ISSN: 2393-8048
International Advance Journal of Engineering, Science and Management (IAJESM)
Multidisciplinary, Multilingual, Indexed, Double Blind, Open Access, Peer-Reviewed, Refereed-International Journal.
SJIF Impact Factor =8.152, July-December 2025, Submitted in October 2025

Phytochemical Investigation, TLC And HPTLC Study of Atylosia Lineata

Ms. Pogula Swetha, Ph.D Research Scholar, Department of Pharmacy, Shri Jagdishprasad Jhabarmal Tiberewala University, Jhunjhunu, Rajasthan
Dr. Satish Pavuluri, Department of Pharmacy, Shri Jagdishprasad Jhabarmal Tiberewala University, Jhunjhunu, Rajasthan
Dr. P. Raja Sridhar Rao, Department of Pharmacy, Shri Jagdishprasad Jhabarmal Tiberewala University, Jhunjhunu, Rajasthan

Abstract

Atylosia lineata is belongs to the family Fabaceae, it is available in the Grasslands and forests of the Western and south India, the leaves were collected from Coimbatore local market, the collected plant materials were authenticated by Prof. Odhelu Department of Botany, Kakatiya University, sample spaceman was stored in Department library for further reference,10 kgs of leaves were collected, shade dried and made into fine powder form, the powder was subjected to extraction by different solvents, Petroleum ether, Methanol and Aqueous extract were mixed with activated charcoal and filtered it in order to remove the intense coloure caused by the pigments, the petroleum ether, Methenol extracts were dried by vaccum distillation, whereas Aqueous extract was added n – butanol it will form the Azeotropic mixture and make distillation process, then it was subjected to rota evaporator for complete removal of moisture, the both dried extracts were kept in desecrator for avoid absorption of moisture, now these extracts were subjected to primary phytochemical investigations, here we find the above extracts were showing positive results for Alkaloids, Saponins, Flavonoids and Phenolics and negative results for Glycosides, in further study these extracts were subjected to Thin layer chromatography, in petroleum ether extract yellow color spots at the top indicates phenolic and orange colour spots indicates the flavonoid compounds the R_f value was calculated as 0.13, 0.412, in Methanol extract yellow color spots at the top indicates flavonoids and orange colour tailing indicates the phenolic compounds the R_f value was calculated as 0.23, 0.34, in aqueous extract yellow color spots at the top indicates flavonoids and orange color spots with florescent tailing indicates the phenolic compounds, the R_f value was calculated as 0.32, 0.37. HPTLC finger print analysis of ethyl acetate fraction of methanolic extract gave 6 peaks, among 6 peaks 4 the peak rf value and 6 th peak rf value very close to the Gallic acid and Quercetin respectively. HPTLC finger print analysis of ethyl acetate fraction of methanolic extract gave 8 peaks, among 8 peaks 4, 6, 7^{th} peak rf values were compared with reference standard in 7 th rf value was very close to the Phenolic Compound Thymol,

Key Words: Atylosia lineata, Phytochemical investigation, TLC, HPTLC

Introduction

In india drugs of herbal origin have been used in traditional system of medicines, such as Ayurvada, Siddha, Unani and folk medicines are the major system of the indogenous medicines since ancient time. The Ayurveda system of medicine uses about 700 species, Unani 700, Siddha 600, amchi600, and modern medicine around 30 species. The Rigveda (5000BC) has recorded 67 medicinal plants, Yajurevada 81, species Atharvanaveda (4500-2500BC) 290 species. Charaka samhita (700BC) and Sushruta samhitha (200 BC) had described properties and uses of 1100 and 1270 species respectively, in compounding of drugs; these are still used in the classical formulations

Medical practitioners have prescribed drugs from herbal origin as a systemic medicine in India over the centuries. Many of the modern drugs are mainly based on synthetic chemical compounds, however have been found to have harmful side effects on the human system. This has triggered extensive research and development in the field of herbal medicine. In fact, there is growing demand for herbal medicines in the most of the developed and developing countries of the world today. Although, there are several medicinal plants mentioned in the Ayurveda, Siddha, Unani, Homeopathy, Naturopathy and folklore medicine are used as household remedies, other medicinal knowledge and practices all over the globe. The chemical

investigations, biochemical studies or pharmacological studies were not fully established. Such studies may yield useful results in our quest to discover new compounds,

Selection of plant:

Based on the survey of literature, the following plants was selected for investigation

Plant profile

Atylosia lineata, is a tree in the family Fabaceae. It can grow up to 2 m tall. It has aromatic leaves that are used for culinary and medicinal purposes. It is thought to have been one of the major sources of the medicinal plant leaves.

