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Original Article

Detection of HPTLC Fingerprint in Aerial Parts of *Aerva lanata* Linn. HPTLC Finger Print Profile and Evaluation of *in vitro* Antioxidant Activity of *Aerva lanata* Linn. Juss

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ABSTRACT

Background: Natural products enhance the standard of living, which greatly contributes to maintaining human health. Associated oxidative stress-mediated problems have been treated using *Aerva lanata* Linn. Juss in traditional and folkloric medicine. **Objectives:** The research aims to analyze the phytochemical profile using HPTLC of MEAL (methanol extracts of *Aerva lanata*) and AEAL (aqueous extracts of *Aerva lanata*) and to determine*in vitro* antioxidant capacity of MEAL and AEAL. The use of HPTLC fingerprint analysis will aid in identifying and monitoring drug effectiveness and ensuring therapeutic efficacy. **Materials and Methods:** Total phenol content, free radical scavenging efficiency of DPPH (1,1 diphenyl 2 picryl hydrazyl), ferric ion reducing antioxidant, and phosphomolybdate assays were used to determine *in vitro* antioxidant activity at various concentrations. **Results:** The number of phyto-constituents in MEAL at 375 nm and 550 nm was found to be more than AEAL. MEAL had a, stronger DPPH free radical scavenging capacity, higher phenol content, lower ferric ion antioxidant potential, and lower phosphomolybdenum assay. The natural phytoconstituents present in the plant extracts are responsible for antioxidant activity. As sample concentration increases, the antioxidant activity becomes more effective. **Conclusion:** The outcome results of the present investigation suggested that aerial parts of*Aerva lanata* could provide a source of natural antioxidants.

Keywords: *Aerva lanata*, DPPH , HPTLC, Physicochemical parameters, Fingerprint profile, *in vitro* antioxidant activity.

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INTRODUCTION

Natural product helps to keep people healthy by improving their standard of life. The need for medicinal plants is increasing as people become more aware of natural products and realize that they have fewer adverse effects than synthetic compounds, are more readily available, and are more cost-effective. World Health Organization (WHO) estimates more than eighty percent of people depend on traditional medicines only for their basic health care requirements in developing countries. Alkaloids, flavonoids, and terpenoids are bioactive molecules found in plants that are rich in antioxidants and have different structures depending on the presence of various functional groups in the molecules, which makes a significant contribution to the antioxidant potential.[1,2] In addition to food additives commonly used (butylated hydroxytoluene, propyl gallate, and butylated hydroxyanisole) and vitamins such as ascorbic acid, tocopherol, carotene, specific spices, and herbs were used as antioxidants.[3] As a result, plants' antioxidants and various phytocompounds are used to prevent oxidative stress and the disorders that come with it.[4,5] Excessive Reactive Oxygen Species (ROS) and free radicals will decrease antioxidants' potency in today's lifestyle. Even though ROS functions as a second messenger can cause inflammation, neurodegenerative diseases, atherosclerosis, cataracts, cancer, aging, protein oxidation, lipid peroxidation, and DNA mutation in pathological as well as physiological pathways.[6-12] The natural antioxidant present in the human body eliminates free radicals and prevents oxidative stress and associated complications. Antioxidant found in nature plays an important function in health care.[13] The plant's natural antioxidant sources are phenolic acids, flavonoids, anthocyanins, tannins, and carotenoids.[14,15]

TLC with improved resolutions differs from TLC solely in pore size and particle size of the sorbent is a significant improvement. HPTLC can serve as an essential tool for qualitative and quantitative analysis of phytoconstituents in herbal extracts, identification of synthetic drugs and herbal formulation, herbal extract and formulation standardization, adulteration detection, stability studies, and quantification of natural as well as synthetic drugs in formulations. Analytical method validation is essential in

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drug discovery, pharmaceutical research, and production because HPTLC can produce chromatographic fingerprinting which can be processed and viewed as an electronic image.[16] *Aerva lanata* Linn. Juss, belonging to the Amaranthaceae family, is a plant of importance that contains phytoconstituents and has an extensive range of pharmacological applications. The plant is widely grown in tropical India, as well as in wastelands. It is widely grown also in Sri Lanka, tropical Africa, Arabia, Java, and the Philippines.[17]

Aerva lanata Linn. Juss is used to treat anthelmintic, urolithiasis, Anti-inflammatory, diuretic and nephroprotective activities were reported in rats.[18-22] *Aerva lanata* plant had better antimicrobial activity.[23] *Aerva lanata* alcoholic extracts were used for diabetes treatment in rats.[24] The petroleum ether extract, partially filtered, was reported cytotoxic.[25] Leaf and roots of aqueous and alcoholic extracts were found to have hepatoprotective activity.[26] Aerial parts of *Aerva lanata* Linn. Juss ex Schult were used to treat Dalton's ascetic lymphoma.[27] *Aerva lanata* Linn has been used traditionally and in folk for treating diabetes.[28] Using HPTLC techniques, the MEAL and AEAL were utilized to determine antioxidant activity (*in vitro*) and phytochemical profiles. The current study was undertaken to quantify the contents of phenolic content, ferric ion reducing antioxidant, phosphomolybdate assays, and HPTLC fingerprint analysis of Methanol (MEAL) and Aqueous (AEAL) extracts of aerial parts of *Aerva lanata* Linn. and to assess the *in vitro* anti-oxidant potential for scientific validation.

