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IMPACT OF *ACORUS CALAMUS AND FERULA ASAFOETIDA* IN ANTI SNAKE ENVENOMATION STUDIES-A PHYTOCHEMICAL, SPECTROSCOPIC AND CHROMATOGRAPHIC PERSPECTIVE

Anoop M.*, Haritha Venugopal[1] and Venugopal K. G.[2]

*Associate Professor, Dept. of Biotechnology and Research KVM College of Science & Technology Cherthala Kerala.
[1]Dept of Biotechnology & Research, KVM College of Science & Technology, Cherthala, Kerala.
[2]Associate Professor, Dept. of Homeopathic Pharmacy, Sarada Krishna Homeopathic Medical College, Kulasekharam, Tamil Nadu.

***Corresponding Author: Dr. Anoop M.**
Associate Professor, Dept. of Biotechnology and Research KVM College of Science & Technology Cherthala Kerala.
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ABSTRACT
The scheme of examining the bioactive components in *Acorus calamus and Ferula asafoetida* plants alone and in combination with other plants were presented in this work that extrapolates the idea behind traditional anti snake envenomation practices using the selected medicinal plants through a preliminary piece of phytochemical ,chromatographic and spectroscopic analysis. The Rf values depicted in the ranges of 0.9 and 0.75, 0.645 for acetone extracts of *Acorus calamus and Ferula asafoetida* respectively that indicated the presence of a component that can stimulate the anti venom approach. The UV-VIS spectroscopic bands clearly represented presence of terpenoid class of compounds in the ranges specially in 200-320 nm for *Ferula asafoetida* acetone extracts and 500-700 nm ranges for the aqueous extract of *Acorus calamus*. The dispense of brown color ring formation stipulated the existence of terpenoid classes as secondary metabolite. These preliminary assays extended the way for further future interpretations of the residence of active principles that may be assayed through FTIR and mass spectrometer studies. The work aimed a Homoeopathic formulation that may be triggered through an ultradilution technique and nanoparticulate formulations from a purified bioactive extract that may be a constructive approach in traditional snake envenomation management practices.

KEYWORDS: *Acorus calamus, Ferula asafoetida,* TLC, Phytochemical, Spectroscopic Homoeopathy.

INTRODUCTION
One of the main properties of plant includes their medicinal properties. Some plants are rich in certain secondary metabolites like terpenoids, alkaloids etc. which are very helpful in curing diseases.(A. Cristina Figueiredo et al; 2008). These secondary metabolites have been used for centuries due to their large biological activities. Plant based products has a wide application in various industries like pharmaceutical industry, cosmetic industry, chemical industry etc.(F. Bourgaud et al; 2001). The change in climate and poor waste management has increased an overlap between humans and poisonous snakes (M Anoop et al, 2020). The secondary metabolites were elucidated with phytochemical analysis and the study aims in investigation and contemplation of active principles in traditional anti snake envenomation practices.

Acorus calamus commonly known as sweet flag. Its rhizome has got many medicinal properties. They are used in the treatment of mental ailments like epilepsy and memory disorders, chronic dysentery and diarrhea, cough, asthma and glandular and abdominal tumours. Rhizomes are used in the form of powder, balms, enemas and pills. (Pulok Kumar Mukherjee et al; 2007). The dried rhizomes are used in the treatments of diseases like bronchitis inflammation of gastric membrane, arthritis, cough, stroke, cold, head ache, nausea, stomach ache, sinuses and also for kidney and liver troubles (Amrita devi Khwairakpam et al; 2017)

Ferula asafoetida commonly known as asafoetida. It is oleo-gum-resin. It is obtained from the exudates of the roots of asafoetida. Traditionally asfaoetida have been used in the treatment of diseases like nervous disorder, asthma, epilepsy, microbial infections, ear ache and intestinal parasites (Faegheh Farhadi et al; 2019). They are also used as flavouring agents in food. It is specially considered as an ailment for women. It is used as a medicine for several problems such as unwanted abortions, unusual pain, sterility and also for difficult and excessive menstruation (Augustine Amalraj et al; 2016).