Collection of plant

Leaves were collected during November 2023 from Warangal. market –India and stored in sterile plastic bag and the identification of plant species was done at Kakatiya University,

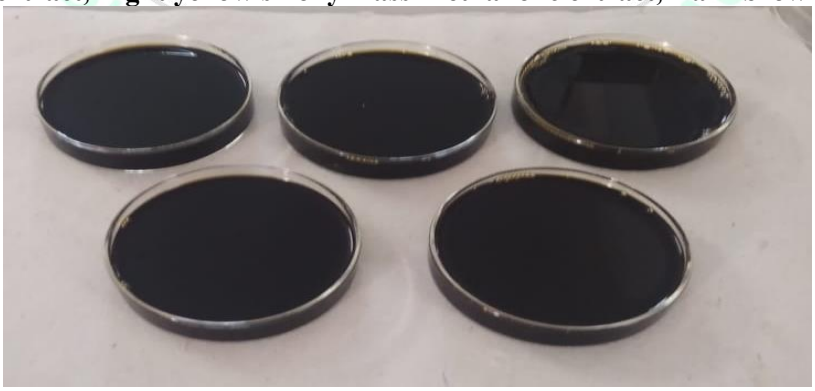
Extraction

Successive soxhlations solvent extraction of dried powder leaves of Atylosia lineata with Petroleum ether, Methanol (95%) and Aqueous, the percentage of yield was found to be very less in non-polar solvents Petroleum ether (3.2%), while Methanol, Aqueous extract showed relatively high yield (10% and 16.6%)

The three extracts based on polarity, petroleum ether, Methanolic and aqueous extracts by soxhlations (by percolation) were prepared separately. The percentage of yield, color, consistency and odor of all above extracts are recorded

Extracts	Color and consistency	Oduor	%w/w yield
Petroleum ether extract	Light yellowish oily mass	Pleasant	3.2
Methanolic extract	Dark brownish powder	Sweet, Pleasant	10
Aqueous extract	Dark browm sticky mass	Pleasant	16.6

Petroleum ether extract, Light yellowish oily mass Methanolic extract, Dark brownish sticky mass



Aqueous extract, Dark brownish dry mass

Methods & Results: Phytochemical investigation for various extracts

The three extracts were subjected to Primary Phytochemical test for active compounds

ISSN: 2393-8048
International Advance Journal of Engineering, Science and Management (IAJESM)
Multidisciplinary, Multilingual, Indexed, Double Blind, Open Access, Peer-Reviewed, Refereed-International Journal.
SJIF Impact Factor =8.152, July-December 2025, Submitted in October 2025

TEST	PE	ME	AE
Alkaloids a) Dragendorffs test b) Mayers test c) Wagners test d) Hagers test	+ - - +	+ - + +	+ - + +
Carbohydrates	+	+	+
Glycosides	-	-	-
Steroidal	+	-	+
Saponins	+	+	++
Flavonoids	+ +	+ ++	+ +
Phenol	+	+ +	+ +
Tannins	+	+	+

Keywords: '- 'absent. '+' presence, '++' more clarity, '+++' significant.
ME: Methanolic extract; PE: Petroleum ether; AE: Aqueous extract

Thin layer chromatography

TLC profile of Petroleum ether extract

TLC profile of the petroleum ether extract showed spots after acid spray and heated up to 70^0C , whereas single spot was seen, when observed under UV light at 254 nm, before acid spray using benzene as mobile phase. The color of the spots and Rf values are recorded in the following table.