MATERIALS AND METHODS

Collection and authentication

Aerva lanata Linn Juss was collected from different parts of Tirunelveli district in Tamil Nadu, India, around the third week of November. Dr Shiddamallayya N, Regional Research Institute (Ayurvedic) Assistant Director, Bangalore, carried out authentication as well as identification, and a voucher specimen (RRCBI-5588) was retained for future reference in the department.

Preparation of herbal extracts

About 500 g of *Aerva lanata* (aerial parts) were properly weighed and ground into a coarse powder, then extracted with solvents of increasing polarity such as petroleum ether, chloroform, acetone and methanol in Soxhlet extractor and extracted with water for 7 days by maceration. Marc was collected after each extraction and before being packed in an extractor, it should be dried in the air. The extracts were collected from a rotary evaporator; it is then filtered, concentrated, dried and stored in a desiccator under reduced pressure. The extracts prepared from methanol and water was marked as MEAL and AEAL respectively.

Physicochemical parameters

Foreign organic matter, ash values such as acid-insoluble ash, water-soluble ash, alcohol-soluble extractive value, water-soluble extractive value, total ash and Loss on Drying (LOD) were among the physicochemical characteristics that were investigated.[29]

Preliminary phytochemical analysis

Phytochemical analysis was performed to detect phytochemicals such as flavonoids, alkaloids, terpenoids and tannins.[30,31]

HPTLC fingerprint analysis

To ensure that crude drugs are correctly identified, standardization is necessary.[32] The sample solution MEAL and AEAL has been prepared and configured to ensure high-quality fingerprinting. The MEAL and AEAL of 100 mg were dissolved in 1 mL of methanol and water, and then centrifuged for 5 min at 3000 rpm. These solutions were used as samples to be tested for HPTLC analysis. A Linomat 5 automatic applicator with a 100 µL Hamilton syringe, a twin trough plate development chamber, a Camag TLC scanner 3, and win CATS software were used in the CAMAG (Muttenz, Switzerland) HPTLC system. HPTLC precoated silica gel 60 F_{254} plate was spotted with the appropriate volume of MEAL (2 µL) and AEAL (2 µL) in the shape of bands with a bandwidth of 5 mm. Mobile phase systems were viz. Toluene: ethyl acetate: methanol: glacial acetic acid (55: 35: 10: 2) and Toluene: ethyl acetate: methanol: glacial acetic acid (45: 35: 20: 2) used for development. Saturation of the glass chamber was carried out by mobile phase for twenty min at room temperature (30.5°C). After development, the mobile phase was evaporated by using an air dryer from the HPTLC plate for 10 min. 10% v/v methanolic sulphuric acid was used as a spraying agent and incubated at 110°C for 10 min. The plate which was air-dried was examined for the detection of secondary metabolites at 254 nm, 366 nm and visible light. Densitometry scanning was carried out by using tungsten and D_2 (deuterium lamp) in absorbance mode at 375 nm and 550 nm for MEAL.

Estimation of total phenolic content

The total phenolic content of MEAL and AEAL was performed by a slightly modified Folin-Ciocalteu (FC) reagent method.[33] Specified concentration MEAL and AEAL (200 µL) was added to Folin-Ciocalteu (FC) reagent concentration and incubated for 10 min. 1.25 mL of aqueous sodium carbonate (Na_2CO_3) and 1 mL of distilled water were added to reaction mixtures and incubated for 90 min at 37°C, measured the absorbance by UV spectrophotometer at wavelength 760 nm and carried out the blank using distilled water. Gallic acid was employed as a reference standard that is measured in milligrams per Gallic Acid Equivalent (GAE).

Free radical scavenging activity (DPPH)

The antioxidant activity of MEAL, as well as AEAL was carried out by DPPH free radical scavenging activity with minor modification to determine the antioxidant activity of MEAL as well as AEAL.[34] Dissolve a specific concentration of 100-500 µL of MEAL as well as AEAL in ethanol, free radical scavenging activity was performed. DPPH (0.004%) dissolved in ethanol and 1 mL of the above solution was added to MEAL, AEAL and ascorbic acid (standard) individually. The absorbance was measured spectrophotometrically wavelength of about 517 nm after incubating in the dark for 30 min. The degree of change of colour from purple to yellow reveals how effective MEAL and AEAL are at scavenging free radicals. Greater free radical scavenging activity was associated with lower reaction absorption.

$$\text{DPPH free radical scavenging activity (\%)} = \frac{\text{Control Absorbance - Test Absorbance}}{\text{Control Absorbance (Ac)}} \times 100$$

Blank absorbance (1 mL of DPPH solution in 1 mL of ethanol).