MATERIALS AND METHODS

Collection of samples

Rhizome of *Acorus calamus* and resin of *Ferula asafoetida* were collected from traditional ayurvedic medical shops and were dried thoroughly under sunlight and were powdered finely and stored in an air tight glass jar.

Cold Extraction

2.5g of powdered *Acorus calamus* and *Ferula asafoetida* were macerated separately in petroleum ether, acetone, ethanol-acetone, chloroform, ethyl acetate, methanol and water for 24 hours in the increasing order of their polarity. The collected samples were evaporated and filtered using what man filter paper No 1.

Thin layer chromatography

The solvent system used as the mobile phase is chloroform: ethyl acetate: acetic acid in the ratio 6.5:3.5:0.5. The two plant extracts in seven different solvents were spotted on TLC silica plate and R_f values were calculated.

Uv-Visible Spectroscopy

The Optical Density values were recorded with the help of an UV-Vis spectrophotomer in varying wavelengths from 280nm-750nm in 50nm intervals for *Acorus calamus* and *Ferula asafoetida* macerated in petroleum ether, acetone, ethanol-acetone, water and Optical Density values were recorded with the help of an UV-Vis spectrophotomer in varying wavelengths from 300nm-500nm in 30nm intervels for Acorus calamus and Ferula asefoetida macerated in chloroform, ethylacetate, methanol and water.

Phytochemical Analysis

Acetone extracts of *Acorus calamus* and *Ferula asafoetida* were assayed for phytochemical tests that is flavanoid, phenol alkaloid and terpenoid tests under to trace out the standard bioactive compounds which might be showing the potential in snake venom treatments.

Test for flavonoids

Alkaline reagent test: 1ml of extract was mixed with 2ml of 2% of solium hydroxide solution. An intense yellow colour were formed which turn colourless on addition of few drops dilute acid which indicate the presence of flavanoid.

Test for phenol

1ml of extract was mixed with 2 ml of 2% ferric chloride solution. A Blue, Green or black colouration indicate the presence of phenols, tannins.

Test for alkaloids

1ml of plant extract was mixed with 2ml of Wagner's reagent (disslove 2 gm of iodine and 6 gm of potassium iodide in 100 ml of water) and shaken well. Reddish brown precipitate indicate the presence of alkaloids.

Test for terpenoids

0.1 ml of plant extract was mixed with 0.5 ml chloroform and 1.25 ml of Conc.H_2SO_4 carefully added to form a layer. Presence of reddish brown colour at the interface gives the positive result.

