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QUANTITATIVE STUDIES ON AHIPHENA BIJA CURNA (*PAPAVER SOMNIFERUM* LINN.) HPTLC TECHNIQUE AND PHYTOCHEMICAL STUDIES

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ABSTRACT

Objective: To study HPTLC and Phytochemical studies of Ahiphena bija curna (*Papaver somniferum* Linn.) **Results:** HPTLC Densitometric scan at **254**nm shows **5** spots with R_f **0.30** (has maximum area of 78.03%), at **366**nm densitometric scan shows **2** spots with R_f **0.44** (which had maximum area 92.05%), at **620**nm densitometric scan shows **9** spots with R_f **0.11** (which had maximum area 21.90%) were noted. Phytochemical studies shows presence of Alkaloids, Proteins, Amino acids, Flavonoids, Xanthoproteins, Saponins and Glycosides with P^H of **5**.

KEYWORD: Ahiphena bija curnam, *Papaver somniferum*, HPTLC, Phytochemical.

I. INTRODUCTION

High performance thin layer chromatography

Aim: To study the Quantitative determination and Phytochemical characteristics of the sample Ahiphena bija curnam.

Particulars of sample: Ahiphena bija curnam for the given sample, (*Papaver somniferum* Linn.), HPTLC was performed, Photodocumentation, R_f values and densitogram's were documented. S.D.M Centre for Research in Ayurveda and allied sciences, UDIPI (Karnataka).

II. METHODOLOGY

HPTLC Analysis of *Papaver somniferum* Linn. sample.

Sample details: 1gm of Ahiphena bija curnam.

Test solution: 1gm of Ahiphena bija curnam (*Papaver somniferum* Linn.) was suspended in 20 ml Ethanol macerated at room temperature with intermittent soaking. After 24hrs it was filtered through filter paper and extract was used for it.

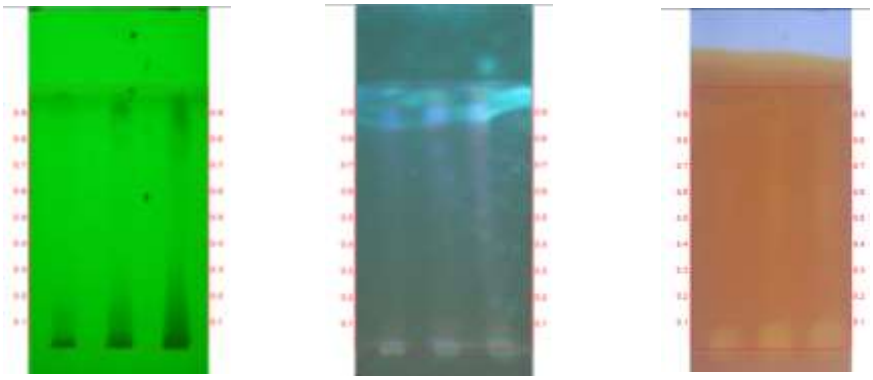
Stationary phase: 3, 6, 9µl of each of the above extract was applied on a Pre-coated Silica gel F254 on Aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator.

Mobile phase: The plate was developed in Toluene: Ethyl acetate: Formic acid: Methanol (5.5: 3.0: 1.0: 0.5).

Development: The developed plates were visualized in short UV, long UV and then derivatised with Dragendroff's reagent and scanned under UV 254nm, 366nm and 620nm (Post derivatisation).

HPTLC Instrumentation: Densitometric scan.

Derivatization: Derivatised with Dragendroff's reagent.



Short UV **Long UV** **Post derivatization**

Image No. 1: HPTLC photo documentation of ethanol extract of Ahiphena bija (*Papaver somniferum*)

Track 1 - Ethanol extract of Ahiphena bija (*Papaver somniferum*)– 3µl

Track 2 - Ethanol extract of Ahiphena bija (*Papaver somniferum*)– 6µl

Track 3 - Ethanol extract of Ahiphena bija (*Papaver somniferum*)– 9µl

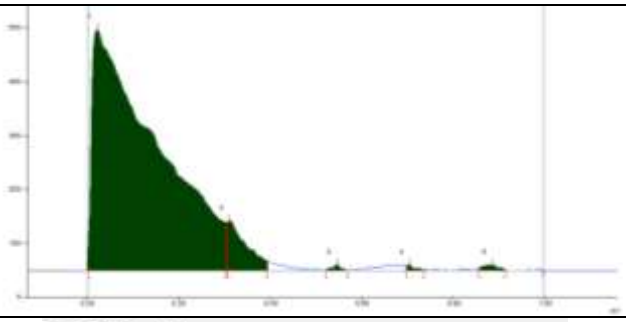
Solvent system – Toluene: Ethyl acetate: Formic acid: Methanol (5.5: 3.0: 1.0: 0.5)

Table 1: R_f values of sample of Ethanol extract of Ahiphena bija (*Papaver somniferum*).