Ferric ion reducing antioxidant power assay

Ferric ion-reducing antioxidant power (FRAP) assay was carried out using a technique with small alterations.[35] MEAL and AEAL antioxidant power assay were determined by adding the various concentrations (100 µL to 500 µL) in 2.5 mL of 0.2 mM phosphate buffer (pH 7.4) and 2.5 mL of potassium ferricyanide (1% w/v) and it was incubated for 20 min at 50ºC. To the above solution, 2.5 mL of trichloroacetic acid (10% w/v) was added and centrifuged 15 min at 3000 rpm. Measured the absorbance spectrophotometrically at a wavelength of 700 nm using 2.5 mL of distilled water and 0.5 mL of ferrous chloride (0.1% w/v). A reference standard (ascorbic acid) was used.

Phosphomolybdenum assay

The antioxidant activity of phosphomolybdenum (PM) assay was carried out by adding MEAL and AEAL in quantities ranging from 100 µL to 500 µL to 3 mL of distilled water and add 1 mL of molybdate reagent solution in each tube. Incubated at 95ºC for 90 min. Then kept at room temperature for about 25 min before being spectrophotometrically evaluated at a wavelength of 695 nm against ascorbic acid (standard).[36]

RESULTS

Phytochemical screening

Preliminary phytochemical analysis reveals the presence of phytoconstituents such as alkaloids, phenol, saponins, flavonoids and reduced sugar in MEAL and AEAL. Table 1 summarizes the results of *Aerva lanata* physicochemical parameters.

Total phenolic content

MEAL was found to have a higher phenolic content than AEAL (Table 4). The presence of phenolic content in plant extracts is attributable to antioxidant activity.

DPPH assay

The antioxidants activity was made easy and rapid by the use of 2,2-diphenylpicrylhydrazyl (DPPH). The percentage of inhibition was found to be increased significantly, when the concentration of MEAL and AEAL increased. Free radical scavenging activity (DPPH) of MEAL was found to be higher than AEAL. Ascorbic acid was used as standard as mentioned in Table 5.

Ferric ion reducing antioxidant power assay

The presence of antioxidants in MEAL as well as AEAL would result in an electron being donated to reduce ferricyanide Fe^{3+} to ferrocyanide Fe^{2+}. The FRAP assay was performed in MEAL and AEAL along with standard ascorbic acid. As indicated in Figure 5, MEAL possessed stronger ferric ion-reducing antioxidant activity than AEAL as compared to ascorbic acid (standard).

Phosphomolybdenum assay

MEAL and AEAL and ascorbic acid (standard), were used in the phosphomolybdenum (PM) assay. MEAL exhibited greater activity than AEAL (Figure 6).

DISCUSSION

Medicinal plants were a major source of potentially bioactive components that can be used to generate novel chemotherapy drugs. To determine the purity as well as the quality of a powdered crude drug, number of soluble constituents, and physicochemical parameters. Phytochemical screening was carried out in aerial parts of *Aerva lanata*. The MEAL and AEAL were found to contain alkaloids, tannins, flavonoids and saponins according to a preliminary phytochemical analysis. The higher phenolic content

Table 1: Physicochemical Parameter of *Aerva lanata*.

Parameter	Results (*n*=3, Mean)
Ash values	
Total ash (% w/w)	8.4238±0.01
Acid insoluble ash (% w/w)	1.8274±0.03
Water soluble ash (% w/w)	3.0549±0.05
Extractive values	
Alcohol soluble extractives (% w/w)	1.551±0.12
Water soluble extractives (% w/w)	1.0936±0.07
Foreign organic matter (% w/w)	0.53±0.03
Moisture content (% w/w)	4.03±0.15

Mean±standard deviation, *n*=3.

of MEAL and AEAL in general may be a major contributing component and rationale for antioxidant activity.[37]

Medicinal plant adulteration and inappropriate medication handling can be identified using physicochemical characteristics. Total ash analysis is required to assess the purity and quality of drugs. Total ash, acid-insoluble ash, and water-soluble ash were three different ash value determination methods. Total ash that contains physiological as well as non-physiological ash is usually made up of phosphates, carbonates, silica and silicates. High ash values may be indicated by adulteration, contamination, substitution, or carelessness in the manufacture of crude drugs. A significant quantity of insoluble acid ash suggested contamination by silica. If moisture is present, moulds and bacteria will degrade the drugs, and its active components will be enzymatically destroyed.

MEAL achieved the best results in the following solvent systems viz. toluene: ethyl acetate: methanol: glacial acetic acid (55:35:10:2). Subsequently the plates were scanned and visualized in wavelength of about 254 nm, 366 nm and visible range once after sprayed with sulphuric acid. The HPTLC images in Figures 3 and 4 reveal that all phytoconstituents were separated clearly with no diffusivity or tailing. The presence of ten and nine phytoconstituents was detected in HPTLC fingerprints scanned for MEAL and AEAL around 375 nm as mentioned in Table 2 and Figure 1. The presence of twenty and seventeen phytoconstituents was detected in an HPTLC fingerprint scanned for MEAL and AEAL around 550 nm as mentioned in Table 3 and Figure 2. HPTLC can be helpful as a phytochemical marker and found to be more effective in the field of plant taxonomy and also for determining the identity of secondary metabolites in plants.[38]

Table 2: HPTLC fingerprint of MEAL at 375 nm.

