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ISOLATION AND PURIFICATION OF ALKALOIDS FROM *ACHYRANTHES ASPERA* AND *CISSUS QUADRANGULARIS* BY HPLC

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Methanolic extracts of *Achyranthes aspera* (seeds) and *Cissus quadrangularis* (stem) were evaluated for the presence of alkaloids by TLC. As the TLC showed positive results for the presence of alkaloids, further purification of total alkaloids was done using HPLC. HPLC profile of sample *Achyranthes aspera* revealed 2 major and 1 minor peaks with retention time 5.194 min, 5.567 min and 6.447 min respectively and their respective concentrations are 33.475 µg/ml, 29.963 µg/ml and 14.888 µg/ml respectively. HPLC profile of *Cissus quadrangularis* revealed 1 major and 3 minor peaks with retention time 5.549min and 6.036 min ,6.531 min, 7.655 min respectively and their respective concentrations are 38.417 µg/ml, 9.241 µg/ml, 18.412 µg/ml , 15.213 µg/ml (respectively. This study may give a scientific basis to plants already used for traditional purposes to treat various ailments and metabolic disorders which have not been evaluated.

Keywords: *Achyranthes aspera, Cissus quadrangularis,* TLC (Thin layer chromatograpy) , HPLC (High performance liquid chromatography), Alkaloid

Plants have been an integral component of traditional medicine across the continents since time immemorial. Medicinal plants have their values in the substance or substances present in various plant tissues generally known as phytochemicals. These produce definite physiological action in the human body. A wide range of organic compounds are responsible for the biological activity of herbs. They are traditionally classified as primary and secondary metabolites. Alkaloids, selected for this study are included in secondary metabolites. The alkaloids are one of the most significant groups of secondary metabolites that mostly contain basic nitrogen atoms. These are found in living organisms and are of various structural types, biosynthetic pathways and pharmacological activities. Many alkaloids have been used for hundreds of years in medicine and continue to be of great interest today. Alkaloid containing plants have been used by humans since ancient times for therapeutic purposes. Many researches have been in the support of antimicrobial activity, antioxidant activity, free radicals scavenging activity of alkaloids. Plant-based medicinal agents like alkaloids offer an alternative approach to the management of obesity also. Therefore determination of total alkaloids is very important related to the quality of medicinal plants. Keeping this in view, present study is designed to investigate the presence of total alkaloids in selected plants.

MATERIALS AND METHODS

Collection and identification of plants

Cissus quadrangularis (stem) was collected from various parks of Bikaner where it was cultivated as an ornamental plant whereas seed samples of *Achyranthes aspera* (seeds) were purchased from the shop of herbal medicine and were identified by a well known taxonomist of Bikaner. The fresh sample of *Cissus* stem and seeds of *Achyranthes* was dried separately and used for further analysis.

Extraction Procedure :

Cissus quadrangularis and *Achyranthes aspera* were extracted using 95% methanol. Crude extract formation was done under following steps of extraction -

- **Crude Extract preparation using 95% Methanol**

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Figure-1: High performance liquid Chromofogram of Cissue alkaloids

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Acquired by	: Admin
Sample Name	: Cissus alkaloids
Sample ID	: Alkaloids Cissus
Tray #	: 2
Vail #	: 1
Injection Volume	: 20 µL
Conc.	:
Data File Name	: Cissus alkaloids data new.lcd
Method File Name	: Cissus alkaloids Meth.lcm
Report File Name	: Default.lcr
Data Acquired	: 1/3/2016 11:10 AM
Data Processed	: 1/3/2016 11:35 AM