Extract	Adsorbent	Solvent system	Observation / R_f values	
			Under u v light 254 nm	After acid spray and heated at 70°C
Petroleum ether extract	Silica Gel 60GF 254 Precoated plate	Benzene	1 spot: 0.13 (yellow color)	spots: 0.412 (brown), (orange),

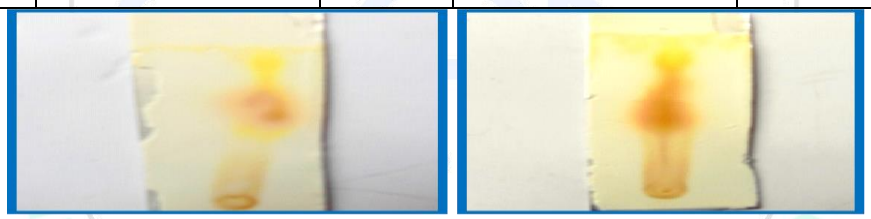


Plate I & II

 EXTRACT I (PEE) (Plate- I & II): was eluted in Benzene solvent the extract was shows presence of compounds the yellow color spots at the top indicates phenolic and orange color spots indicates the flavonoid compounds the R_f value was calculated as 0.13, 0.412

b) **TLC profile of Methanolic extract**

TLC profile of the Methanolic extract showed three spots after acid spray and heated up to 94^0C, whereas single spot was seen, when observed under UV light at 254 nm, before acid spray using Toluene: Chloroform (70:30) as mobile phase. The color of the spots and Rf values are recorded in the following table

Extract	Adsorbent	Solvent system	Observation / R_f values	
			Under u v light 254 nm	After acid spray and heated at 95°C
Methanolic extract	Silica Gel $60GF_{254}$ Precoated plate	Toluene: Chloroform (70:30)	spot 1: 0.27 (deep brown)	2 spots: 0.23, 0.34,

ISSN: 2393-8048
International Advance Journal of Engineering, Science and Management (IAJESM)
Multidisciplinary, Multilingual, Indexed, Double Blind, Open Access, Peer-Reviewed, Refereed-International Journal.
SJIF Impact Factor =8.152, July-December 2025, Submitted in October 2025

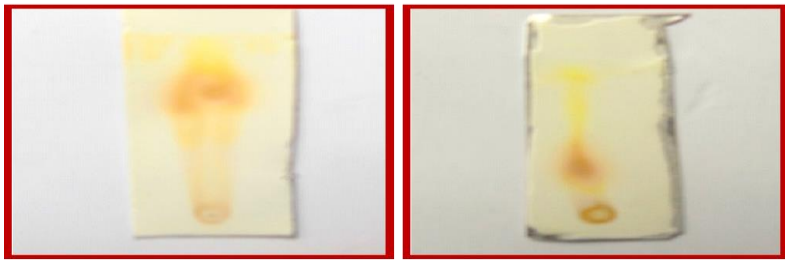


Plate III & IV

 EXTRACT II (ME) (Plate – III&IV): was eluted in 70% toluene in 30% chloroform that is (70:30) the extract was shows presence of compounds the yellow colour spots at the top indicates flavonoids and orange colour tailing indicates the phenolic compounds the R_f value was calculated as 0.23, 0.34

C) TLC profiles 0f aqueous extract

TLC profile of aqueous extract showed 2 spots under UV light at 254 nm and 2 florescent spots at 360 nm using Benzene: Toluene (3:6) as mobile phase. The R_f values are recorded in the following table.

Extract	Adsorbent	Solvent system	Observation / R_f values	
			Under u v light 254 nm	Under u v light 360 nm
Aqueous extract	Silica Gel 60GF 254 Precoated Plate	Benzene: Toluene (4:6)	2 spots: 0.19, 0.22	2 florescent spots: 0.32,0.37

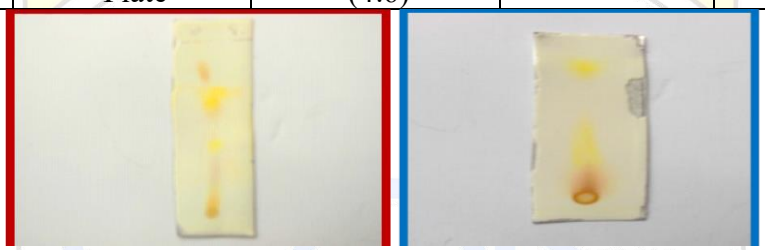


Plate V& VI

 EXTRACT III (AE)(Plate – V&VI): was eluted in 4% Benzene in 6% Toluene that is (40: 60) the extract was shows presence of compounds the yellow color spots at the top indicates flavonoids and orange color spots with florescent tailing indicates the phenolic compounds