Extracts	Solvent system	Number of Peaks	R_f Value	Percentage Area
MEAL	Toluene: ethyl acetate: methanol: glacial acetic acid (55: 35: 10: 2)	10	0.14, 0.22, 0.28, 0.33, 0.43, 0.54, 0.57, 0.61, 0.77, 0.93.	38.32, 14.09, 6.24, 5.92, 13.11, 3.64, 1.70, 2.41, 12.40, 2.15.
AEAL	Toluene: ethyl acetate: methanol: glacial acetic acid (55: 35: 10: 2).	09	0.09, 0.23, 0.29, 0.35, 0.49, 0.57, 0.63, 0.77, 0.94.	33.56, 18.95, 5.71, 6.63, 15.55, 4.33, 2.60, 10.62, 2.05.

Table 3: HPTLC fingerprint of MEAL at 550 nm.

Extracts	Solvent system	Number of Peaks	R_f Value	Percentage Area
MEAL	Toluene: Ethyl acetate: methanol: Glacial acetic acid (55: 35: 10: 2).	20	0.06, 0.08, 0.10, 0.16, 0.18, 0.20, 0.26, 0.30, 0.33, 0.44, 0.46,0.48, 0.53, 0.61, 0.67, 0.71, 0.74, 0.89, 0.95, 0.98.	20.63, 2.38, 3.07, 5.24, 1.79, 2.00, 4.80, 3.43, 1.67, 14.47, 1.40, 2.38, 5.36, 9.20, 3.96, 2.05, 1.73, 13.60, 0.57, 0.18.
AEAL	Toluene: Ethyl acetate: methanol: Glacial acetic acid (55: 35: 10: 2).	17	0.12, 0.09, 0.09, 0.18, 0.20, 0.22, 0.29, 0.35, 0.43, 0.48, 0.53, 0.63, 0.70,0.73, 0.91, 0.93, 1.00.	0.02, 0.07, 26.25, 2.06, 1.52, 0.87, 5.40, 2.82, 11.50, 4.88, 6.05, 13.06, 1.17, 1.47, 20.55, 0.71, 0.99.

The developed HPTLC method was found to be more accurate and suitable for determining the number of components, it can also be used as an analytical tool in regular research to determine the loss or variation of phytoconstituents in crude extracts of medicinal plants. The HPTLC fingerprint profiles that were developed will be useful for Quality Control (QC) and standardization of herbal drugs by manufacturers, as well as in the identification of species from adulterants in systematic plant investigation. Based on HPTLC studies, MEAL and AEAL are composed of secondary metabolites with therapeutic value, which supports their use as antioxidants.

The antioxidant activity of phenol compounds are linked to their reduction-oxidation property in absorbing and neutralizing free radicals, singlet and triplet oxygen quenchers and degrading peroxides.[39] The DPPH assay was used widely to assess *in vitro* antioxidant activity of pure components and plant extracts.[40,41] Simultaneously, as the MEAL and AEAL

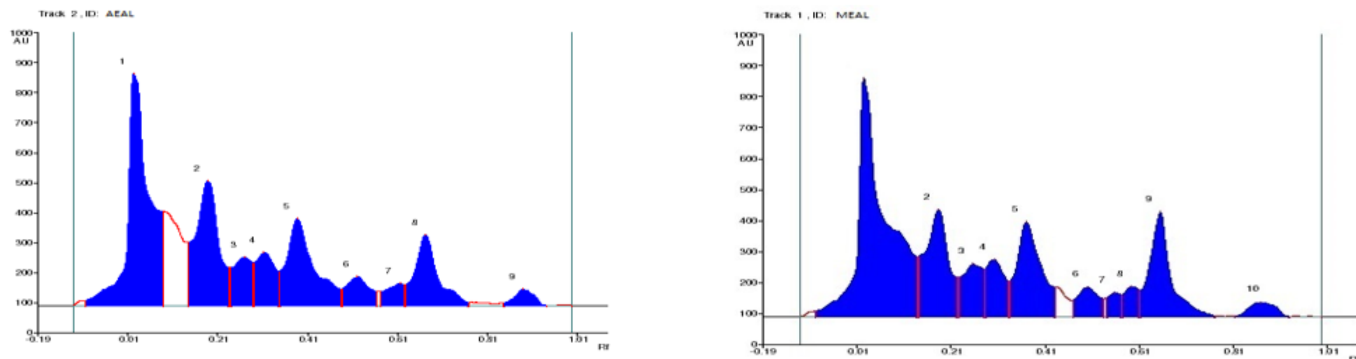

Figure 1: Comparative chromatogram of MEAL and AEAL at 375 nm.