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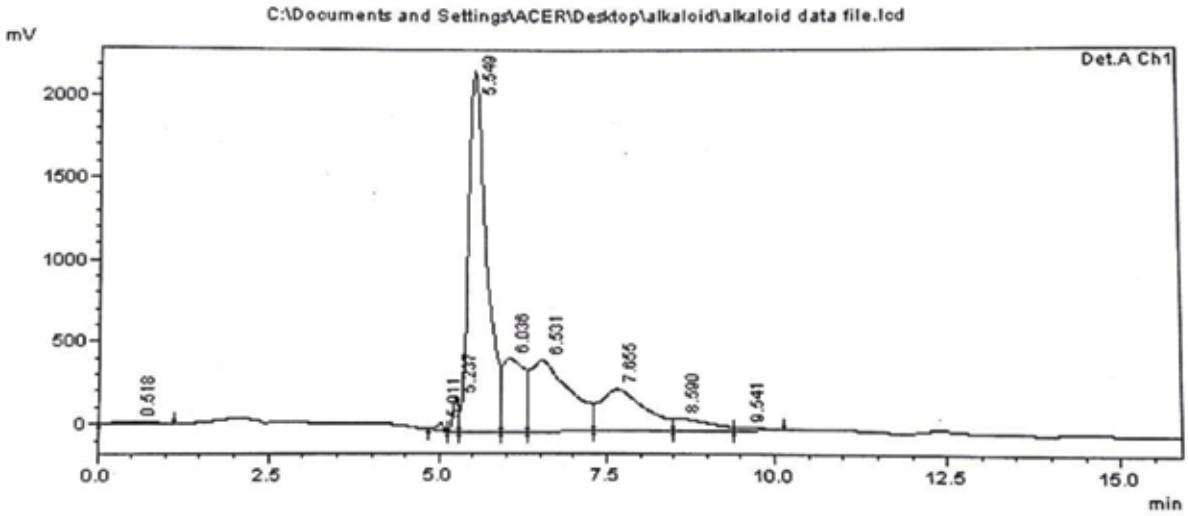


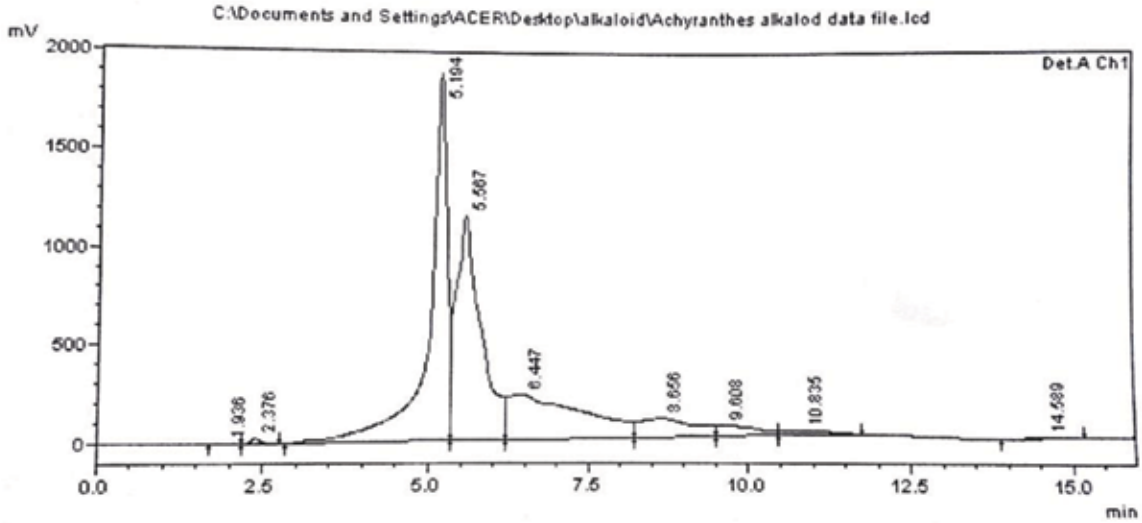
Peak	Name	Retention Time	Area(mm^2)	Conc.(µg gm^{-1})
1.	-	0.518	0.0534	0.346
2.	-	5.011	0.315	1.203
3.	-	5.237	1.257	3.189
4.	RT5.549	5.549	41.186	**38.417**
5.	-	6.036	9.799	**9.241**
6.	RT6.531	6.531	17.976	**18.412**
7.	RT7.655	7.655	11.936	**15.213**
8.	RT80590	8.590	2.604	6.111
9.	-	9.541	0.575	2.012

