Phanda in

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the Samhithas(1) .Arka kalpana was introduced in Ayurveda in the later part of development.Arka Prakasha is the book of Arka kalpana but there is no explanation given about the author of the text. It is conveyed in the form of a conversation between Ravana and his wife Mandoodari(2).

Arka kalpana is a unique preparation in which volatile oils and active principles of herbal drugs are extracted through distillation method. The product obtained is known as arka. Arka kalpana plays a vital role in Ayurvedic treatment nowadays because of its specific qualities like increased shelf life, good palatability, easy administration for patients who hesitate to take medicines and quick action(3,4) .

Vasa (Adathoda vasica Nees) of Acanthaceae family is a well-known drug used in respiratory ailments in Ayurvedic treatment. The active constituents in it have mucolytic, expectorant and bronchodilator action. Nebulization plays an important role in reducing the symptoms of respiratory diseases(5,6) .A great number of studies has been carried out on different dosage forms of Vasa but studies on arka kalpana are not found.The arka kalpanas are used by various medical practitioners for the treatment of ophthalmologic diseases, colicky pain and paediatric ailments.But the reference standard of these arkas is still not formulated.With this objective, we standardised the Vasa arka (leaf of Adathoda vasica Nees) by preliminary physico-chemical and chromatographical analysis so that it can be used as a nebulising agent by virtue of its special qualities as arka. It will be a boon to this era of increasing range of respiratory disorders as well as an initiative to conduct more researches in this field

Methodology:-

Materials & Methods:-

Study design:

Physicochemical analysis, Chromatography

Study setting:

1. Department of Rasa sastra And Bhaishajya Kalpana , Vaidyaratnam Ayurveda College Ollur, Thrissur, Kerala.
2. Department of Dravyaguna Vijnana, Vaidyaratnam Ayurveda College Ollur, Thrissur, Kerala.
3. Quality Testing Laboratory,Vaidyaratnam Oushadhashala Pvt limited, Ollur , Thaikattusery ,Thrissur, Kerala (AYUSH accredited lab)
4. CARe KERALAM, KINFRA Small Industries Park, Nalukettu road, Koratty

The methods followed in this study are divided into pharmaceutical study and analytical study.

Materials:-

• Name of the drug : Vasa (Adathoda □□□□□□ Nees)

• Collection of the drug :

Fresh leaves of Vasa are taken, washed and cleaned.And it is taxonomically authenticated by a botanist.

Preparation of Vasa arka:

According to Arka Prakash(7)

The leaves of Vasa are made into paste and soaked in two times of water for one day. Again two times of water is added to the drug on the next day and is mixed well. Then the mixture is transferred in the arka yantra and the distillate is collected.This distillate is then kept in airtight bottles as 'arka'.

Preparation of Vasa swarasa:

According to Bhaishajya kalpana(8)

The freshly collected drug is washed thoroughly and is squeezed through a thin cloth after crushing. The juice thus obtained is called swarasa.

Methods:-

The following parameters will be carried out.

• Organoleptic characters :

(1) Colour

(2)Odour

(3)Taste
(4)Consistency
• pH
•volatile matter
• Specific gravity at 25ºC
• clarity test
• Identifications (TLC)

Alkaloids:
Mayer's test: One or two drops of Mayer's reagent [HgCl2 (1.36 g) was added to the acidified plant extract, dissolved in 60 ml of distilled water and mixed with a solution of 5 g of KI in 10 ml of water. The formation of a white precipitate indicated the presence of alkaloids.

Lead acetate test for tannins:
Lead acetate test : A few drops of basic aqueous lead acetate solution are added to 1 ml aqueous solution of the crude extract using a test tube. A reddish-brown voluminous precipitate indicates the presence of tannin.

Lead acetate test for flavonoids:
Lead-acetate test : Using a test tube, add a few drops of basic aqueous lead acetate solution to 1 ml of alcoholic extract. The appearance of a large reddish-brown precipitate indicates the presence of flavonoids.