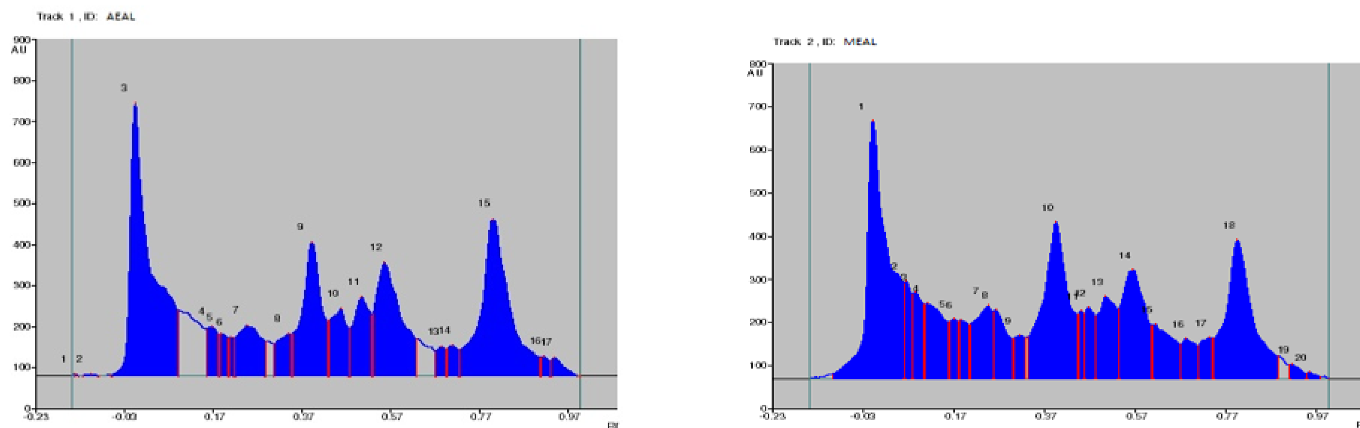

Figure 2: Comparative chromatogram of MEAL and AEAL at 550 nm.

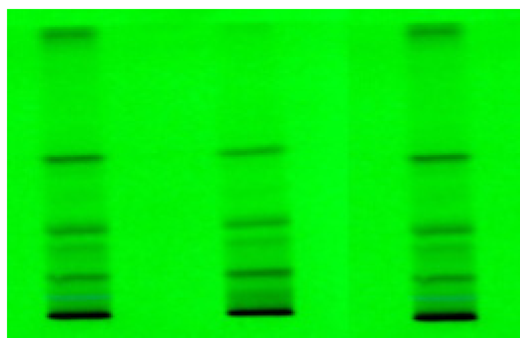

Figure 3: HPTLC fingerprint of MEAL at 254 nm, where T1, T2 and T3 are the concentration of 1 mg/mL solution in methanol are the concentrations of 1 mg/mL solution in methanol developed on a silica gel G60 F_{254} plate in triplicates that was eluted with Toluene: Ethyl acetate: Methanol: Glacial acetic acid (55: 35:10: 2) as the mobile phase.

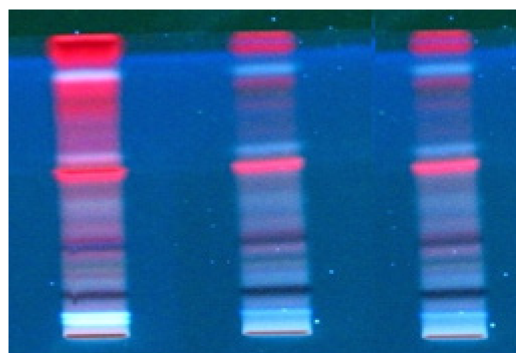

Figure 4: HPTLC MEAL fingerprint profile at 366 nm, where T1, T2 and T3 are the concentrations of 1 mg/mL solution in methanol developed on a silica gel G60 F_{254} plate in triplicates that were eluted with Toluene: Ethyl acetate: Methanol: Glacial acetic acid (55: 35: 10: 2) as the mobile phase.

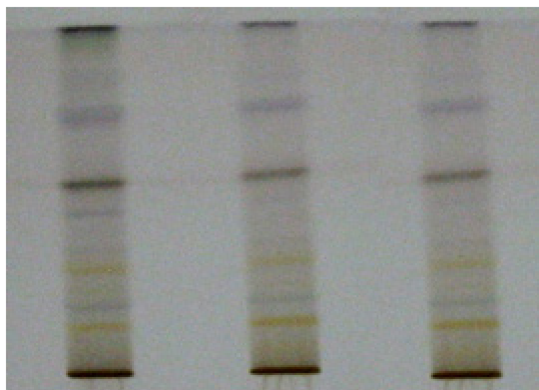

Figure 5: HPTLC MEAL fingerprint profile at visible region, where T1, T2 and T3 are the concentrations of 1 mg/mL solution in methanol developed on a silica gel G60 F_{254} plate in triplicates that were eluted with Toluene: Ethyl acetate: Methanol: Glacial acetic acid (55: 35: 10: 2) as the mobile phase.

