QUALITATIVE AND QUANTITATIVE STUDY OF PHYTOCHEMICALS IN ROOTS EXTRACT OF *AERVA LANATA*

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Abstract

Phytochemicals have vital properties to prevent or treat various common diseases. Identifying & isolating these phytochemicals will definitely help the human civilization. Thus, this study paper deals with exploring the phytochemicals & performing qualitative & quantitative estimation of the same. The roots of *Aerva lanata* was collected & subjected to extraction by solvents like chloroform, ethyl acetate, methanol and water by maceration method. Further qualitative & quantitative studies were done on the same. The results revealed chloroform, ethyl acetate, methanol and water observed to have 2.32%, 2.90%, 8.14%&3.44% respectively. The chloroform extract found to have presence of tannin only. In case of ethyl acetate phenol & tannin tested positive. The methanolic extract yielded somewhat grater number of phytoconstituents which are namely flavonoid, phenol & tannin. Finally, the aqueous extract tested positive for flavonoid & tannin. Results of TLC for phenol & flavonoid confirmed that along with the standard used for comparison, other classes of phenol & flavonoids are present in the *Aerva lanata* extract. Total phenolic content for *Aerva lanata* methanolic extract estimated to be 1.380 mg/100mg while the ethyl acetate & aqueous extract contain 0.866 & 0.613 mg/100 mg of phenol respectively. Total flavonoid content was estimated only in methanolic extract which was observed to be 1.280 mg/ 100 mg. From the obtained data it can be summarized that plenty of bioactive compounds are present in *Aerva lanata* root which can be further analysed for pharmacological activities.

Keywords: Herbal medicines, *Aerva lanata,* Thin layer chromatography, Total phenol content, Total flavonoids content, Phytochemicals, Medicinal plant.

Introduction

It is well acknowledged that plants constitute a crucial part of the biodiversity of the earth and one of its most important natural resources.The history of human civilization can be traced back to the dawn of the healing arts. Some of the plant's chemical constituents, which have a clear physiological effect on the human body, have medical significance (Piero*et al*.,2012; Prakash Sharma, 2014).

Primary and secondary metabolites known as phytochemicals are found naturally in many areas of plants and act as a plant's defense mechanism against numerous pathogens. Primary metabolites (carbohydrates, lipids, and proteins) are directly involved in plant development and mechanism. Secondary metabolites, such as alkaloids, phenolics, sterols, steroids, essential oils, lignins, and tannins, among others, are thought of as the end products of primary metabolites and are engaged in metabolic activity (Ali and Alqurainy, 2006; Velu e*t al*., 2018;Frisvad*et al*., 2007). Phytochemicals are naturally occurring chemical substances that are physiologically active and are present in plants. They shield plant cells from environmental dangers such pollution, stress, dehydration, UV exposure, and pathogenic attack. These substances, also referred to as secondary plant metabolites, are advantageous for human health. They are believed to work as synergistic agents, enabling the body to utilize nutrients more effectively. Low toxicity, low cost, easy accessibility, and biological properties like antioxidant activities, antimicrobial effects, modulation of detoxification enzymes, stimulation of the immune system, reduction of platelet aggregation, modulation of hormone metabolism, and antineoplastic properties are some of the advantages of phytochemicals.Phytochemicals have vital properties to prevent or treat various common diseases, even though they are not necessary nutrients or needed by the human body to sustain life. Numerous studies have been conducted to demonstrate the health advantages of phytochemicals as a result of this feature (Mendoza & silva, 2018; Nyamai *et al*., 2016).

The plant *Aerva lanata* (Linn) Juss. ex. Schult is widely used in urinary disorders in southern part of India as a source of Pashanabheda. It is commonly known as Gorakha ganja a member of Amaranthaceae, usually found as weed on mountains and bare ground. It is an herb which trails on the ground with many branches and leaves are alternately arranged with fine hairs above and with wooly beneath. Flowers are greenish white in clusters. Since years many researches have been carried out to elicit the diuretic & anti-urolithic activity of this plant. Besides, it has been proven for many more pharmacological activities like anti-diarrhoeal, anti-hyperglycameic, anti-oxidant, anti-helmentic, and analgesic. In addition, various phyto chemical investigations reveal the presence of steroids, tannins, flavonoids, nutrients, terpenoids in different parts of the plant (Nagaratna*et al*., 2014; Bitastaand Madan, 2016; Athiraand Nair, 2017). Thus, this study paper deals with exploring the phytochemicals & performing qualitative & quantitative estimation of the same.