Molisch test - carbohydrates:
Two ml of the sample solution is placed in a test tube. Add two drops of Molisch's reagent (a solution of naphthol in 95% ethanol). The solution is then slowly poured into a tube containing 2 ml of concentrated sulfuric acid so that two layers are formed.
Purple colour formation denotes carbohydrates

Terpenoids or steroids:
A red color is formed when a chloroform solution of the steroid is treated with concentrated sulfuric acid.

Saponins:
Saponins were indicated by the formation of a foam that persisted for a long time when the sample was mixed with water and shaken well.

Glycosides:
Picric acid test: **Reducing** sugars react with **picric acid (a toxic** yellow crystalline **solid),** also **known** chemically as 2,4,6-trinitrophenol **(TNP),** to form red **picramic acid.**

Phenols:
Folin ciocalteu reagent test.

TLC:
Vasa Swarasa
Test solution,
Extract 1 g of sample with 10 µL of methanol, filter, concentrate and perform thin layer chromatography. Spread 5 µl (Track 1, 2, 3) of the extract on an HPTLC plate and develop the plate 8 cm using a mixture of chloroform: methanol: ammonia (90:10:1). After development, allow the plate to dry and examine under UV light at 254 nm and 366 nm.

Visualization
Observe the plate under UV light at 254 nm and 366 nm. Record the Rf value and color of the separated bands. After viewing, spray the plate with anisaldehyde-sulfuric acid reagent and heat at 105 0C until the color of the lines appears. Record the Rf value and color of the zones..

Vasa Arka

Test solution,

10g sample evaporated to dryness and reconstituted with chloroform filter, Concentrate and carry out the thin layer chromatography. Apply 10µl(Track 1,2,3) of the extract on HPTLC plate and develop the plate to a distance of 8 cm using Toluene:Chloroform:Methanol(6:3:1).After development allow the plate to dry in air and examine under ultraviolet light 254 nm & 366 nm

Visualization

Observe the plate under UV light at 254 nm and 366 nm. Record the Rf value and color of the separated bands. After viewing**,** spray the plate with anisaldehyde-sulfuric acid reagent and heat at 105 0C until the color of the lines appears**.** Record the Rf value and color of the bands.

Reference

The ayurvedic pharmacopoeia of india Quality standards of Indian medicinal plants.

Result:-

Table 1:- Showing the organoleptic parameter of Vasa swarasa & Vasa arka.

ORGANOLEPTIC CHARACTERS	VASA SWARASA	VASA ARKA
(1) Colour	Fresh leaves of Vasa has been taken, crushed and juice is extracted Green blackish colour swarasa obtained.	Colour less
(1) Odour	No characteristic odour	No characteristic odour
(1) Taste	Bitter	Bitter

Table 2:- Showing physiochemical parameters.

PHYSICOCHEMICAL PARAMETERS	VASA SWARASA	VASA ARKA
(1) pH	7.83	7.34 (In house validated method)
(1) Total suspended solids	7.28% (As per API)	0.04% (As per API)
(1) Specific gravity	1.0338 (As per API)	0.9966 (As per API)
(1) Phenol	1.36% (Folin cio calteu Method by UV)	0.01% (Folin cio calteu Method by UV)

Table 3:- Showing Phytochemical Screening.

PHYTOCHEMICAL SCREENING	VASA SWARASA	VASA ARKA	TEST METHOD
Alkaloids	Present	Absent	Mayer's reagent test
Flavonoids	Present	Absent	Lead acetate test
Glycosides	Present	Absent	Picric acid test
Phenol	Present	Present	Folin ciocalteu reagent test
Saponins	Absent	Absent	Foam test
Tannins	Present	Present	Lead acetate test
Carbohydrates	Present	Present	Molisch's method

Terpenoids	Absent	Absent	Salkowski reaction test
Steroids	Absent	Absent	Salkowski reaction test

TLC of Vasa Swarasa

Allow it to dry in air after development and examine under 254 nm & 366 nm.The major spots found at 254 nm are Rf 0.37(Black).The major spots are found at 366 nm are Rf 0.10(Red),Rf 0.12(Blue),Rf 0.98(Red). The plate is sprayed with Anisaldehyde- sulphuric acid reagent followed by heating at 105 0C for about 10 minutes. The result shows major spots at Rf 0.10(Blue),Rf 0.95(Blue).