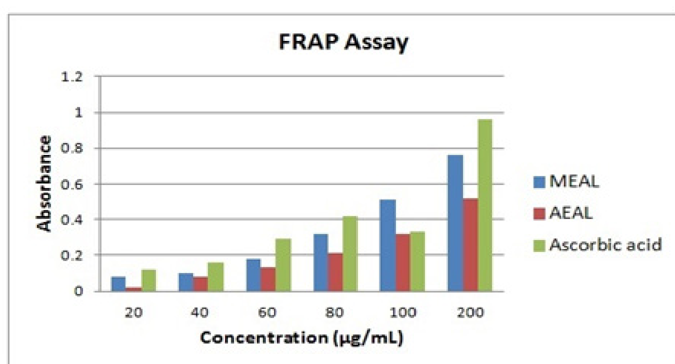

Figure 6: Ferric reducing antioxidant power (FRAP) assay.

Table 4: Total phenol content of MEAL and AEAL.

Extracts	Total phenol content (mg/kg)
MEAL	56.82 ± 0.21
AEAL	48.51 ± 0.25
Gallic acid	81.95 ± 0.35

Mean ± standard deviation, *n*=3. The measurements were conducted in triplicates.

concentrations increased from 100 to 500 µg / mL, the percentage of free radical scavenging (DPPH) effect has been increased.

The diphenyl picrylhydrazine, which is yellow in color, was produced when MEAL and AEAL reduced the stable free radical of DPPH. This shows that certain chemical compounds found in the MEAL and AEAL are capable of giving hydrogen to a free radical in order to eliminate the odd electron that provides the radical its ability to react. MEAL shows more scavenging effect as compared to that of AEAL. It is a highly reactive metal that catalyzes oxidative reactions in proteins, lipids and other cell components.[42] In the FRAP assay, the production of a ferrous ion ferrozine complex was used to evaluate MEAL's metal chelating capacity. Chelating compounds that form a σ metal bond have

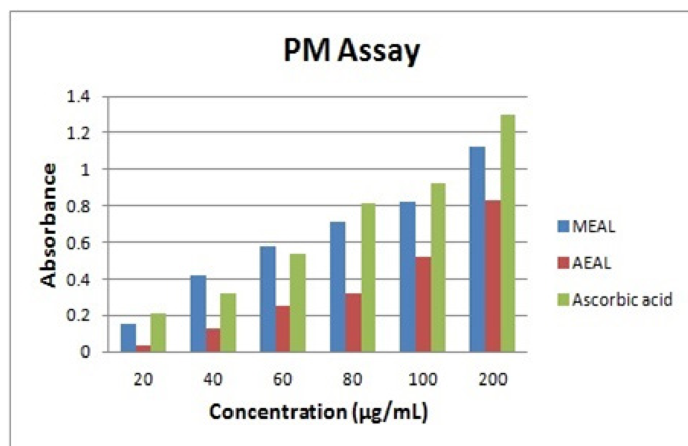

Figure 7: Phosphomolybdenum (PM) assay.

Table 5: Free radical scavenging activity (DPPH) of MEAL and AEAL.

Concentration (µg/mL)	Percentage of Inhibition		
	MEAL	AEAL	Ascorbic acid
100	55.12±0.13	32.75±0.13	60.42±0.18
200	64.12±0.29	46.12±0.18	68.57±0.11
300	70.24±0.19	60.05±0.22	76.03±0.13
400	75.16±0.12	71.69±0.15	83.15±0.14
500	79.86±0.15	75.40±0.17	90.18±0.19

Mean±standard deviation, *n*=3.

been found to be effective secondary antioxidants since they lower the redox potential by stabilizing the metal ion oxidized form.[43] As compared to ascorbic acid, the reference standard, the percentage inhibition of MEAL iron binding ability was found to be higher than that of AEAL (Figure 6). MEAL has significant reducing power because it has a variety of mechanisms, including ones that prevent chain initiation, degradation of peroxides, reducing capacity, and scavenging of free radicals. PM assay is a quantitative method for analyzing the antioxidant efficacy of plant extracts. In comparison to the ascorbic acid reference standard, the reduction capacity of MEAL was shown to be higher than that of AEAL (Figure 7).

CONCLUSION

HPTLC fingerprint was considered as one of the potential approaches for quality control of medicinal plants as well as the identification, to assess the bioactive principle and standardization. Though, more studies using marker compounds were required before quantitative estimation could be performed. The antioxidant potential of plants also indicates their therapeutic value. *In vitro*, tests show MEAL is a rich source of natural antioxidants that may help to protect against oxidative stress. It is

necessary to investigate the phytoconstituents that are the reason for antioxidant activity and to determine the exact mechanism of action for antioxidant activity.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

ACKNOWLEDGEMENT

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ABBREVIATIONS

HPTLC: High-performance thin layer chromatography; **MEAL:** Methanol extracts of *Aerva lanata*; **AEAL:** Aqueous extracts of *Aerva lanata*; **DPPH:** 1,1 diphenyl 2 picryl hydrazyl; **FRAP:** Ferric ion-reducing antioxidant power; **PM:** Phosphomolybdenum.

