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RESEARCH ARTICLE

PHYSICAL AND PHYTOCHEMICAL ANALYSIS OF AN AYURVEDIC POLYHERBAL FORMULATION.

Dr.Anjali.P.[1] and Dr.Deepa M.S.[2]

1. MD Scholar,Department of Dravyagunavijnana,Govt.Ayurveda College,Thiruvananthapuram.
2. Associate Professor, Department of Dravyagunavijnana,Govt.Ayurveda College,Thiruvananthapuram.

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Abstract

Amruthotharam kwatha(AK) is an Ayurvedic polyherbal formulation indicated in the treatment of fever mentioned in traditional Ayurvedic textbooks such as Chikitsa Manjari & Sahasrayogam.It comprises of 3 drugs viz;Tinospora cordifolia(Willd) Miers,Terminalia chebula Retz. and Zingiber officinale Rosc. in the ratio 6:4:2.Since physical and phytochemical analysis of this formulation has not been reported till date, in the present study, an attempt has been made to analyze the preliminary physical and phytochemical parameters of this formulation. The parameters assessed were specific gravity, pH, total solids, total ash, acid insoluble ash, qualitative analysis of alkaloids, steroids, phenols, flavanoids, tannins and saponins.TLC and HPTLC analysis were done using the solvent system Toluene:Ethyl acetate:Methanol:Formic acid in the ratio 25:15:2:2.

Introduction:-

Amruthotharam kwatha (AK) is an Ayurvedic polyherbal formulation mentioned in traditional Ayurvedic texts like Sahasrayogam ("Sahasrayogam,2015,p.29") and Chikitsa Manjari ("Chikitsamanjari , 2008.p.58") which is widely used in the treatment of fever. It comprises of 3 drugs, viz; *Tinospora cordifolia*(Willd) Miers(*Amrutha*),*Terminalia chebula* Retz. (*Harithaki*) and *Zingiber officinale* Rosc.(*Nagara*) in the ratio 6:4:2. The physical and phytochemical parameters of *Amruthotharam kwatha* has not been reported till date. Thus the present study aims to evaluate the physical and phytochemical parameters of *Amruthotharam kwatha*.

Materials And Methods:-

Collection of Plant Materials

The plant materials used in this research, fresh stem of *Tinospora cordifolia*(Willd) Miers (*Amrutha*), the mature fruits of *Terminalia chebula* Retz. (*Harithaki*)and dried rhizomes of *Zingiber officinale* Rosc.(*Nagara*) were collected from a local drug store located at Thiruvananthapuram,Kerala and were dried well. All the three ingredients were finely powdered and mixed together in the ratio of 6:4:2 and this was used for the preparation of the decoction.(Fig:1)

Corresponding Author:-Dr.Anjali.P.
Address:-MDScholar,DepartmentofDravyagunavijnana,Govt.Ayurveda College,Thiruvananthapuram.

Fig1:-Ingredients of *Amruthotharam kwatha* mixed in the ratio 6:4:2

Preparation of Decoction (*Kwatha*)

Fresh *Amruthotharam kwatha* was prepared by boiling 12 g of the above mentioned powder in 200 ml water and the filtrate was collected.

Pharmacognostical evaluation

Macroscopic evaluation

Macroscopic evaluation is an important part in pharmacognosy. It includes evaluation of organoleptic characters like colour, odour, taste, consistency etc.("Kokate et al,2014").Since majority of information on the identity, purity and quality of a material can be drawn from this information, this quickest and simplest evaluation is of primary importance in drug evaluation.

The sample was subjected to macroscopic evaluation by observation with naked eyes and by tactile and other sensory inspection.

Physical and Phytochemical evaluation

AK was subjected to preliminary physicochemical and phytochemical analysis as per standard procedures described in API ("API,2007"). Physical characters like total solids, pH, specific gravity, total ash, acid insoluble ash were estimated.Qualitative analysis of the sample for the presence of alkaloids, steroids, phenols, flavanoids, tannins and saponins were also done.TLC and HPTLC were also performed.

Reagents used

Concentrated Sulphuric acid, Magnesium ribbon, Neutral Ferric chloride, Sodium bicarbonate, Dragendroff's reagent, Dilute HCl, Mayer's reagent, Lead acetate.

Apparatus

Silica crucible, bunsen burner, measuring jars, beakers, conical flask, funnel, glass rods, watch glass, electronic balance, pH analyser, pycnometer.

Preparation of Methanolic Extract of AK for TLC and HPTLC

10 ml of AK was taken in a beaker and was kept on a water bath at a temperature of 80° C for 2 hrs until it became dry. This residue obtained was dissolved in 10 ml methanol by continuous stirring by a glass rod. It was further filtered using Whatman no.1 filter paper and the filtrate obtained was made upto 10 ml in a volumetric flask. This was used for TLC and HPTLC analysis.

Results and Discussion:-

Results of Macroscopic Evaluation

On organoleptic evaluation , AK was found to be a brown decoction with liquid consistency,extreme bitter taste and with a characteristic odour.