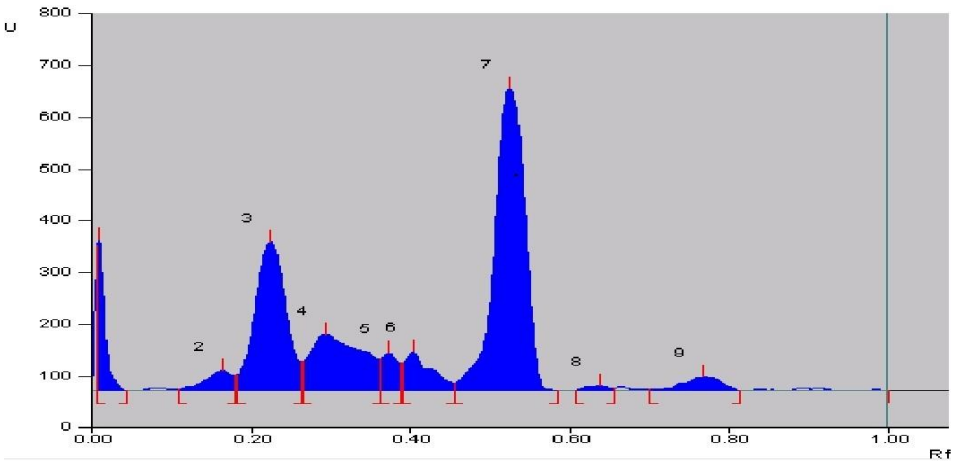
HPTLC Finger print analysis

1. The characteristic HPTLC finger print profile of the chemical constituents in the methanolic extract has been developed in solvent system Benzene: Toluene: Acetic acid (3:6:1). The developed plates were photographed under normal light, in UV chamber at 254 nm and at 365 nm

HPTLC Finger print profile of Methanolic extract in solvent system Benzene: Toluene: Glacial Acetic Acid (3:6:1). HPTLC Chromatogram: a, b and c.

a) Normal light b) at UV Light 254 nm c) at UV Light 365 nm

ISSN: 2393-8048
International Advance Journal of Engineering, Science and Management (IAJESM)
Multidisciplinary, Multilingual, Indexed, Double Blind, Open Access, Peer-Reviewed, Refereed-International Journal.
SJIF Impact Factor =8.152, July-December 2025, Submitted in October 2025

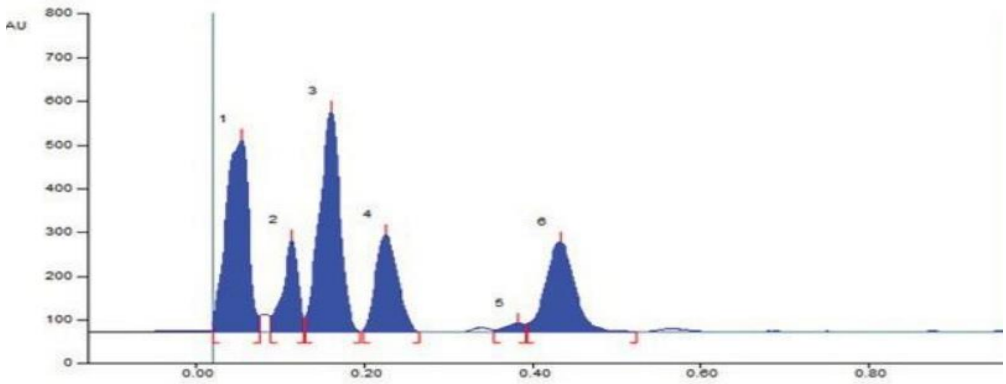


Peak	Start Rf	Max. Rf	Max. Height
1	0.01	0.01	292.4
2	0.11	0.16	39.4
3(Gallicacid)	**0.18**	**0.22**	**287.2**
4	0.26	0.29	109.1
5	0.36	0.37	73.1
6	0.39	0.4	74.9
7(Quercetin)	**0.46**	**0.52**	**584.5**
8	0.61	0.64	10.3
9	0.7	0.77	26.4