Figure-1: High performance liquid Chromofogram of Achyranthes alkaloids

DEPARTMENT OF BIOCHEMISTRY, S. P. MEDICAL COLLEGE, BIKANER

Acquired by	: Admin
Sample Name	: Achyranthes alkaloids
Sample ID	: Alkaloids Achy.
Tray #	: 2
Vail #	: 1
Injection Volume	: 20 µL
Conc.	:
Data File Name	: Achyranthes alkaloids data new.lcd
Method File Name	: Achyranthes alkaloids Meth.lcm
Report File Name	: Default.lcr
Data Acquired	: 11/2/2016 2:15 PM
Data Processed	: 11/2/2016 2:28 PM

<Chromatogram>



Peak	Name	Retention Time	Area(mm^2)	Conc. (µg gm^{-1})
1.	-	1.936	0.010	0.021
2.	-	2.376	0.261	1.114
3.	RT5.194	5.194	38.229	**33.475**
4.	RT5.567	5.567	32.232	**26.963**
5.	RT6.447	6.447	19.481	**14.888**
6.	-	8.656	0.223	1.001
7.	-	9.608	1.012	5.214
8.	-	10.835	0.367	1.964
9.	-	14.589	0.397	2.103

Fresh plant material was collected dried separately and grounded finely. Then the known quantity of samples were filled in thimble directly and were placed in soxhlet apparatus and extracted separately in methanol for 24 hrs followed by drying and concentration of extracts. These methanolic extracts of selected plants were subjected to analysis for the presence of alkaloids by TLC.

Separation, Identification and Confirmation of Alkaloids

Each of the crude extract was applied separately on the pre-activated glass plates coated with silica gel G. The solvent system used for TLC was chloroform: methanol (15:1). The TLC plate was then viewed under UV light (254 nm) for fluorescence. The distance of each spot from the point of origin was recorded. The spots were sprayed with Dragendorff's reagent and then observed. Browinsh orange spots indicated the presence of alkaloids. The Rf values of the sample were computed as follows:

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solute}}$$

Purification, Qualitative and quantitative analysis of Alkalaids by HPLC

1gm of selected plant samples were kept overnight in HPLC grade methanol for extraction. These extracts were sonicated for 20 minutes. 20µl of this extract was passed through .45µm filter and that filtrate was used for HPLC analysis. Qualitative and quantitative HPLC analysis of the sample was performed according to the method of borde *et.al.* (2014). The HPLC system (Shimadzu lab chromo 2010 HT HPLC) was used. The software package used for analyzing results was Shimadzu lab chromo HPLC control and auto-sampling. Chromatographic analysis was carried out using a C-18 column at ambient temperature. Running conditions included: injection volume 20µl; mobile phase: methanol: water (60:40), flow rate 0.50 ml/min; and detection at 205nm.