Material & Methods

Collection of plant material

Roots of *Aerva lanata* Linn were collected from local area of Bhopal in month of January, 2022. Drying of fresh plant parts was carried out in sun but under the shade.

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered stems (Khandelwal, 2005; Starmans & Nijhuis, 1996).

Defatting of Plant Material

62gram of roots of *Aerva lanata* Linn were coarsely powdered and subjected to extraction with petroleum ether in maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by macerationmethod

Defatted powdered roots of *Aerva lanata* Linn were exhaustively extracted with successive solvent like chloroform, ethyl acetate, methanol and water by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts (Mukherjee, 2007).

Phytochemical screening

Phytochemical examinations were carried out extracts as per the following standard methods (Kokate, 1994: Pandey & Tripathi, 2014).

Separation and Identification of phytoconstituents by TLC

Thin layer chromatography is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase. Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system toluene: ethyl acetate: formic acid (5:4:1) solvent system used for flavonoids and toluene: ethyl acetate: formic acid (7:5:1) solvent system used for phenol (Patel *et al*., 2017). After pre-saturation with mobile phase for 20 min for development were used. The movement of the active compound was expressed by its retention factor (Rf), values were calculated for different samples (Sherma & Fried, 2003).

Total phenol content estimation

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Parkhe

and Bharti, 2019). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti, 2019).10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Results & Discussion

The chloroform extract found to have percentage yield of 2.32% which is lowest of all. A Bit good results are seen in case of ethyl acetate with percentage yield of 2.90%. The aqueous extract posses 3.44% yield. The methanol extract has highest percentage yield of 8.14%. The phytochemical screening of *Aerva lanata* was then performed to detect the phytochemical constituents. The chloroform extract found to have presence of tannin only. In case of ethyl acetate phenol & tannin tested positive. The methanolic extract yielded somewhat greater number of phytoconstituents which are namely flavonoid, phenol & tannin. Finally, the aqueous extract tested positive for flavonoid & tannin.

According to study conducted by Battu and Kumar the chloroform extract of the entire *A. lanata* plant only tannins, alkaloids, and flavonoids in addition to carbohydrates and the lack of alkaloids, proteins, saponins, and resins (Battuand Kumar,2012).

Similarly according to Yamunadevi*et al*. methanolic extract of *A. lanata* has reported the presence of flavonoids and glycosides as in the current study, they have also shown the presence of terpenoids and alkaloids (Yamunadevi*et al*., 2011).

After phytochemical screening Thin layer chromatography was performed. TLC for flavoinoid was performed by using quercetin as flavonoid. The Rf value obtained for Quercetin standard was 0.72. For methanolic extract of *Aerva lanata,* range of Rf values are obtained when visualized in short UV. The Rf value obtained confirmed the compound present in extract are part of large flavonoid groups. Additionally, TLC for phenol was performed for methanol, ethyl acetate & aqueous extract. The Rf value obtained for gallic acid standard was 0.58. From the Rf values obtained for all the three extract it

was evident that ethyl acetate extract contain phenol similar to gallic acid.

Total phenolic content for *Aerva lanata* methanolic extract estimated to be 1.380 mg/100mg while the ethyl acetate & aqueous extract contain 0.866 & 0.613 mg/ 100 mg of phenol respectively. Total flavonoid content was estimated only in methanolic extract which was observed to be 1.280mg/ 100 mg. In same way study conducted by Bahar *et al*., 2013 noticed that *Aerva lanata* extracts in methanol and petroleum ether had total phenol concentrations of 108.9125 mg/ml and 147.5025 mg/ml, respectively.