Short UV	Long UV	Post derivatization
0.90 (Green)	0.90 (F. blue)	0.90 (Orange red)

*F – Fluorescent; L –Light; D – Dark

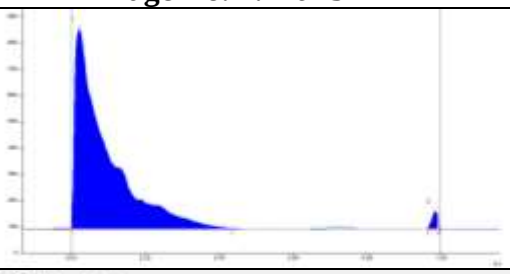
Densitometric scan of Ethanol extract of Ahiphena bija (*Papaver somniferum*)



Track 3, ID: Ahiphena beeja

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	9.1 AU	0.02 Rf	447.0 AU	78.83 %	0.30 Rf	88.9 AU	45192.4 AU	93.85 %
2	0.31 Rf	89.3 AU	0.31 Rf	92.4 AU	16.13 %	0.40 Rf	17.9 AU	2843.7 AU	5.85 %
3	0.52 Rf	0.7 AU	0.55 Rf	11.6 AU	2.03 %	0.57 Rf	0.0 AU	145.0 AU	0.30 %
4	0.70 Rf	0.1 AU	0.71 Rf	11.2 AU	1.96 %	0.74 Rf	0.8 AU	135.4 AU	0.28 %
5	0.86 Rf	4.5 AU	0.89 Rf	10.6 AU	1.85 %	0.92 Rf	0.2 AU	253.1 AU	0.52 %

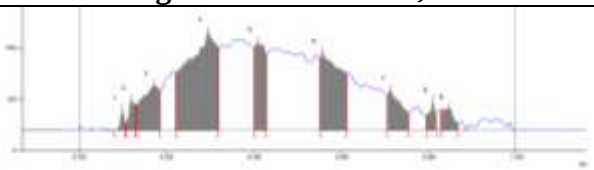
Image No. 2: At 254nm



Track 3, ID: Ahiphena beeja

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	23.5 AU	0.02 Rf	759.6 AU	92.85 %	0.44 Rf	1.8 AU	46910.7 AU	98.62 %
2	0.97 Rf	0.9 AU	0.99 Rf	65.6 AU	7.95 %	0.99 Rf	57.2 AU	655.2 AU	1.38 %

Image No. 3: At 366nm, flu



Track 3, ID: Ahiphena beeja

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.08 Rf	0.2 AU	0.10 Rf	19.5 AU	4.45 %	0.11 Rf	7.6 AU	129.3 AU	1.06 %
2	0.11 Rf	7.8 AU	0.12 Rf	29.9 AU	6.81 %	0.13 Rf	25.1 AU	307.4 AU	2.53 %
3	0.13 Rf	24.5 AU	0.17 Rf	43.7 AU	9.97 %	0.19 Rf	39.3 AU	1264.9 AU	10.40 %
4	0.22 Rf	56.6 AU	0.30 Rf	96.1 AU	21.90 %	0.32 Rf	81.0 AU	4700.8 AU	38.64 %
5	0.40 Rf	80.5 AU	0.41 Rf	86.1 AU	19.62 %	0.43 Rf	74.1 AU	1546.0 AU	12.71 %
6	0.55 Rf	69.7 AU	0.56 Rf	75.3 AU	17.16 %	0.61 Rf	55.5 AU	2577.9 AU	21.19 %
7	0.71 Rf	34.3 AU	0.71 Rf	39.3 AU	8.96 %	0.76 Rf	17.5 AU	903.6 AU	7.43 %
8	0.80 Rf	14.8 AU	0.81 Rf	27.6 AU	6.30 %	0.82 Rf	17.1 AU	310.5 AU	2.55 %
9	0.83 Rf	19.8 AU	0.85 Rf	21.2 AU	4.83 %	0.87 Rf	8.0 AU	425.4 AU	3.50 %

Image No. 4: At 620nm (Post derivatisation with Dragendroff's reagent)