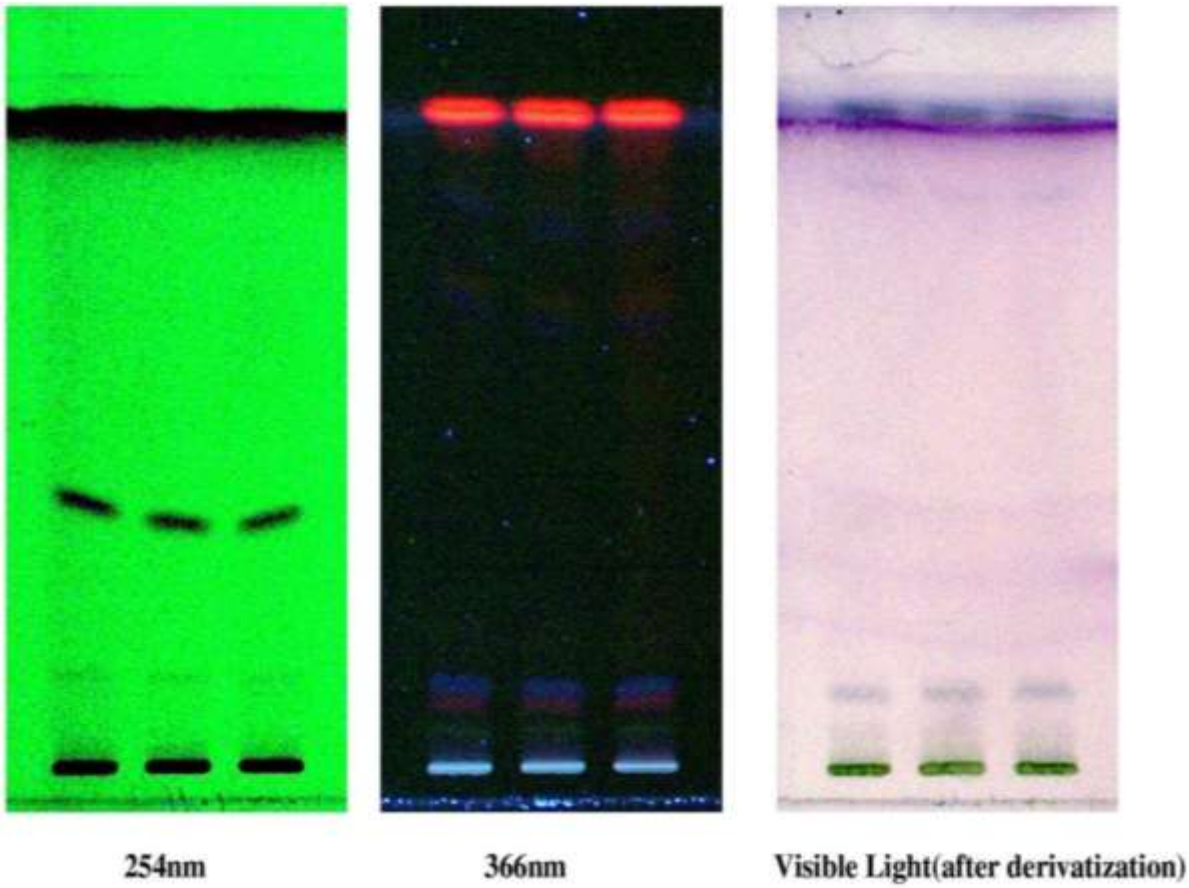

Fig 1:- Thin Layer Chromatography of Vasa Swarasa.