SUMMARY

Aerva lanata Linn. Juss, belonging to the Amaranthaceae family, is a plant of importance that contains phytoconstituents and has been used traditionally for oxidative stress. HPTLC analysis was carried out to find the number of phytoconstituents in the plant and *in vitro* antioxidant activity. The finding of this study gave a scientific base to the traditional claim of *Aerva lanata* in the management of oxidative stress and was also utilized for *in vivo* studies to verify its use in oxidative stress.

REFERENCES

1. Nanfack P, Biapa NPC, Pieme CA, Moor VA, Moukette B, Ngogang YJ. The *in vitro* antisickling and antioxidant effects of aqueous extracts *Zanthoxyllum heitzii* on sickle cell disorder. BMC Complement Altern Med. 2013;13(6):162.
2. Chiocchio I, Mandrone M, Tomasi P, Marincich L, Poli F. Plant secondary metabolites: an opportunity for circular economy. Molecules. 2021;26(2):495. doi: 10.3390/molecules26020495, PMID 33477709.
3. Lourenço SC, Moldão-Martins M, Alves VD. Antioxidants of natural plant origins: from sources to food industry applications. Molecules. 2019;24(22):4132. doi: 10.3390/molecules24224132, PMID 31731614.
4. Dziegielewska-Gesiak S. Metabolic syndrome in an aging society - role of oxidant-antioxidant imbalance and inflammation markers in disentangling atherosclerosis. Clin Interv Aging. 2021;16:1057-70. doi: 10.2147/CIA.S306982, PMID 34135578.
5. Jafri SAA, Khalid ZM, Khan MZ, Jogezai N. Evaluation of phytochemical and antioxidant potential of various extracts from traditionally used medicinal plants of Pakistan. Open Chem. 2022;20(1):1337-56. doi: 10.1515/chem-2022-0242.
6. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nat Rev Drug Discov. 2021;20(9):689-709. doi: 10.1038/s41573-021-00233-1, PMID 34194012.
7. Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P, D'Alessandro AG. Free radical properties, source and targets, antioxidant consumption and health. Oxygen. 2022;2(2):48-78. doi: 10.3390/oxygen2020006.
8. Jena AB, Samal RR, Bhol NK, Duttaroy AK. Cellular Red-Ox system in health and disease: the latest update. Biomed Pharmacother. 2023;162:114606. doi: 10.1016/j.biopha.2023.114606, PMID 36989716.
9. Rivas-Arancibia S, Guevara-Guzmán R, López-Vidal Y, Rodríguez-Martínez E, Zanardo-Gomes M, Angoa-Pérez M, *et al.* Oxidative stress caused by ozone exposure induces loss of brain repair in the hippocampus of adult rats. Toxicol Sci. 2010;113(1):187-97. doi: 10.1093/toxsci/kfp252, PMID 19833740.
10. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev. 2010;4(8):118-26. doi: 10.4103/0973-7847.70902, PMID 22228951.
11. Kıran TR, Otlu OK, Karabulut AB. Oxidative stress and antioxidants in health and disease. Lab Med. 2023;47(1):1-11. doi: 10.1515/labmed-2022-0108.
12. Perrone S, Manti S, Petrolini C, Dell'Orto VG, Boscarino G, Ceccotti C, *et al.* Oxygen for the newborn: friend or foe? Children (Basel). 2023;10(3):579. doi: 10.3390/children10030579, PMID 36980137.
13. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr J. 2016;15(1):71. doi: 10.1186/s12937-016-0186-5, PMID 27456681.
14. Sidhu JS, Zafar TA. Bioactive compounds in banana fruits and their health benefits. Food Qual Saf. 2018;2(4):183-8. doi: 10.1093/fqsafe/fyy019.
15. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, *et al.* Natural antioxidants in foods and medicinal plants: extraction, assessment and resources. Int J Mol Sci. 2017;18(1):96. doi: 10.3390/ijms18010096, PMID 28067795.
16. Balekundri A, Mannur V. Quality control of the traditional herbs and herbal products: a review. Futur J PharmSci. 2020;6(1):67. doi: 10.1186/s43094-020-00091-5.
17. Krishnamurthi A. The wealth of India. Council of Scientific and Industrial Research, new-Delhi. 2003;1:92.
18. Rajesh R, Chitra K, Paarakh PM. *In vitro* anthelmintic activity of aerial parts of *Aerva lanata* Linn. Juss. Int J Pharm Sci Drug Res. 2010;2(4):269-71.
19. Dinnimath BM, Jalalpure SS, Patil UK. Antiurolithiatic activity of natural constituents isolated from *Aerva lanata*. J Ayurveda Integr Med. 2017;8(4):226-32. doi: 10.1016/j.jaim.2016.11.006, PMID 29169771.