Table No. 1: % yield of *Aerva lanata*Linn

S. No.	Extracts	% Yield (W/W)
1.	Chloroform	2.32%
2.	Ethyl acetate	2.90%
3.	Methanol	8.14%
4.	Aqueous	3.44%

Table No. 2: Result of phytochemical screening of *Aerva lanata* Linn

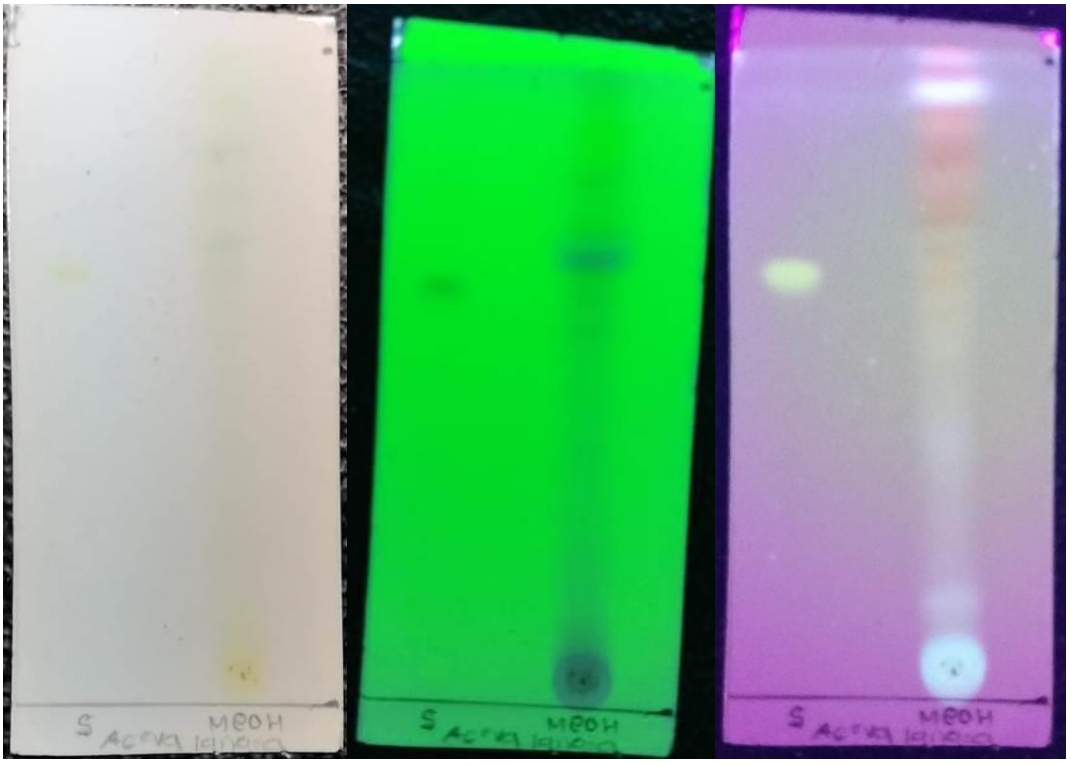
S. No.	Constituents	Chloroformextract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	**Alkaloids** Hager's Test:	-ve	-ve	-ve	-ve
2.	**Glycosides** Conc. H_2SO_4 Test:	-ve	-ve	-ve	-ve
3.	**Flavonoids** Lead acetate Test: Alkaline Reagent Test:	-ve -ve	-ve -ve	-ve +ve	-ve -ve
4.	**Diterpenes** Copper acetate Test:	-ve	-ve	-ve	-ve
5.	**Phenol** Ferric Chloride Test: FolinCiocalteu Test:	-ve -ve	-ve +ve	-ve +ve	-ve +ve
6.	**Proteins** Xanthoproteic Test:	-ve	-ve	-ve	-ve
7.	**Carbohydrate** Fehling's Test:	-ve	-ve	-ve	-ve
8.	**Saponins** Froth Test:	-ve	-ve	-ve	-ve
9.	**Tannins** Gelatin Test:	+ve	+ve	+ve	+ve
10.	**Sterols** Salkowski's Test:	-ve	-ve	-ve	-ve

[+ve =positive; -ve= negative]

Table No. 3:Identification of phytoconstituents by TLC of *Aerva lanata*Linn

TLC of Flavonoids		
S. No.	**Mobile phase** Toluene: Ethyl acetate: Formic acid	***Rf*value**

	(5:4:1)	
1.	**(Quercetin)** Dis. travel by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV = 1 No. of spot at normal light= 1	 Long- 0.72 Short- 0.72 Normal- 0.72
2.	**(Methanol extract)** Dis. travel by mobile phase= 5cm No. of spot at long UV = 4 No. of spot at short UV = 5 No. of spot at normal light= 2	 Long-0.1, 0.2, 0.8, 0.84 Short- 0.8, 0.86, 0.98, 0.9, 0.96 Normal- 0.8, 0.98
	Spot Sequence	
	Quercetin	1st
	Methanolic extract	2nd



