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Phytochemical Screening and Thin Layer Chromatography of Daemia *Extensa* (Jacq.) R. Br Stem

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***Abstract*: The present study deals with the phytochemical screening and thin layer chromatographic studies of Daemia extensa stem extract belonging to family Asclepiadaceae. 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, ethanol and methanol extracts. Phytochemical screening reflects presence of alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, flavanoids, quinones and terpenoids shows different types of results in different solvents extracts. Among these phytochemical screening, Alkaloids, Cardiac glycosides, phenol, Tannins, Phytosterol, Carbohydrates, proteins Flavanoids were present in all solvent extracts whereas Saponin, Terpenoids were absent in all extracts. A large number of solvent systems were tried to achieve a good resolution. Finally, the solvents hexane: ethyl acetate: Methanol (8:1:1) was used. Thin layer chromatographic studies of the hexane extract of *Daemia extensa* Solvent system I, four spots were observed having different Rf values. In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 2 spot detected. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 2 spot detected Rf value. In 0.21, 0.26. Solvent system IV Hexane: Ethyl acetate: Acetic acid (3:6:1), only 1 spots were visible Rf value0.15. In solvent system V Hexane: Ethyl acetate: Methanol (2:7:1), 1 spots was obtained. The TLC studies of Chloroform, Ethyl Acetate, Ethanol and Methanol also studied by using different solvent systems. The present investigation provides evidence that solvent extracts of Daemia extensa stem contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases, specially in cough syrup.**

***Keywords*: Daemia extensa, phytochemicals, TLC.**

1. Introduction

Daemia extensa is also known as Pergularia daemia. The plant belongs to family Asclepiadaceae. The plant having twining herb grows on other plants for support. It secretes milky juice after plucking of leaves. Leaves are ovate and heart shaped, with are about 10-12 cm long, cover of soft hair. It showed anti helminthic property. P. daemia is also useful as antipyretic and anti-inflammatory purpose (Sathish et al., 1998). It is effective to reduce sugar level in diabetes (Wahi et al., 2002). Latex of *D. extensa* useful in toothache. The leaves of *D. extensa* contain alkaloids, terpenoids, flavenoid, phenols and proteins (Nithyatharani and Kavitha, 2018). Ethanol extract of D. extensa reveals the presence of alkaloids, flavenoid, steroids, saponin and steroids, by GCMS analysis (Sridevi et al., 2014). The *Daemia extensa* showed activities against anthelmintic, asthma, uterine complaints, eye problems, ulcers, anti-diabetic and hepataoprotective. All these medicinal properties are due to presence of secondary metabolites in plants (Kirtikar, K. R. & Basu, B. D, 1999; Suresh kumar, S.V. & Mishra, S.H. 2006; Wahi et al., 2002).The present studies focus on Phytochemicals investigation and Thin Layer Chromatography of Daemia extensa stem extracts.

Scientific Classification of *Daemia extensa*.(Jacq) R. Br.

Table 1

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub-class	Asteridae
Order	Gentianales
Family	Asclepiadaceae
Genus	*Daemia/ Pergularia*
Species	*Extensa/ daemia* (Forsk) Chiv

2. Material and Methods

1) Collection of plant

Daemia extensa Plant stems were collected from the Aurangabad Region Maharashtra region India in the month of September and October 2020. The plant voucher specimens identification was done with the help of Prof. and Head Dr. Dhabe Department of Botany Dr. Babasaheb Ambedkar University, Aurangabad. The obtained voucher specimen

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Table 2
The percentage yield of different extracts of *Daemia extensastem*

S. no	Solvent	Colour of extracts	Yield of extracts in gram	Percentage yield
1	Hexane	Yellow	4.080	2.04%
2	Chloroform	Brown	1.720	0.86%
3	Ethyl Acetate	Dark brown	3.750	1.85%
4	Methanol	Light brown	5.020	2.51%
5	Ethanol	Dark brown	4.080	2.04%

Table 3
Phytochemical activity of Selected Plant *Daemia extensa* Extracts

S. no	Phytochemicals	Tests	Hexane	Chloroform	Ethyl Acetate	Ethanol	Methanol
1	Alkaloids	Mayers test	+++	+	++	++	+++
2	Cardiac Glycosides	Keller-kellani test	++	++	++	++	++
3	Saponins	Foam test	-	-	-	-	-
4	Phenol	Lead acetate test	+++	+++	+++	+++	+++
5	Tannin	$FeCl_3$ test	+	+	+	+	+
6	Phytosterol	LibermannBuchard test	+	+	+	+	+
7	Carbohydrates	Fehiling test	++	++	+	+	+
8	Protein	Biuret test	+	+	+	+	+
9	Flavonoids	Shinoda test	++	++	++	++	++
10	Quinones	NaOH test	+	+	+	+	+
11	Terpenoids	$CHCl_{3test}$	-	-	-	-	-

number was 641.

2) Preparation of plant extract

Stem were collected in bulk, washed, shade dried, macerated and extracted with hexane, chloroform, ethyl acetate, Ethanol 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.

3) 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 of Practical Pharmacognosy By (Kokate C.K.,1994).

3. Thin Layer Chromatography

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 scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC. The applied sample volume 1-micro litre by using capillary at distance of 1.5 cm at 2 tracks. In the twin trough chamber with different solvent system. A large number of solvent systems were tried to achieve a good resolution. Finally, the solvents hexane: ethyl acetate: Methanol (8:1:1) was used. Thin layer chromatographic studies of the hexane extract of *Daemia extensa* Solvent system I. Hexane: Acetic acid: Methanol (7:2:1), 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: Methanol (2:7:1). After pre-saturation with mobile phase for 10 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 (Rf), values were calculated for different samples.