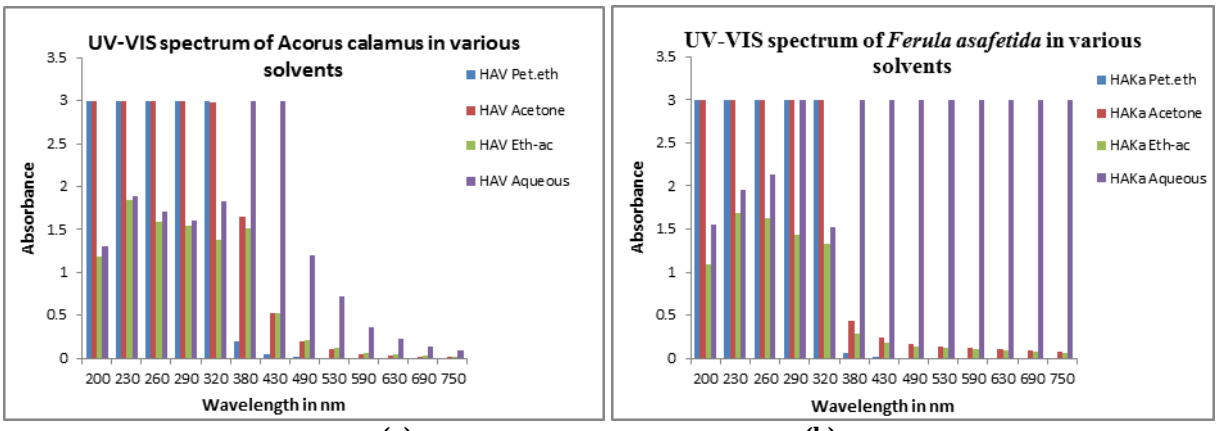
Terpenoid test

0.5ml of plant extract was mixed with 2 ml chloroform and 5 ml of Conc.H_2SO_4 carefully added to form a layer. Presence of reddish brown color at the interface gives the positive results. Terpenoid test was carried out for *Acorus calamus* and *Ferula asafoetida* in all selected seven different solvents petroleum ether, acetone, ethanol-acetone, chloroform, ethyl acetate, methanol and water denoted by its polarity.

RESULTS

UV-Visible Spectroscopy

The Optical Density values were recorded with the help of an UV-Vis spectrophotomer in varying wavelengths from 280nm-750nm in 50nm intervals for *Acorus calamus* and *Ferula asafoetida* macerated in petroleum ether, acetone, ethanol-acetone, water.



(a) **(b)**

Fig. 1: Graph showing (a) UV-VIS spectrum of *Acorus calamus* in various solvent (b) UV-VIS spectrum of *Ferula asafoetida* in various solvents.

Thin Layer Chromatography

Thin layer chromatography of two plant extracts in seven different solvents was spotted on TLC silica plate and calculated Rf values are given below.

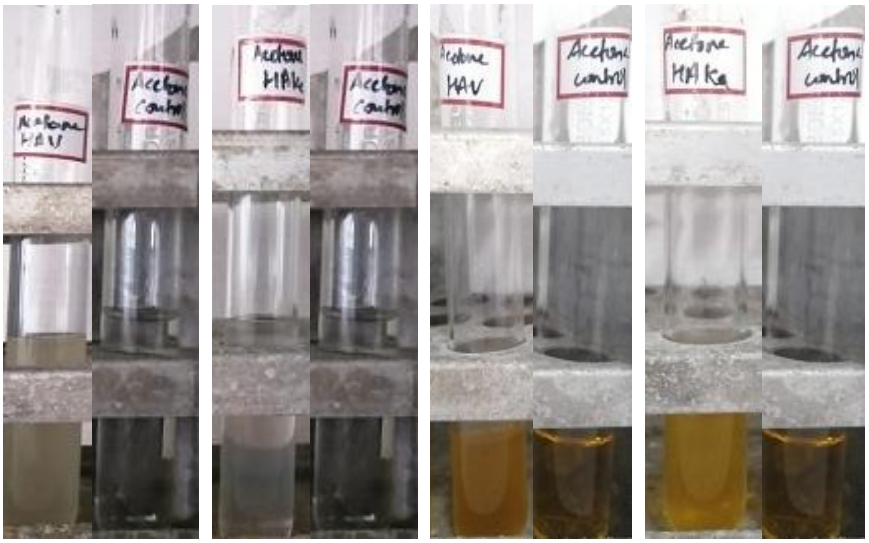
Table 1: R_f values depicting various solvent extracts of *Acorus calamus* and *Ferula asafetida.*

	Petroleum ether	Acetone	Ethanol-acetone	Chloroform	Ethyl acetate	Methanol	water
Acorus calamus	0.937	0.75	0.916	0.9	0.975	0.9	0.978
	0.791	0.645					
Ferula asafoetida	0.9	0.9	0.938	0.9	0.9	0.979	0.9