Data analysis

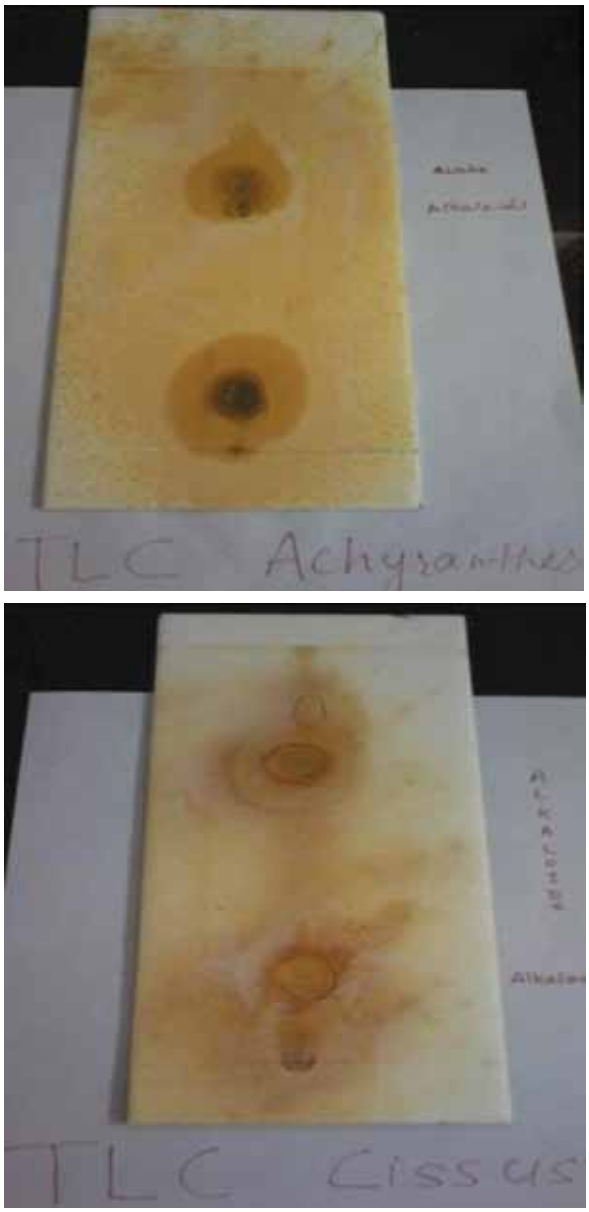
Retention time and concentration of alkaloids was identified by using HPLC software (lab chromo HPLC control and sampling).

RESULTS AND DISCUSSION

Since ancient times, humans have derived many traditional medicines from herbs and plants. Traditional medicines are perceived as competent, safe and cost effective. Furthermore, medicinal plants are an integral part of research and development in the pharmaceutical industry. Medicinal plants have their values in the substances present in various plant tissues with specific physiological action in the human body. Medicinal plants like *Achyranthes aspera* (Apamarga) and *Cissus quadrangularis* (Hadjod) have been selected for present study of presence of alkaloids by TLC and further purification for total alkaloids by HPLC.

TLC analysis of medicinal plants

The isolation and partial purification of alkaloid-rich fraction of *Achyranthes aspera* (seeds) and *Cissus quadrangularis* (stem) were done using Thin-Layer Chromatography. Three florescent spots (two big one small) in *Cissus quadrangularis* and Two florescent spots in *Achyranthes aspera* were identified. Two brownish orange spots were identified after spraying showing the presence of alkaloid content in each plant sample. (Fig: 1). After the positive results for the presence of alkaloids by TLC, further purification of total alkaloids were done using HPLC.



(Figure-1)

HPLC analysis of samples

HPLC analysis of samples revealed that the highest peaks were present in *Cissus quadrangularis* with retention time 5.549 min. HPLC profile of *Cissus quadrangularis* reveled 1 major and 3 minor peaks with retention time 5.549min and 6.036 min ,6.531 min, 7.655 min respectively and their respective concentrations are 38.417 µg/ml, 9.241 µg/ml, 18.412 µg/ml , 15.213 µg/ml respectively. In the HPLC profile of *Achyranthes* reveled 2 major and 1 minor peak with retention time(Rt) 5.194 min, 5.567 min and 6.447 min respectively and their respective concentrations are 33.475 µg/ml, 29.963 µg/ml and 14.888 µg/ml respectively.

As a whole, the results showed that these plants contain significant amount of total alkaloids which are therapeutically important. In pharmaceutical sciences, they serve as raw materials for the formulation of new and effective drugs, so this study would yield a significant contribution to plants with medicinal use. In the field of medicine, alkaloids are a part of every medicinal scientist's resources and play an important role in treating diverse diseases. This study would serve as a guide for researchers in their researches on the use of indigenous materials in their community.

Conclusion

In this study, total alkaloid content from methanol extracts of *Achyranthes aspera* (seeds) and *Cissus quadrangularis* (stem) was investigated. Alkaloid containing plants have many therapeutic properties like antimicrobial activity, antioxidant activity, free radicals scavenging activity. This study is given a scientific basis to plants already used for traditional purposes to treat various ailments and metabolic disorders.

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