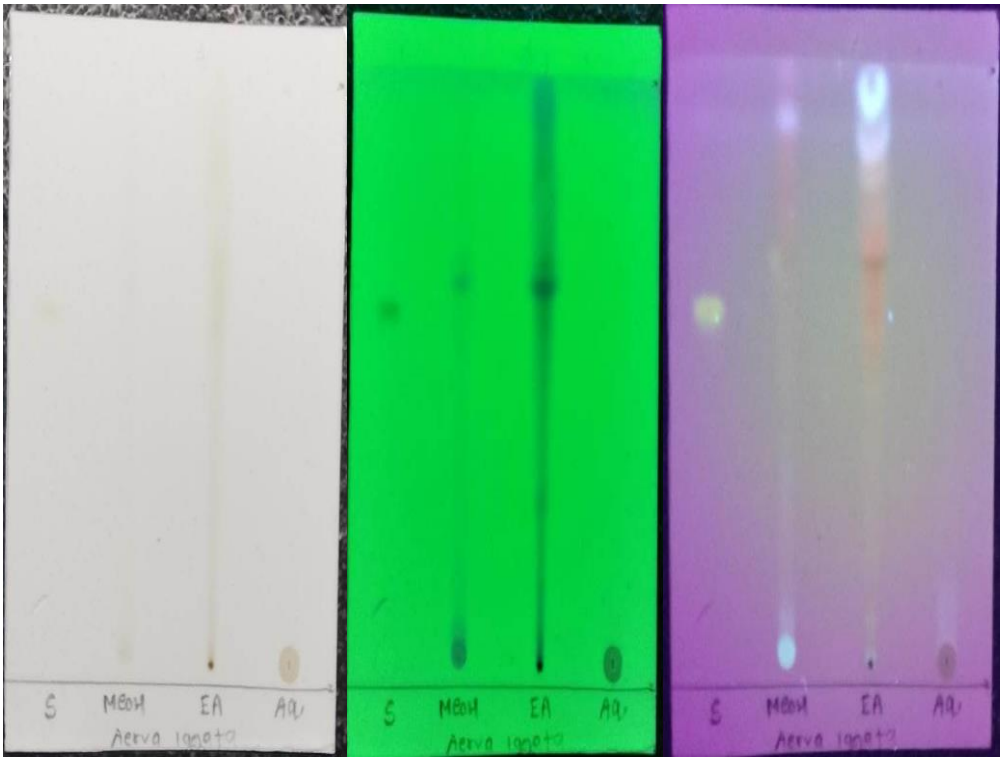
Normal light Short UV Long UV

Figure 1:TLC of Flavonoids

Table No. 4: Identification of phytoconstituents by TLC of *Aerva lanata*Linn

TLC of Phenol		
S. No.	**Mobile phase** Toluene: Ethyl acetate: Formic acid (5:4:1)	***Rf*value**
1.	**(Gallic acid)** Dis. travel by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV = 1	 Long- 0.58 Short- 0.58

	No. of spot at normal light= 1	Normal- 0.58
2.	**(Methanol extract)** Dis. travel by mobile phase= 5cm No. of spot at long UV = 1 No. of spot at short UV = 1 No. of spot at normal light= 1	 Long-0.1 Short- 0.64 Normal- 0.74
3.	**(Ethyl acetate extract)** Dis. travel by mobile phase= 5cm No. of spot at long UV = 5 No. of spot at short UV = 5 No. of spot at normal light= 1	 Long-0.58, 0.72, 0.76, 0.94, 0.98 Short- 0.1, 0.24, 0.64, 0.8, 0.82 Normal- 0.92
4.	**(Aqueous extract)** Dis. travel by mobile phase= 5cm No. of spot at long UV = 1 No. of spot at short UV = 5 No. of spot at normal light=1	 Long- 0.42 Short- 0.42 Normal- 0.42
	Spot Sequence	
	Gallic acid	1st
	Methanolic extract	2nd
	Ethyl acetate extract	3rd
	Aqueous extract	4th



Normal light Short UV Long UV

Figure 2:TLC of phenol

Table No. 5: Estimation of total phenolic and flavonoids content of *Aerva lanata*Linn

S. No.	Extract	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1	Ethyl acetate	0.866	-
2	Methanol	1.380	1.280
3	Aqueous	0.613	-

Conclusion

Nevertheless, the conventional medical system offers physiologically active chemicals that are attractive sources of possible secondary metabolites that can be employed as pharmaceutical substances. Finding the antibacterial and antioxidant components that may be used in herbal formulations is the goal of further research. The outcome of this research offered an important phytomarker for the identification and description of *A. lanata*. Additionally, pharmacological research will isolate, characterize, describe, and clarify the structure of the bioactive substance.

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