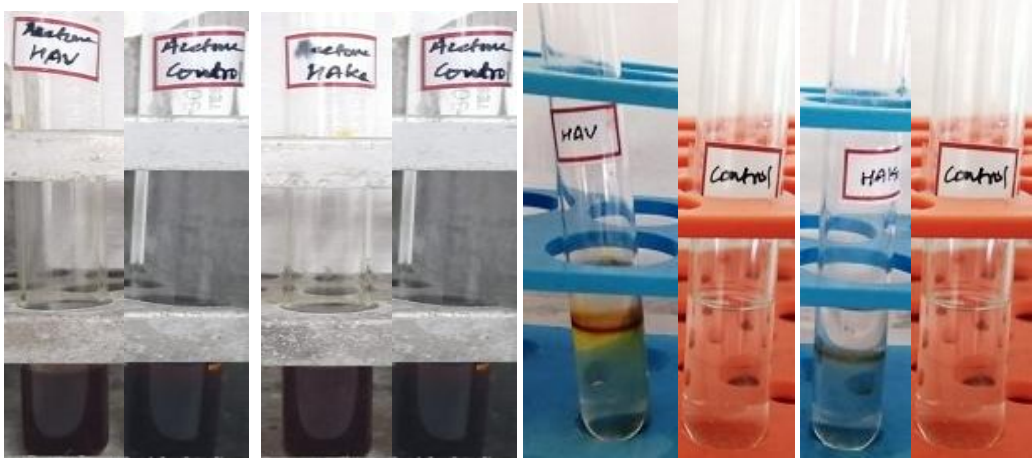
Phytochemical Analysis

Table 2: Qualitative flavonoid, phenolics, alkaloid and terpenoid test for acetone extracts of *Acorus calamus* and *Ferula asafetida*.

	Flavanoid	Phenolic	Alkaloid	Terpenoid
Acorus calamus	-	-	-	+
Ferula asafoetida	-	-	-	Slight reddish brown colour appeared



(a) (b) (c) (d)

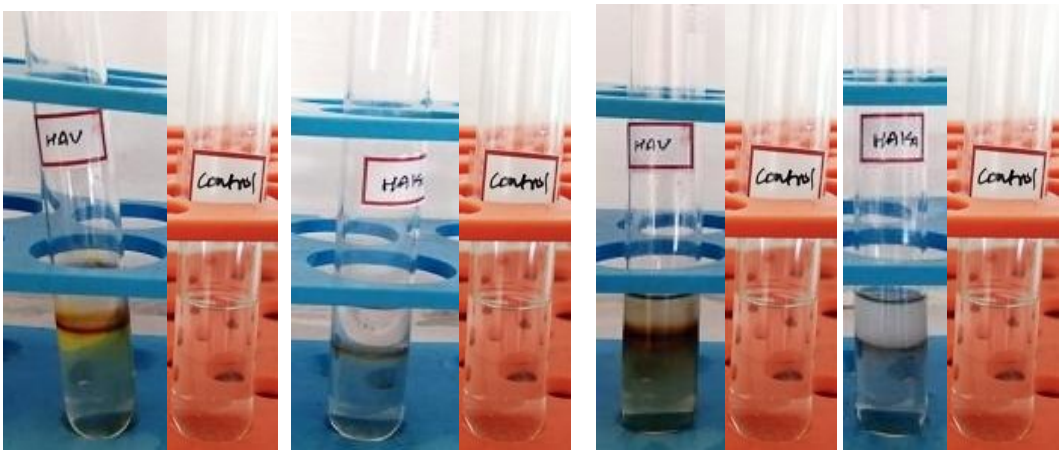


(e) (f) (g) (h)

