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PHYTOCHEMICAL SCREENING AND THIN LAYER CHROMATOGRAPHIC STUDIES OF *AERVA LANATA* ROOT EXTRACT

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Abstract: India being a rich and varied flora of medicinal plants since the Vedic period. The present study deals with the phytochemical screening and thin layer chromatographic studies of *Aerva lanata* root extract belonging to family Amaranthaceae. Phytochemical screening determination by some chemical tests and thin layer chromatographic study was carried out by using various solvent system of varying polarity of hexane, chloroform, ethyl acetate, acetone and methanol extracts. Phytochemical screening reflects presence of alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, amino acids, flavanoids, quinones and terpenoids shows different types of results in different solvents extracts. Thin layer chromatographic studies of the *Aerva lanata* root extracts constituted different colored phytochemical compounds with different R_f values. The chloroform and methanol extracts in the drug is carried out to establish the biomarker compound. The result obtained in present study indicated *Aerva lanata* root as a rich source of natural antioxidants, and provides evidence that solvent extract of *Aerva lanata* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

Keywords: *Aerva lanata*, Root extract, Phytochemical, Amaranthaceae, Retention factor, TLC Studies

I. INTRODUCTION

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. Medicinal plants are great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food plants. They also sometimes added to foods meant for pregnant women and nursing mothers for medicinal purposes as reported by Okwu, D. E. and Hill A.F. [1, 2, 3]. Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Different parts of the plants like bark, roots, leaves, exudates etc. are used as per medicinal properties proposed by Perumal Samy R. and Gopala Krishnakone P. [4].

Aerva lanata is an important medicinal plant, found throughout tropical India as a common weed in fields and waste land in [5]. Because of its reputation in folk medicine, *Aerva lanata* has become the subject of intense pharmacological and chemical studies for the last 30 years. Traditionally, root of *Aerva lanata* are used as sap for eye-complaints, an infusion is given to cure diarrhoea, kidney stone and in snake bite treatment.

In addition to the traditional uses, the plant is reported for a number of pharmacological activities viz., anti-helminthic, demulcent, anti-inflammatory in [6], diuretic in [7] expectorant, hepatoprotective in [8] and

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nephroprotective in [9], anti-diabetic in [10], anti-hyperglycaemic, anti-microbial, cytotoxic in [11], urolithiatic, hypoglycaemic, anti-hyperlipidemic, anti-parasitic and anti - helmenthic activities. In order to identify the bioactive compounds responsible for the above pharmacological activities, phytochemical studies have been carried out by several researchers with the report of phenolic compounds in[12]. *Aerva lanata* plant root was selected for this study is based on its traditional medicinal use in [13].

II. MATERIAL AND METHODS

Collection of plant

Aerva lanata Plant roots were collected from the Chintoor mandal, Khammam district of Andhra Pradesh, India in the month of September and October 2012. The plant voucher specimens identification was done with the help of Prof. Vastsavaya.S.Raju Department of Botany, Kakatiya University, Warangal and the same was deposited at Infectious Diseases & Metabolic Disorders Research Lab, Department of Zoology, Kakatiya University, Warangal.

Preparation of plant extract

Roots were collected in bulk, washed, shade dried, macerated and extracted with hexane, chloroform, ethyl acetate, acetone, and methanol. The extract was filtered and it was finally dried at low room temperature under pressure in a rotary vacuum evaporator (Thermotech, buchi type model th-012). The extracts were concentrated, percentage yield calculated and then subjected to phytochemical screening and TLC profiling studies. The dried extract was properly stored in the desiccators for further experiment and analysis.

Phytochemical Screening

Chemical tests for the screening and identification of bioactive chemical constituents like alkaloids, carbohydrates, glycosides, saponins, phenolic compounds, phytosterols, proteins, amino acids, flavonoids, and tannins, in the medicinal plants under study were carried out in extracts by using standard procedure in [14].

Thin layer chromatographic studies

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 Hexane: Acetic acid (9:1) solvent system I, In solvent system II Hexane: Ethyl acetate :Acetic acid (5:4:1), In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1) used. After pre-saturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (R_f), values were calculated for different samples

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front TLC plates}}$$

III. RESULT

Percentage of yield extract

The yield of sequential extracts (g) is shown in [Table 1]. The amount obtained from hexane, chloroform, ethyl acetate, acetone and methanol extracts are 5.020 gm, 4.080 gm, 2.750 gm, 1.720 gm, and 3.750 gm respectively.