TLC of Vasa Arka

Allow it to dry in air after development and examine under 254 nm & 366 nm.The major spots found at 254 nm are Rf 0.99(Black).The major spots are found at 366 nm are Rf 0.87(Red). The plate is sprayed with Anisaldehyde-sulphuric acid reagent followed by heating at 105 0C for about 10 minutes. The result shows major spots at Rf 0.57(Pink),Rf 0.77(Pink),Rf 0.99(Violet).

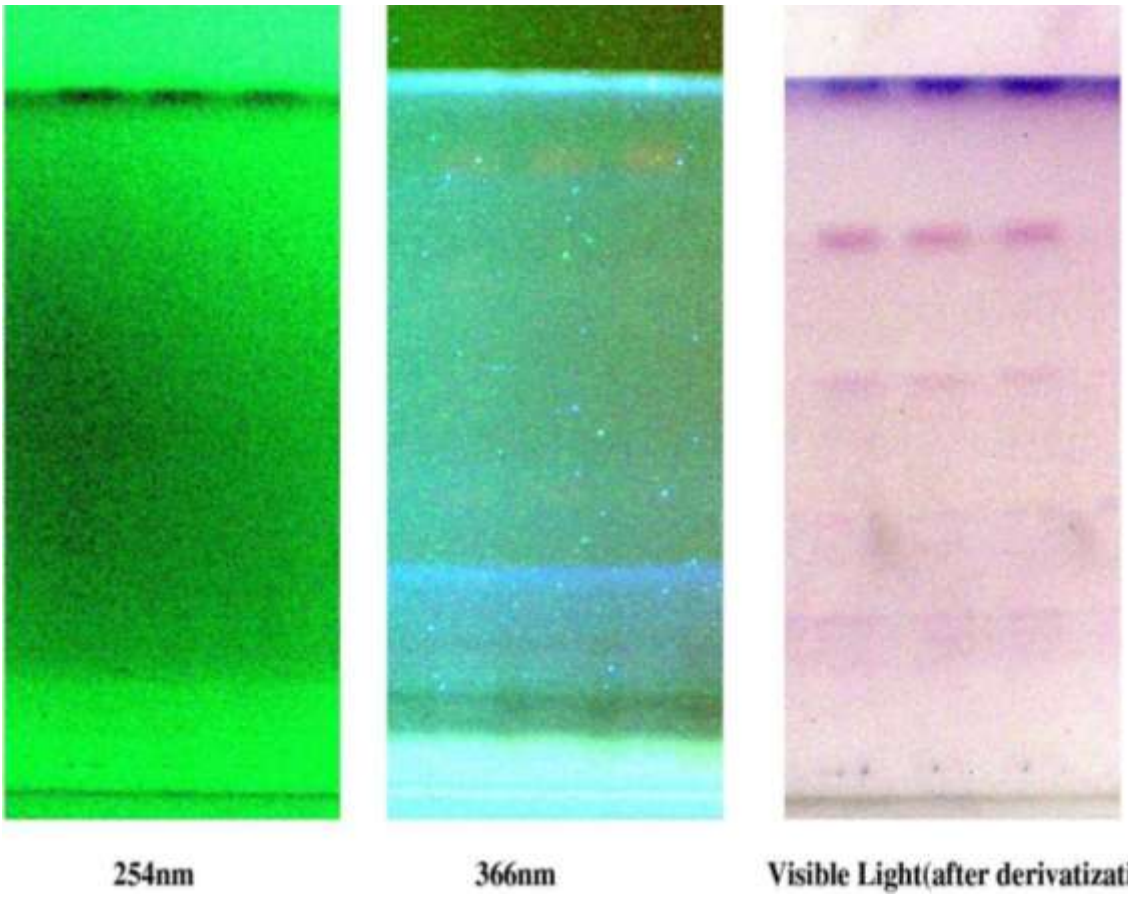

Fig 2:- Thin Layer Chromatography of Vasa Arka.

Discussion:-

Phytochemical screening shows the presence of alkaloids, phenols, flavonoids, glycosides and tannins. Various studies prove that the bronchodilatory of vasaka leaves is due to the two major alkaloids called vasicine and vasicinone. Vasicine also shows strong respiratory stimulant activity.