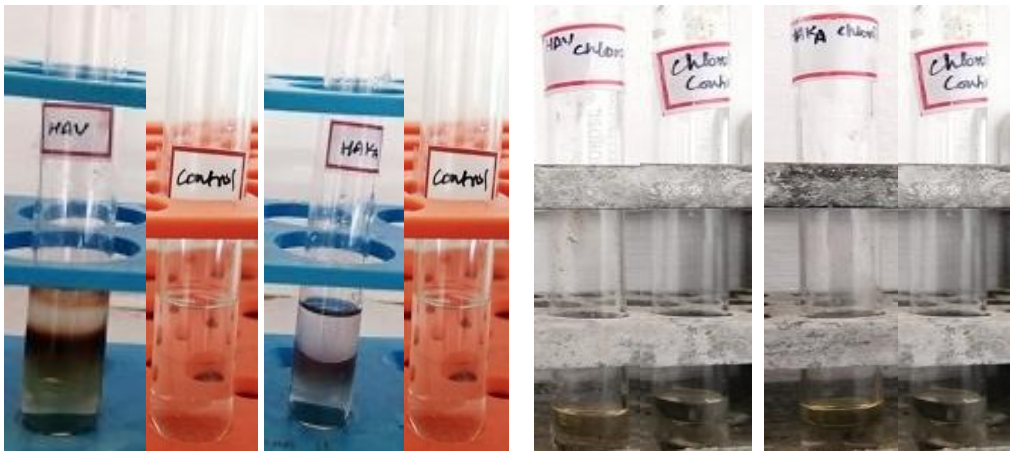
Fig. 2: Image depicting (a) & (b) Flavonoid, (c) & (d) Alkaloid, (e) & (f) Phenolics, (g) &(h) Terpenoid in acetone extract of *Acorus calamus* and *Ferula asafoetida* respectively.

Table 3: Qualitative terpenoid assay for different solvent extracts of *Acorus calamus* and *Ferula asafetida.*

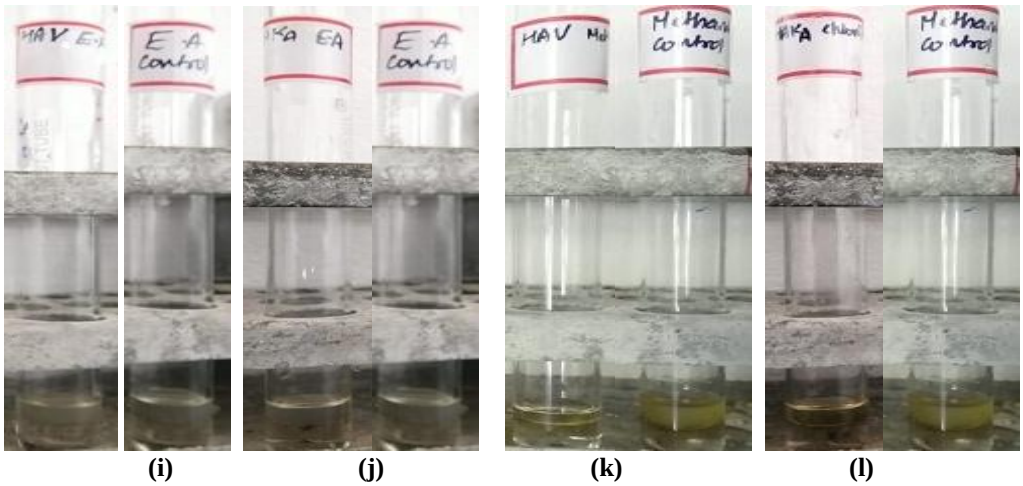
	Petroleum ether	Acetone	Ethanol-acetone	Chloroform	Ethyl acetate	Methanol	Aqueous
Acorus calamus	+	+	+	Very slight reddish brown colour appeared	Very slight reddish brown colour appeared	Very slight reddish brown colour appeared	+
Ferula asafoetida	Slight reddish brown colour appeared	Slight reddish brown colour appeared	–	Very slight reddish brown colour appeared	–	Very slight reddish brown colour appeared	Slight reddish brown colour appeared



