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PHYTOCHEMICAL ANALYSIS AND HPTLC FINGERPRINTING PROFILING OF GNAPHALIUM POLYCEPHALUM MOTHER TINCTURE

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Abstract : Gnaphalium polycephalum is a homeopathic medicinal plant widely used in the management of neuralgic conditions like sciatica. However, there is little scientific information about its phytochemical composition and chromatographic profile. The present study was conducted to investigate the phytochemical constituents and HPTLC fingerprint profile of Gnaphalium polycephalum. Phytochemical screening was first performed on the mother tincture by standard qualitative methods. The chromatographic fingerprint was established by high-performance liquid chromatography (HPLC) analysis. Phytochemical analysis revealed the presence of flavonoids, tannins, and volatile oils. The HPTLC chromatogram showed several peaks at different retention times, indicating the presence of different chemical constituents, providing a characteristic fingerprint profile. The data obtained from this study are useful for the identification, quality control, and standardization of Gnaphalium polycephalum, which can be used as a homeopathic remedy.

IndexTerms **- Gnaphalium polycephalum, phytochemical, HPTLC, sciatica**

INTRODUCTION

The genus Gnaphalium is a variable annual or perennial herb of world-wide distribution. It contains about 200 species of the family Compositae (Asteraceae) of the tribe Gnaphalieae. Plants of the genus Gnaphalium are used traditionally in some Latin American countries for the treatment of stomach diseases, swelling, wounds, prostatism, lumbago, neuritis and angina ache, for the lowering of blood pressure or as diuretic, antipyretic and antimalarial [(1)]. Several previous investigations have studied the phytochemicals present in Gnaphalium species. So far, 257 compounds have been identifying and characterized. The literature review shows the presence of several components mainly in flavonoids, phenolic acids, alkaloids and terpenoids. Some of the components are directly or indirectly related to the pharmacological activities of plants of the genus Gnaphalium [(2)].

Pseudognaphalium obtusifolium (L.) (=Gnaphalium obtusifolium L.) is an important plant used in the practice of traditional medicine among many Native American groups in eastern North America. The plant has long been known by the binomial Gnaphalium obtusifolium L., but the genus has been changed and is currently given as Pseudognaphalium. Pseudognaphalium obtusifolium is a flowering plant belonging, in traditional botanical classification, to the Asteraceae family [(3)]. Michaux's Gnaphalium polycephalum was a replacement name for Gnaphalium obtusifolium — a concept to exclude the aberrant plant of Dillenius, i.e., "G. obtusifolium[(4)].

Gnaphalium polycephalum is remedy of unquestionable benefit for sciatica pain which is associated by numbness of affected part. Sciatica and lumbago with numbness alternating with pain. Intense pain along sciatic nerve [(5)].
There is no unified national quality standard for genus Gnaphalium, and most of them are based on local standards. Moreover, only TLC or HPLC methods were used in some local standards, which might not reflect the comprehensive quality of Gnaphalium. it is necessary to further strengthen the basic research on quality control, such as HPLC fingerprint, multiple component determination [(2)].

In today's analytical environment, HPTLC is a crucial technique that works in tandem with HLPC rather than in opposition to it. Because of its simplicity, adaptability, and dependability, the fingerprint analysis methodology employing HPTLC has emerged as the most effective method for herbal medicine quality control. It can be used as a tool for herbal medication identification, authentication, and quality assurance. The creation of chromatographic fingerprints is crucial for complex herbal medicines' quality control. One of the instruments for the quality assessment is phytochemical evaluation, which includes marker compound analysis using contemporary analytical techniques, chemo profiling, and preliminary phytochemical screening [(6)].

Therefore, the present study was undertaken to evaluate the phytochemical constituents and to develop an HPTLC fingerprint profile of plant Gnaphalium polycephalum.

MATERIALS AND METHODS:

STUDY DESIGN AND SETTING:
This study was designed as an experimental, laboratory-based investigation to evaluate the phytochemical constituents and High-Performance Thin Layer Chromatography (HPLC) fingerprint profile of Gnaphalium polycephalum mother tincture. The study was carried out in a standard analytical laboratory equipped with facilities for phytochemical screening and chromatographic analysis. All procedures were conducted according to established laboratory protocols.

SAMPLE AND REAGENTS:
The homoeopathic mother tincture of Gnaphalium polycephalum was procured from a standard pharmaceutical source. All reagents and solvents used for phytochemical screening were of analytical grade, while solvents used for HPTLC analysis were of HPTLC grade.
The reagents used included methanol, distilled water, hydrochloric acid, sulphuric acid, ferric chloride, Mayer’s reagent, Dragendorff’s reagent, Wagner’s reagent, sodium hydroxide, chloroform, acetic anhydride, and Sudan III reagent.

SAMPLE PREPARATION:
The mother tincture was used directly for preliminary phytochemical screening. For HPLC analysis, the sample was filtered through a 0.45 µm membrane filter to remove particulate matter. The filtered sample was suitably diluted with HPLC-grade methanol and transferred into HPLC vials for analysis.