Results of Physical and Phytochemical Evaluation

Table 1:-Physical parameters of *Amruthotharam kwatha*

Specific gravity	0.1769
pH	4.16
Total solids	0.2212g/10ml
Total ash	0.66%
Acid insoluble ash	0.17%

On analysis of pH, AK was found to be acidic with a pH of 4.16.Total solids was found to be 2.2% of *kwatha*.

Result of Qualitative Analysis

On qualitative analysis, AK showed the presence of alkaloids, steroids, phenols, flavanoids, tannins and saponins which may be responsible for its pharmacological action.

Result of Chromatographic Analysis

TLC (Thin layer chromatography)

The best separation was achieved using Toluene: Ethyl acetate: Methanol: Formic Acid as solvent system in the ratio 25:15:2:2.The plates were viewed through UV-fluorescence viewing cabinet(365 nm) and Rf values were noted.

Fig 2:-Spots obtained in TLC

Table 2:-Rf value and colour of the spots under UV light of Thin Layer Chromatography

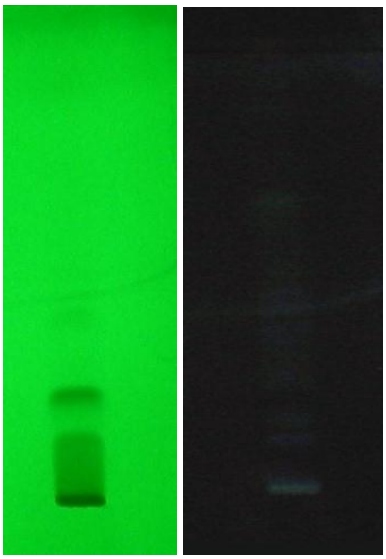
Extract	Spot detection	No. Of spots	Colour of spots	Rf value
Methanolic extract of *Amruthotharam kwatha*	UV(365 nm)	1	Faint blue	0.70

In TLC, one spot was obtained with an Rf value of 0.70.

HPTLC(High Performance Thin Layer Chromatography)

HPTLC analysis of AK was done with Toluene: Ethyl acetate: Methanol: Formic Acid as a solvent system in the ratio 25:15:2:2.

On HPTLC analysis,6 peaks were obtained for AK.



(a) (b)

Fig 3:- (a) HPTLC Profile for *AK* at 254nm
(b)HPTLC Profile for *AK* at 366nm

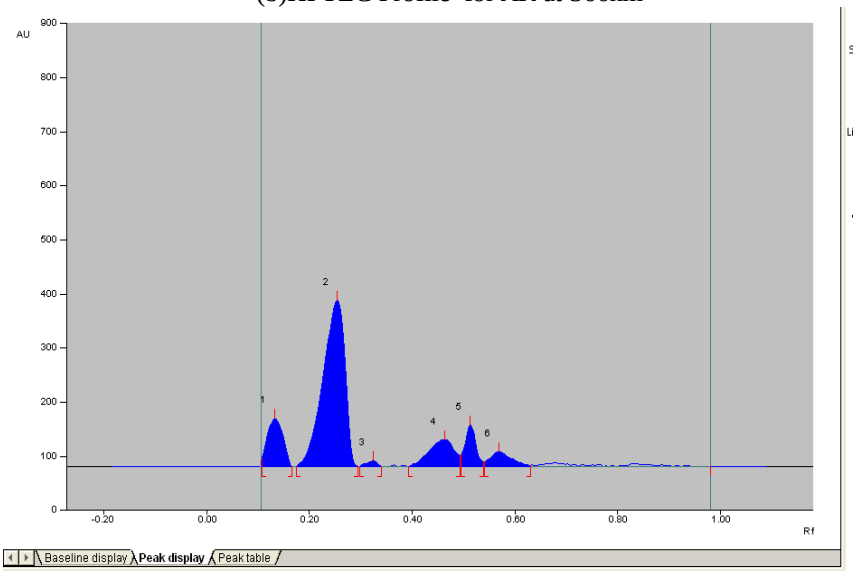


Fig 4:-Peaks obtained for AK

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.11 Rf	14.2 AU	0.13 Rf	89.0 AU	15.82 %	0.17 Rf	0.3 AU	1723.9 AU	12.70 %
2	0.18 Rf	0.1 AU	0.25 Rf	307.5 AU	54.66 %	0.29 Rf	0.1 AU	8404.9 AU	61.92 %
3	0.30 Rf	0.7 AU	0.33 Rf	10.7 AU	1.90 %	0.34 Rf	0.6 AU	148.9 AU	1.10 %
4	0.39 Rf	0.1 AU	0.46 Rf	50.2 AU	8.92 %	0.49 Rf	21.0 AU	1567.8 AU	11.55 %
5	0.50 Rf	21.9 AU	0.51 Rf	77.7 AU	13.82 %	0.54 Rf	8.6 AU	1001.8 AU	7.38 %
6	0.54 Rf	8.7 AU	0.57 Rf	27.5 AU	4.88 %	0.63 Rf	2.7 AU	727.2 AU	5.36 %

Fig 5:-Rf values of AK

Conclusion:-

In the present study, pharmacognostic and preliminary physical and phytochemical analysis were carried out as per API. These results may help in standardisation of AK and in carrying out further research on AK.

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