1) Percentage of yield extract

The yield of sequential extracts (g) is shown in [Table 2]. The amount obtained from hexane, chloroform, ethyl acetate, Ethanol and methanol extracts are as given belows.

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

2) Phytochemical Screening

The present study carried out in the *Daemia extensa* revealed the presence of medicinal active constituents. The phytochemical active compounds of Daemia extensa were qualitatively analyzed for stem and the results are presented in Table 3. In these screening process alkaloids, glycosides, saponins, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, flavanoids, quinones and terpenoids shows different types of results in different solvents extracts. Among these phytochemical screening, Alkaloids, Cardiac glycosides, phenol, Tannins, Phytosterol, Carbohydrates, proteins, Flavanoids were present in all solvent extracts whereas Saponin, Terpenoids were absent in all extracts.

3) Thin layer chromatographic studies

A large number of solvent systems were tried to achieve a good resolution. Finally, the solvents hexane: ethyl acetate: Methanol (8:1:1) was used. Thin layer chromatographic studies of the hexane extract of *Daemia extensa* Solvent system I, four spots were observed having Rf values of 0.18, 0.32, 0.51, 0.42. Hexane: Acetic acid: Methanol (7:2:1), In solvent system II Hexane: Ethyl acetate: Acetic acid (5:4:1), 2 spot detected Rf value 0.12 and 0.31. In solvent system III Hexane: Ethyl acetate: Acetic acid (4:4:2), 2 spot detected Rf value. In 0.21, 0.26. Solvent system IV Hexane: Ethyl acetate: Acetic acid

Table 4
Rf values of TLC solvent systems for different extract of *Daemia extensa* stem.

S.no.	Extract Name	Solvent system I		Solvent system II		Solvent system III		Solvent system IV		Solvent system V	
		No. of spots	Rf Value	No. of spots	Rf Value	No. of spots	Rf Value	No. of spots	Rf Value	No. of spots	Rf Value
1	Hexane	4	0.18 0.32 0.51 0.42	2	0.12 0.31	2	0.21 0.26	1	0.15	1	0.23
2	Chloroform	3	0.41 0.40 0.44	2	0.22 0.19	1	0.18	1	0.12	1	0.24
3	Ethyl acetate	3	0.12 0.32 0.30	3	0.33 0.21 0.18	2	0.23 0.12	2	0.14 0.16	1	0.13
4	Ethanol	2	0.15 0.43	4	0.24 0.26 0.20 0.14	2	0.34 0.35	2	0.07 0.09	3	0.94 0.80 0.81
5	Methanol	1	0.23	1	0.21	1	0.22	2	0.09 0.08	2	0.09 0.07

(3:6:1), 1 spots were visible Rf value0.15. In solvent system V Hexane: Ethyl acetate: Methanol (2:7:1), 1 spots were obtained having Rf of 0.23. The TLC studies of Chloroform extract showed 3 spots in solvent system I, showing Rf values of 0.41, 0.40, 0.44. Solvent system II detected 2 spots of Rf values 0.22 and 0.19. Solvent system III, IV and V 0.18, 0.12 and 0.24 resp. Solvent system I of Ethyl Acetate extract showed 3 spots of Rf values 0.12, 0.32 and 0.30. Solvent system II detected 3 spots of Rf values 0.33, 0.21 and 0.18. Solvent systems III and IV th detected 2 spots of Rf values 0.23 0.12 and 0.14 and 0.16. Solvent system V th showed only 1 spot of Rf value 0.13.

Ethanol extract of *Daemia extensa* in Solvent system –I detected 2 spots with Rf values of 0.15 and 0.43. Solvent system showed four spots 0.24, 0.26, 0.20, 0.14. The IIIrd and IV the solvent system identified 2 spots with Rf values of 0.34, 0.35 and 0.07 and 0.09 resp. The solvent system showed 3 spots of 0.80, 0.81 and 0.94. The solvent system I, II and III rd detected only 1 spot with Rf values 0.23, 0.21 and 0.22 res in Methanol extract. Solvent system IV th and Vth identified 2 spots 0.09, 0.08, 0.09, 0.07 respectively.

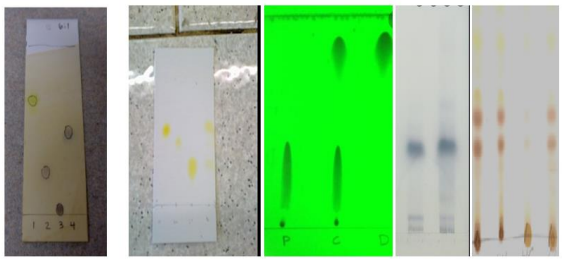

Fig. 1. TLC studies of the Chloroform extract of *Daemia extensa* Solvent system Hexane: Ethyl Acetate and Methano

4. Discussion

A large number of plants produce secondary metabolites such as alkaloids, flavonoids, 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 (Wagner H.et al., 1996: Bhawani et al., 2010)

In the present study, phytochemical screening for all five extracts showed significant indication about the presence of metabolites were found to be present in the all the sequential extracts of Daemia Alkaloids, Cardiac glycosides, phenol, Tannins are present. This 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 Rf values in different solvent system. This variation in Rf 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 Rf values of compounds in different solvent system. Different Rf 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.

5. Conclusion

The plant screening for phytochemical constituents seems 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. The stem of *Daemia extensa* 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 various microbes. Further purification, identification and characterization of the active compounds would be our priority in future studies.

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