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Table 1:-The percentage yield of different extracts of *Aerva lanata* root

S. No	Solvent	Color of extract	Yield of the extract (in gm)	Percentage yield(%w/w)
1	Hexane	White	5.020	2.51%
2	Chloroform	Light brown	4.080	2.04%
3	Ethyl acetate	Light brown	2.750	1.37%
4	Acetone	Light brown	1.720	0.86%
5	Methanol	Dark brown	3.750	1.85%

Phytochemical Screening

The present study carried out in the A*erva lanata* revealed the presence of medicinal active constituents. The phytochemical active compounds of *Aerva lanata* were qualitatively analysed for roots and the results are presented in Table 2. In these screening process alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, amino acids, flavanoids, quinones and terpenoids shows different types of results in different solvents extracts. Among these phytochemical screening, Alkaloids, Saponinis, Tannins, Amino acids, Flavanoids and Terpenoids were present in all solvent extracts where as Phytosterols are present all extracts except methanol, Phenolic compounds are in Ethyl acetate and methanol extracts, proteins and carbohydrates were present in ethyl acetate and methanol extracts, Quinones were found in hexane, acetone, and methanol extracts, Glycosides are absent in all solvent extracts.

Table:-2 Phytochemical constitute of the root extract of *Aerva lanata*

S.No	Phytoconstituents	Tests	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
1	Alkaloids	Mayer's test	+ve	+ve	+ve	+ve	+ve
2	Glycosides	Borntrager's test	-ve	-ve	-ve	-ve	-ve
3	Saponins	Froth forming test	+ve	+ve	+ve	+ve	+ve
4	Phenolic compounds	Lead acetate test	-ve	-ve	+ve	-ve	+ve
5	Tannins	$Fecl_{3\ test}$	+ve	+ve	+ve	+ve	+ve
6	Phytosterols	Libermann-Buchard test	+ve	+ve	+ve	+ve	-ve
7	Corbohydrates	Fehilings test	-ve	+ve	-ve	+ve	+ve
8	Protiens	Biuret test	-ve	-ve	-ve	+ve	+ve
9	Aminoacids	Ninhydrin test	+ve	+ve	+ve	+ve	+ve
10	Flavanoids	Alkaline test	+ve	+ve	+ve	+ve	+ve
11	Quinones	Quinone test	+ve	-ve	-ve	+ve	+ve
12	Terpenoids	Terpenoid test	+ve	+ve	+ve	+ve	+ve

Thin layer chromatographic studies

A large number of solvent systems were tried to achieve a good resolution. Finally, the solvents hexane: ethyl acetate: acetic acid was used. Thin layer chromatographic studies of the hexane extract of *Aerva lanata*. Solvent system I Hexane: Acetic acid (9:1), 3 spots were visible R_f values 0.20, 0.36 and 0.52. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 1 spot detected R_f value 0.90. In solvent system III Hexane: Ethyl acetate: Acetic acid

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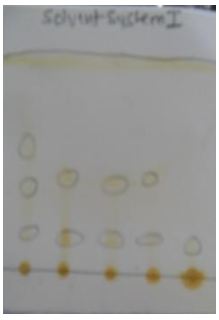
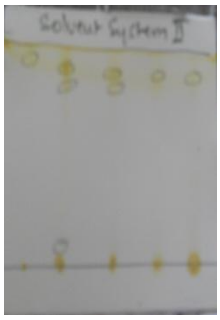
(4:4:2), 1 spot detected R_f value 0.90. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible R_f values 0.07 and 0.81. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 3 spots were obtained having R_f of 0.09, 0.81 and 0.94.

TLC studies of the Chloroform extract of *Aerva lanata*. Solvent system I Hexane: Acetic acid (9:1), 2 spots were visible R_f values 0.14 and 0.40. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 3 spots were detected R_f values 0.10, 0.82 and 0.90. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 2 spots were detected R_f values 0.05 and 0.90. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible R_f values 0.09 and 0.78. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having R_f of 0.18 and 0.94.