The presence of phenolic compounds points towards diverse activities such as anti-inflammatory, anti-ageing, antioxidant and anti-proliferative activities.

Polyphenols, especially flavonoids, phenolic acids and tannins, have the important property of inhibiting α-glucosidase and α-amylase, which are the main enzymes responsible for breaking down dietary carbohydrates into glucose. Plant polyphenols and polyphenol-rich products in foods regulate carbohydrate and fat metabolism, weaken hyperglycemia, dyslipidemia and insulin resistance, improve β-cell function, stimulate insulin secretion, improve adipose tissue metabolism and relieve oxidative stress, stress-sensitive signalling pathways, and inflammatory processes. Phenolic compounds are the most common natural compounds found in plants.

Tannins have astringent properties, they accelerate the healing of wounds and inflamed mucous membranes.

Aluminium chloride colorimetric method was used to estimate the total flavonoid content. Flavonoids are strong antioxidants and are potent free radical scavengers which prevent oxidative cell damage.Flavonoids also possess antibacterial, antiviral, anti-inflammatory, antimutagenic, and anticarcinogenic properties.

The pharmacotherapeutic effects of herbs are mainly due to the presence of various secondary metabolites such as flavonoids, terpenoids, alkaloids, glycosides, phenols, sterols etc.

The preliminary phytochemical analysis is essential in the detection and isolation of bioactive principles and may subsequently lead to drug development. In the present study, the preliminary phytochemical analysis of the swarasa and arka of Vasaka revealed the presence of important antioxidant phytochemicals such as phenolic compounds. The study reveals that vasa swarasa is rich in alkaloids, glycosides, flavonoids and phenols, which may be responsible for its therapeutic effects (Table 3).

Different solvent combinations were tried after continuous trials to develop maximum separation of bands in the TLC system. The most suitable solvent system for Vasa swarasa was found to be Chloroform:Methanol:Ammonia in ratio (90:10:1)., in which maximum separation of the phytocompounds was visualised (Fig. 1). Toluene:Chloroform:Methanol(6:3:1) was found to be suitable solvent system for Vasa arka in which maximum separation of photochemicals was obtained and visualised.Table 4 represents TLC profile of the extract with Rf values and band colour.

Conclusion:-

The number of people being affected with respiratory ailments are increasing day by day, a proper management technique with best effect & least side effect has become a necessity. Since the potency of the nebulizing agent is far more than oral medicine, the aim of my research is to standardise the formulations and compare them preliminarily.

Arka kalpana is a unique preparation in which volatile oils and active principles of herbal drugs are extracted through distillation method. The product obtained is known as arka. Arka kalpana plays a vital role in Ayurvedic treatment nowadays because of its specific qualities like increased shelf life, good palatability, easy administration for patients who hesitate to take medicines and quick action.In clinical practice vasa arka has shown effect similar to swarasa in pacifying kasa and swasa. Comparison of The preliminary phytochemical screening vasa swarasa and arka show wide differences in the constituents.The total phenols could be quantitatively analysed and was present in both formulations. This could explain the anti inflammatory effects of the two kalpanas. Finally on conducting the TLC & phytochemical study of vasa arka, it has been found that the secondary metabolites that are needed for the nebulizing action were present in it. Thus it can be summarised that Vasa arka pocesses signified effects on bronchodilation action & can act as a nebulizing agent. So this study will help to create reference standards for Vasa arka in the pharmaceutical industry so as to maintain a uniformity in manufacturing process and this will be an initiative to conduct more researches on new dosage forms ie, Arka kalpana due to its unique qualities.

Acknowledgement:-

We are thankful to the management, director and staff of various departments of Vaidyaratnam Ayurveda College for their support, advice and guidance which helped us to complete the research on time.

Authors' Contributions

All authors participated equally in the design and implementation of the article.

Funding

Central Council for Research in Ayurvedic Science.

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