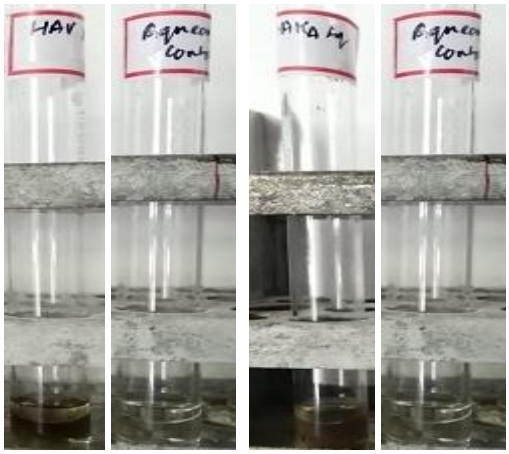
(a) (b) (c) (d)



(e) (f) (g) (h)



(i) (j) (k) (l)



(m) (n)

Fig. 4: Image depicting terpenoid results for (a) & (b) Petroleum ether, (c) & (d) Acetone, (e) & (f)Ethanol–acetone, (g) & (h) Chloroform, (i) & (j) Ethyl-acetate, (k) &(l) Methanol, (m) & (n) Aqueous extracts of *Acorus calamus* and *Ferula asafoetida* respectively.

DISCUSSION

The Thin layer chromatography assay various solvent extracts of *Acorus calamus and Ferula asefoitida* were performed and the Rf values were obtained (Table 1) .The TLC results presented and significant spots at 0.75 and 0.645 in acetone extracts of *Acorus calamus* where as the spots shown in 0.9 ranges of Rf in all the other solvent extracts of *Acorus calamus and*

Ferula asefoitida that prompted the design of further research to in acetone extracts of *Acorus calamus and Ferula asefoitida.* The UV-VIS spectrum too have revealed characteristic peaks in

UV ranges for acetone extracts (Fig 1). *Ferula asefoitida* showed significant peaks for acetone in UV ranges and characteristic bands in visible ranges for aqueous extracts (Fig 1(b)). Qualitative phytochemical analysis presented the presence of phytochemical terpenoid (Table 2) in small amounts as indicated by the appearance of reddish brown color formation (Fig 2 (g) (h)). The results elicited a new way of thought that marked the assay for terpenoids alone in selected solvent extracts (Table 3). The results divulged presence of terpenoids in petroleum ether ,acetone ,ethanol-acetone and aqueous extracts of *Acorus calamus* (Table 3) The terpenoid assay given out the presence of slight reddish brown color that indicated the presence of terpenoids in petroleum ether and acetone extracts of *Ferula asefoitida* The results shown negative for the same phytochemical for ethanol-acetone and ethyl acetate extracts (Table 1) (Fig 4) .The results followed a similar approach that has depicted with Mactrotyloma uniflorum in traditional snake envenomation management practices that too showed a preliminary analysis through spectroscopic chromatographic and phytochemical approach (M Anoop 2020).

CONCLUSION

The acetone extracts of *Acorus calamus and Ferula asefoitida* revealed the presence of bioactive principles as the phytochemical terpenoids. The present results were researched further for structural interpretation to trace the bioactive principle by FTIR and confirmation of the same by LCMS studies that may trigger the formulation of an anti snake envenomation drug .The present result was focused on the development of a homeopathic formulation that may act against snake venom The investigation for bioactive principles in the acetone extracts have to be proceeded yet. The preparation of homeopathic medicines were based upon ultra high dilution technique (Banerjee, 2006). *Acorus calamus and Ferula asefoitida* in combination with other plants have been researched and the bioactive principles were extrapolated from a traditional knowledge of anti snake venom practise for future researches. The effect of *Acorus calamus and Ferula asefoitida* in anti snake venom research were contemplated with traces of its compounds through ultra dilution and nano particle studies. The present research concludes with a qualitative study that provided service quality with low cost and neglected or no side effects.

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