2. HTLC screening profiles of Ethyl acetate fraction of Methanolic extract for flavonoids

TLC profile of the Ethyl acetate fraction of methanolic extract for flavonoids showed 11 spots after acid spray and heated up to 1100C, whereas 16 spots were seen, when observed under UV light at 365 nm, before acid spray using n- Butanol: Acetic Acid: water (4:1:5) as mobile phase.

a) At Normal light **b) At UV Light 254 nm** **c) At UV Light 365 nm**

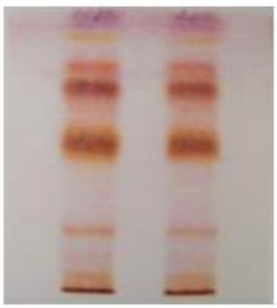


Peak	Rf value	heights	Area %
1(Unknown)	0.05		28.74
2 (Unknown)	0.11		9.06
3(Unknown)	0.16		29.59
4(Gallicacid)	**0.23**		**14.12**
5(Unknown)	0.38		1.13
6(Quercetin)	**0.46**		**17.37**

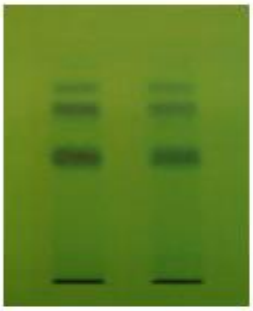
HPTLC finger print analysis of ethyl acetate fraction of methanolic extract gave 6 peaks, among 6 peaks 4 the peak Rf value and 6th peak Rf value very close to the Gallic acid and Quercetin respectively as per the above evidence we are concluded flavonoids are present in this fraction but need further analytical procedures to confirm the flavonoids

3. HTLC screening profile of ethyl acetate fraction of Methanolic extract for Phenolics

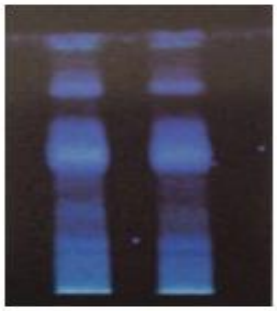
TLC profile of ethyl acetate fraction of methanolic extract for phenolics showed 18 spots after FeCl3 solution spray using Chloroform: Ethyl acetate: Formic Acid (5:4:1).



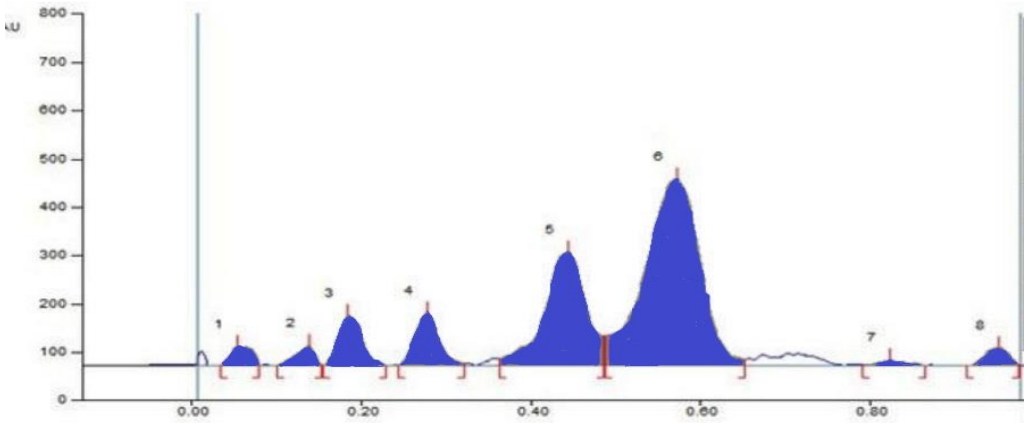
a) At Normal light



b) At UV Light 254 nm



c) At UV Light 365 nm



Peak	Rf value	Heights	Area %
1(Unknown)	0.05	110	2.32
2 (Unknown)	0.14	105	2.09
3(Unknown)	0.19	185	6.41
4(Gallicacid)	**0.28**	**186**	**6.84**
5(Unknown)	0.45	218	25.08
6(Quercetin)	**0.57**	**486**	**53.12**
7(Thymol)	**0.84**	**46**	**0.89**
8(Unknown)	**0.96**	**58**	**2.36**