20. Sharif M, Anjum I, Shabbir A, Mushtaq MN. Immunomodulatory and anti-inflammatory effects of *Aerva lanata* in ovalbumin-induced allergic asthmatic mice. J Ethnopharmacol. 2022;289:115087. doi: 10.1016/j.jep.2022.115087, PMID 35143934.
21. Sridhar N, Kiran BV, Rudrapal M. Diuretic potential of *Aerva lanata* and *Ecbolium ligustrinum* root extracts. Asian J Pharm Res Health Care. 2014;6(2):12-4.
22. Shirwaikar A, Rajendran K, kumar D. C and Bodla R. Effect of *Aerva lanata* on cisplatin and gentamicin models of acute renal failure. J Ethnogr Pharmacol. 2004;90:81-6.
23. Behera PC, Ghosh M. Evaluation of Antioxidant, antimicrobial, and Antiurolithiatic Potential of Different Solvent Extracts of *Aerva lanata* Linn. Flowers. Pharmacogn Mag. 2018;14(53):53-7. doi: 10.4103/pm.pm_60_17, PMID 29576701.
24. Rajesh R, Chitra K, Paarakh PM. Antihyperglycemic and antihyperlipidemic activity of aerial parts of *Aerva lanata* Linn. Juss. in streptozotocin-induced diabetic rats. Asian Pac J Trop Biomed. 2012; 2(2):S924-9.
25. Nevin KG, Vijayammal PL. Effect of *Aerva lanata* on solid tumour induced by DLA cells in mice. Fitoterapia. 2003;74(6):578-82. doi: 10.1016/s0367-326x(03)00148-5, PMID 12946721.
26. Manokaran S, Jaswanth A, Sengottuvelu S, Duraisamy R, Karthikeyan D, Mallegaswari R. Hepatoprotective activity of *Aerva lanata* Linn. against paracetamol induced hepatotoxicity in rats. Res J Pharm Technol. 2008;1(4):398-400.
27. Rajesh R, Chitra K, Padmaa M, Paarakh. Anticancer activity of aerial parts of *Aerva lanata* Linn. Juss ex Schult against Dalton's Ascitic Lymphoma. Eur J Integr Med. 2011;3:245-50.
28. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. New Delhi: Council of Scientific and Industrial Research; 1956. p. 550.
29. Khandelwal KR. Techniques and experiments, practical pharmacognosy. 17th ed, Nirali Prakashan. Pune; 2007;149-56.
30. Kokate CK. Practical pharmacognosy, Vallabh Prakashan. New Delhi; 1994:105-7.
31. Harborne JB. Phytochemical methods. 3rd ed. London: Chapman and Hall; 1998:60.
32. Wagner H, Baldt S, Zgainski EM. Plant drug analysis. Berlin: Springer; 2001:85.
33. Molole GJ, Gure A, Abdissa N. Determination of total phenolic content and antioxidant activity of *Commiphora mollis* (Oliv.) Engl. resin. BMC Chem. 2022;16(1):48. doi: 10.1186/s13065-022-00841-x, PMID 35752844.
34. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, *et al.* Determination of Antioxidants by DPPH radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. Molecules. 2022;27(4):1326. doi: 10.3390/molecules27041326, PMID 35209118.
35. Sudan R, Bhagat M, Gupta S, Singh J, Koul A. Iron (FeII) chelation, ferric reducing antioxidant power, and immune-modulating potential of *Arisaema jacquemontii* (Himalayan Cobra Lily). BioMed Res Int. 2014; 2014:179865. doi: 10.1155/2014/179865, PMID 24895548.
36. Jan S, Khan MR, Rashid U, Bokhari J. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. Osong Public Health Res Perspect. 2013;4(5):246-54. doi: 10.1016/j.phrp.2013.09.003, PMID 24298440.
37. Afolayan AJ, Jimoh FO, Sofidiya MO, Koduru S, Lewu FB. Medicinal potential of the root of *Arctotis arctotoides*. Pharm Biol. 2007;45(6):486-93. doi: 10.1080/13880200701389383.

38. Attimarad M, Ahmed KK, Aldhubaib BE, Harsha S. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. Pharm Methods. 2011;2(2):71-5. doi: 10.4103/2229-4708.84436, PMID 23781433.
39. Munteanu IG, Apetrei C. Analytical methods used in determining antioxidant activity: a review. Int J Mol Sci. 2021;22(7):3380. doi: 10.3390/ijms22073380, PMID 33806141.
40. Bhuiyan MAR, Hoque MZ, Hossain SJ. Free radical scavenging activities of *Zizyphus mauritiana*. World J Agric Sci. 2009;5(3):318-22.
41. Koleva II, Van Beek TA, Linssen JPH. de G root A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal. 2022;13(1):8-17.
42. Collin F. Chemical basis of reactive oxygen species reactivity and involvement in neurodegenerative diseases. Int J Mol Sci. 2019;20(10):2407. doi: 10.3390/ijms20102407, PMID 31096608.
43. Gulcin İ, Alwasel SH. Metal ions, metal chelators and metal chelating assay as antioxidant method. Processes. 2022;10(1):132. doi: 10.3390/pr10010132.

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