III. Interpretations of ahiphena bija (*Papaver somniferum* linn.) curna hptlc results

- At short UV **254**nm **1** band are observed at R_f values of 0.90 with **Green** colour intensity.
- At long UV **366**nm **1** band are observed at R_f values of 0.90 with **Blue** colour intensities of Fluorescent Blue.
- After derivatisation with Dragendroffs reagent, at UV **620** nm, there are **1** band spotted with R_f values of 0.90 with **Orange red** colour intensity.
- Densitometric scan at **254**nm shows **5** spots, out of which maximum area of 78.03% is seen with the R_f value of 0.00, Next to that with the maximum area of 16.13% is observed with the R_f value 0.31
- Densitometric scan at **366**nm shows **2** spots, out of which maximum area of 92.05% is seen with the R_f value of 0.00, Next to that with the maximum area of 7.95% is observed with the R_f value 0.97.
- Densitometric scan at **620**nm shows **9** spots, out of which maximum area of 4.45% is seen with the R_f value of 0.08, Next to that with the maximum area of 6.81% is observed with the R_f value 0.11.

Table 2: R_f Value and % Area of Ahiphena bija Sample at 254nm.

Peak No	R_f Value	Area (AU)	% Area (AU)
1	0.30	45192.4	93.05
2	0.40	2843.7	5.85
3	0.57	145.0	0.30
4	0.74	135.4	0.28
5	0.92	253.1	0.52

Table 3: R_f Value and % Area of Ahiphena bija Sample at 366nm.

Peak No	R_f Value	Area (AU)	% Area (AU)
1	0.44	46910.7	98.62
2	0.99	655.2	1.38

Table 4: R_f Value and % Area of Ahiphena bija Sample at 620nm.

Peak No	R_f Value	Area (AU)	% Area (AU)
1	0.11	129.3	1.06
2	0.13	307.4	2.53
3	0.19	1264.9	10.40
4	0.32	4700.8	38.64
5	0.43	1546.0	12.71
6	0.61	2577.9	21.19
7	0.76	903.6	7.43
8	0.82	310.5	2.55
9	0.87	425.4	3.50

Phytochemical study

The present trial drug was subjected to preliminary phytochemical screening for the detection of various plant constituents present. The Phytoconstituents are responsible for the desired Therapeutic properties. General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them.

Tests for alkaloids

- **Mayer's test:** To 1 ml of the extract, ml of Mayer's reagent was added, the formation of full white precipitate confirmed the presence of Alkaloids.

Test for carbohydrates

- **Benedict's test:** To 5ml of Benedict's reagent, 1ml of extract solution was added and boiled for 2 minutes and cooled. Formation of a red precipitate showed the presence of carbohydrates.

Test for tannins

- To 1 ml of the extract, ferric chloride was added, formation of a dark blue or greenish black colour product showed the presence of tannin.

Test for proteins

- **Biuret test:** To 1 ml of the extract, 1ml of 40% sodium hydroxide solution was added followed by 2 drops of 1% copper sulphate solution. Formation of a violet colour showed the presence of Proteins.

Test for amino-acids

- **Ninhydrin test:** Heat 3ml test solution and 3 drops 5% Ninhydrin solution in boiling water bath for 10 minutes, Purple or Bluish colour appears.

Test for xanthoprotein test

- **Xanthoprotein test:** To 1 ml of the extract, 1ml of concentrated nitric acid was added. A white precipitate is formed, it is boiled and cooled. 20% of sodium hydroxide or ammonia is subsequently added; orange colour indicated the presence of aromatic Amino acids.

Tests for steroids

- **Salkowski test:** Dissolve the extract in chloroform and equal volume of concentrate sulphuric acid. Shake well. Chloroform layer appears red and acid layer appears greenish

yellow represented the Steroid components in the tested extract.

Test for saponins

- **Foam test:** About 1ml of methanol extract was diluted separately with distilled water to 20 ml, and shaken in a graduated cylinder for 15 minutes. A 1% 1cm layer of foam indicated the presence of Saponins.

Test for glycosides

- **Keller killiani test:** The extract was dissolved in acetic acid containing traces of ferric chloride and it was then transferred to a test tube containing Sulphuric acid. At the junction, formation of a reddish or reddish brown colour, which gradually became blue. Conformed the presence of Glycosides.

Test for starch

- **Iodine test**

Mix 3ml of test solution and few drops of dilute iodine solution. Blue colour appears; it disappears on boiling and reappears on cooling. 2. Tannic acid test for Starch: with 20% tannic acid, test solution gives ppt.

Table No. 5: Results of phytochemical analysis of Ahiphena bija curna.