HPTLC finger print analysis of ethyl acetate fraction of methanolic extract gave 8 peaks, among 8 peaks 4, 6, 7th peak rf values were compared with reference standard in 7 th rf value was very close to the Phenolic compound Thymol, as per the above evidence we are concluded Phenolic compounds are present in this fraction but need further analytical procedures to confirm the Phenols

Conclusion

- The primary Phytochemical investigation revealed that the Atylosia lineata having the different chemical compounds
- The thin layer chromatography indicates that methanolic extract had a greater number of active constituents
- HPTLC study indicates that Ethyl acetate fraction of Methanolic extract shown confirmation of flavonoids and Phenolic compounds
- Based on above evidences Atylosia lineata was identified as one of the impartments medicinal plants for the treatment of the diseases
- Hence required further study to know what are the flavonoids and Phenolic compounds and their Pharmacological effects

References

1. http://www.botany.hawaii.edu/faculty/carr/ar.htm.
2. http://www.encyclopedia.com/arum-family-luaraceae.
3. Bose TK, Kabir J, Moity TK, Parthasarathy VA, Soni MG, Vegetable crops vol.2: 1989.
4. Kirtikar KR, Basu BD, Indian Medicinal Plants. Vol. IV, II ed. Dehradun: International Book Distributors; 1987. 2609.
5. Kirian JC, Plants that Heal. Vol.2. Puna, India: Oriental Watchman Publishing House: August 2007.
6. The Ayurvedic Pharmacopoeia of India. Part I, Vol.III. I ed. Ministry of Health and family, The controller of Publishers, Civil Lines. p.205.
7. Shankar Goapl. Medicinal plants. New Delhi: Oxford and IBH publishing co.pvt. Ltd: 2000. p.53.
8. Pullaiah T. Medicinal Plants In India. Vol-1. New Delhi. Regency Publication: 2002. p. 49.
9. Parameshwar Pabba NR, Ramesh E, Arjun N. HYPOGLYCEMIC AND ANTI-LIPIDEMIC EFFECTS OF HYDRO-ETHANOLIC EXTRACT OF HELCTERES ISORA LINN. Inventi Impact: Ethnopharmacology. 2010 Oct 21.
10. Kapoor LD. Ayurvedic Medicinal Plants. I ed. India: Replika Press Pvt.Ltd; 2005. p. 25.
11. Guha Bakshi DN, Sensarma P, Pal DC. A Lexicon of Medicinal plants In India. I ed. India-Calcutta: Published by Naya Prakash; 1999. p. 127.
12. Yoganarasimhan SN, Flora of Goorg' – Karnataka: Reginol Research Center (Ayur) Jayanagar Bangalore. Vimasat Publishers; June 1990. p.489
13. Vayaskara NS, Moos, Ayurvedic Flora Medica, II ed. Kottayam S, India: Vaidyasarathy press (P) Ltd; 1978. p. 35 - 38.
14. Siraj Vk, Indira Balachandran, Ayurvedic Drugs and their plantsources: New Delhi, Bombay, Calcutta : Oxford and IBH Publishing Co. Pvt. Ltd : 1994. p. 457 - 459.
15. Narayan Das Prajapati, Purohit SS, Arun K Sharm, Tarun Kumar AH. Book of Medicinal Plant – A complete source book. Jodhpur: Agrobios (India); 2004. p. 41.
16. Anonymous, the Wealth of India – Raw Material, Vol-I, A. New Delhi. p.233 - 234.
17. Kakkar KK and Chakre OJ. Glossary of Indian medicinal plants with active principle. Part 1 Publication and information directorate; 1992. p.59.
18. Warrier PK, Ramankutty C, Indian Medicinal plants, New Delhi, 1995, 3, 132.