TLC studies of the Ethyl acetate extract of *Aerva lanata*. Solvent system I Hexane: Acetic acid (9:1), 2 spots were visible R_f values 0.10 and 0.40. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 2 spots were detected R_f values 0.82 and 0.90. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 1 spot detected R_f value 0.85. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible R_f values 0.07 and 0.81. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having R_f of 0.03 and 0.94.


Solvent system I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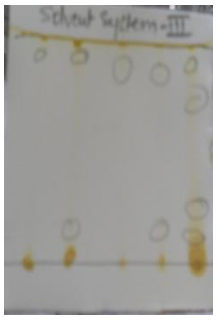
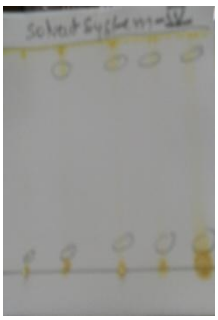

Solvent system III


Solvent system IV


Solvent system V

TLC studies of the Acetone extract of *Aerva lanata*. Solvent system I Hexane: Acetic acid (9:1), 2 spots were visible R_f values 0.16 and 0.44. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 1 spot detected R_f f value 0.82. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 2 spots were detected R_f values 0.85. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible R_f values 0.05 and 0.90. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having R_f of 0.09 and 0.80.

TLC studies of the Methanol extract of *Aerva lanata*. Solvent system I Hexane: Acetic acid (9:1), 1 spot detected R_f value 0.10. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 1 spot detected R_f value 0.92. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 4 spots were detected R_f values 0.05, 025, 080 and 0.90. In solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), 2 spots were visible R_f values 0.10 and 0.81. In solvent system V Hexane: Ethyl acetate: Acetic acid (2:7:1), 2 spots were obtained having R_f of 0.09 and 0.81 (Table 3).

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Table:-3 R_f values of TLC solvent systems for different extract of *Aerva lanata root*

S.No	Extract name	Solvent system I		Solvent system II		Solvent system III		Solvent system IV		Solvent system V	
		No. of spots detected	R_f Value	No. of spots detected	R_f Value	No. of spots detected	R_f Value	No. of spots detected	R_f Value	No. of spots detected	R_f Value
1	Hexane extract	3	0.20 0.36 0.52	1	0.90	1	0.90	2	0.07 0.81	3	0.09 0.81 0.94
2	Chloroform extract	2	0.14 0.40	3	0.10 0.82 0.90	2	0.05 0.90	2	0.09 0.78	2	0.18 0.94
3	Ethyl acetate extract	2	0.10 0.40	2	0.82 0.90	1	0.85	2	0.07 0.81	2	0.03 0.94
4	Acetone extract	2	0.16 0.44	1	0.82	2	0.05 0.90	2	0.03 0.81	2	0.09 0.80
5	Methanol extract	1	0.10	1	0.92	4	0.05 0.25 0.80 0.90	2	0.10 0.81	2	0.09 0.81

IV DISCUSSION

A large number of plants produce secondary metabolites such as alkaloids, flavanoids, phenols, terpenoids, steroids and quinines that are used in pharmaceuticals, cosmetics and pesticide industries. Thus the present study confirms the traditional medical practice and previous pharmacological observations and supplement treatment for other health problems such as allergic reactions, arthritis, some malignancies, and diseases resulting from hormone deficiencies or abnormal production *etc*: in [15, 16]. In the present study, phytochemical screening for all five extracts showed significant indication about the presence of metabolites. Alkaloids, Saponinis, Tannins, Amino acids, Flavanoids and Terpenoids, were found to be present in the all the sequential extracts of *Aerva lanata* root. The results of the present study also supplement the folkloric usage of the studied plants which possess several known and unknown bioactive compounds with bio-activity. By isolating and identifying these bioactive compounds new drugs can be formulated to treat various diseases and disorders.

TLC profiling of all 5 extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gives different R_f values in different solvent system. This variation in R_f values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the R_f values of compounds in different solvent system. Different R_f values of the compound also reflect an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

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V. CONCLUSION

The plant screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. These findings suggested that *Aerva lanata* root could be a potential source of natural antioxidant having great importance as therapeutic agent and preventing oxidative stress related degenerative diseases. The root of *Aerva lanata* can provide lead molecules which could be useful substrate for the synthesis of new broad spectrum antibiotics for the treatment of infections caused by the organisms. Further purification, identification and characterization of the active compounds would be our priority in future studies.

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