S. No.	Phytochemical	Test Name	Ahiphena bija curna + Present, - Absent
1.	Alkaloids	Mayer's Test	+
2.	Carbohydrates	Benedict’s Test	-
3.	Reducing sugars	Benedict’s Test	-
4.	Proteins	Biuret Test	+
5.	Proteins	Lead acetate test	+
6.	Amino acids	Ninhydrin test	+
7.	Xanthoproteins	Xanthoprotein test	+
8.	Steroids	Salkowski reaction	+
9.	Glycosides	Keller Killiani test	+
10.	Saponins	Foam test	+
11.	Flavonoids	Flavonoid test	+
12.	Tannins	Ferric chloride test	-
13.	Triterpenoids	Salkowski reaction	-
14.	Starch	Iodine test	-
15.	P^H	Blue litmus test	5

RESULTS AND OBSERVATIONS

1. **Ahiphena bija curna extract** showed **presence** of **Alkaloids, Proteins, Amino acids, Flavonoids, Xanthoproteins, Saponins, Steroids,** and **Glycosides.**

Image No. 5: Filtration of Soaked Ahiphena bija curna through Filter paper.

Image No. 6: p^H-5.

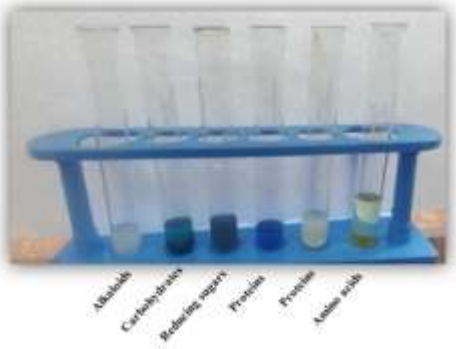


Image No. 7: Phytochemical study of Ahiphena bija curna.

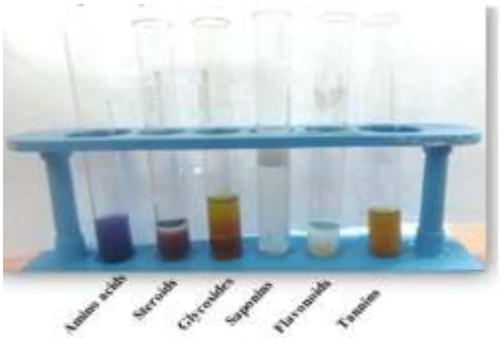


Image No. 8: Phytochemical study of Ahiphena bija curna paper.

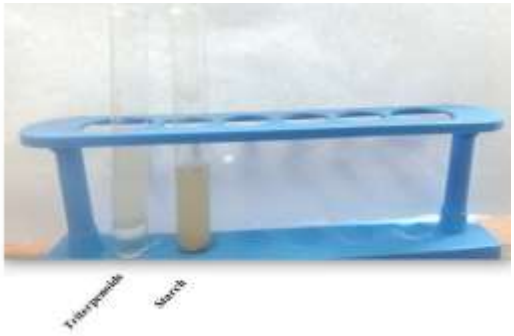


Image No. 9: Phytochemical study of Ahiphena bija curna.

IV. DISCUSSION

At short UV **254**nm **1** band was observed at R_f values of 0.90 with **Green** colour intensity. At long UV **366**nm, **1** band was observed at R_f values of 0.90 with **Blue** colour intensities of Fluorescent Blue. After derivatisation with Dragendroffs reagent, at UV **620**nm, there was **1** band spotted with R_f values of 0.90 with **Orange red** colour intensity. Densitometric scan at **254**nm shows **5** spots, out of which maximum area of 78.03% is seen with the R_f value of 0.00, Densitometric scan at **366**nm shows **2** spots, out of which maximum area of 92.05% is seen with the R_f value of 0.00, Densitometric scan at **620**nm shows **9** spots, out of which maximum area of 4.45% is seen with the R_f value of 0.08. **Ahiphena bija curna extract** showed **presence** of **Alkaloids, Proteins, Amino acids, Flavonoids, Xanthoproteins, Saponins, Steroids,** and **Glycosides.** Presence of these Phytochemical the drug **Ahiphena bija curna extract** acts as Anti-inflammatory, Analgesic, Antifungal, Antimicrobial and Antioxidant property.

V. CONCLUSION

The HPTLC showed **1** band at **254**nm, **1** band at **366**nm and **1** band at **620**nm bands on densitometer derivatisation. There were **5** peaks at **254**nm and **2** peaks at **366**nm in the sample and **9** peaks at **620**nm. The HPTLC studies characterization and Phytochemical studies are helpful in standadization and identification of chemical constituents present in the drug.

VI. ACKNOWLEDGEMENT

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VII. REFERENCES

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