PHYTOCHEMICAL ANALYSIS:
Preliminary phytochemical analysis was carried out using standard qualitative chemical tests to detect the presence of various bioactive constituents.
The following tests were performed:

- Sterols- Salkowski’s test, Libermann-Burchard;s test
- Carbohydrates- Molisch’s, Fehling’s, Benedict’s, Ruthenium red, Iodine tests
- Proteins- Biuret, Nihydrin, xanthoproteic, Millon’s, picric acid test and Tannic acid tests
- Alkaloids – Mayer’s, Dragendorff’s, and Wagner’s tests
- Glycosides – Borntrager’s, Modified Borntrager’s, Keller–Killiani, Legal and Baljet tests
- Saponins – Foam test and hemolysis test
- Tannins – Lead acetate test and ferric chloride test

- Flavonoids – Alkaline reagent test, acid test, ammonia test, Shinoda test, and vanillin-HCl test
- Terpenoids – Liebermann–Burchard test
- Volatile oils – Sudan III test

The presence or absence of phytoconstituents was determined based on characteristic color changes or precipitate formation.

HPTLC FINGERPRITNING:
Instrumentation and Chromatographic Conditions:

High Performance Thin Layer Chromatography (HPTLC) analysis was performed using CAMAG HPTLC system supplied by Anchrom Enterprises, Mumbai, India. The system consisted of a Linomat 5 sample applicator, twin trough glass chamber, TLC Scanner, and WinCATS software for data acquisition and processing.

Materials:

- Pre-coated silica gel 60 F254 HPTLC plates (10 × 10 cm or 20 × 10 cm)
- Mobile phase: Toluene : Ethyl acetate
- Sample solution
- Standard reference compound
- Methanol or suitable solvent for sample preparation

PROCEDURE:
High-Performance Thin Layer Chromatography (HPTLC) analysis was carried out using pre-coated silica gel 60 F254 plates as the stationary phase. The plates were pre-washed with methanol and activated at 110°C for 5–10 minutes prior to use. The mobile phase consisting of toluene, ethyl acetate, and formic acid in the ratio of 6:4:0.3 was prepared and used for chromatographic development. A CAMAG twin trough chamber was saturated with the mobile phase for 20–30 minutes using a filter paper liner. The sample was applied as bands on the plate using a CAMAG Linomat 5 applicator under a stream of nitrogen gas, maintaining standard application parameters. The plate was developed by ascending technique until the solvent front migrated approximately 70–80 mm. After development, the plate was air-dried and observed under UV light at 254 nm and 366 nm. Densitometric scanning was performed using a CAMAG TLC Scanner controlled by WinCATS software, and chromatographic data including Rf values, peak area, and peak height were recorded. The chromatograms were documented and used to establish the fingerprint profile of the sample.

RESULTS:

PHYTOCHEMICAL ANALYSIS:
The results of preliminary phytochemical analysis are presented in **Table 1**

Table 1: Phytochemical Analysis of Gnaphalium Polycephalum Mother Tincture [7]

F.NO	TEST	RESULTS
1.	**TEST FOR STEROLS**	
	a. Salkowski's test	-
	b. Libermann- Burchard's test	-
2.	**TEST FOR CARBOHYDRATES**	
	a. Molisch's test	-
	b. Fehling's test	-
	c. Benedict's test	-
	d. Mucilage test (Ruthenium red test)	-
	e. Iodine test	-

3.	**TEST FOR PROTEINS**	
	a. Biuret test	-
	b. Ninhydrin test	-
	c. Xanthoproteic test	-
	d. Millon's test	-
	e. Picric acid test	-
	f. Tannic acid test	-
4.	**TEST FOR ALKALOIDS**	
	a. Mayer's test	-
	b. Dragendroff's test	-
	c. Hager's test	-
	d. Wagner's test	-
5.	**TEST FOR GLYCOSIDES**	
	a. Anthraquinone glycosides	
	i) Borntrager's test	-
	ii) Modified Borntrager's test	-
	b. Cardiac glycosides	
	i) Keller Killiani test	-
	ii) Legal Test	-
	iii) Baljet test	-
	c. Cyanogenetic glycoside	NA
	d. Coumarins glycoside	NA
6.	**TEST FOR SAPONINS**	
	a. Foam or Froth test	-
	b. Hemolysis test	NA
7.	**TEST FOR TANNINS**	
	a. Lead acetate test	+
	b. Ferric chloride test (FeCl3)	+
8.	**TEST FOR FLAVONOIDS**	
	a. Alkali test	+
	b. Acid test	+
	c. Alkali in Acid test	+
	d. Ammonia test	+
	e. Vanillin-HCl test	+
	f. Shinoda test	+
9.	**TEST FOR TERPENOIDS**	
	Liebermann-Burchard test	-
10.	**TEST FOR VOLATILE OILS**	
	Sudan III test	+

(+) Presence; (-) Absence; NA – Not Applicable

The analysis revealed the presence of tannins,flavonoids, and volatile oils, while other phytochemical constituents were absent.

Representative test reactions are shown in **Figure 1, 2 and 3.**

a b

Figure 1: Test for Tannins

Figure 2: Test for Volatile Oils (Sudan III test)

a b c d e f

d. Ammonia Test shows yellow stain on filter paper

Figure 3: Test for Flavonoids

HPTLC FINGERPRINTING:

The HPTLC chromatogram of Gnaphalium polycephalum mother tincture is shown in **Figure 4,** and corresponding peak data is shown in **Figure 5.**

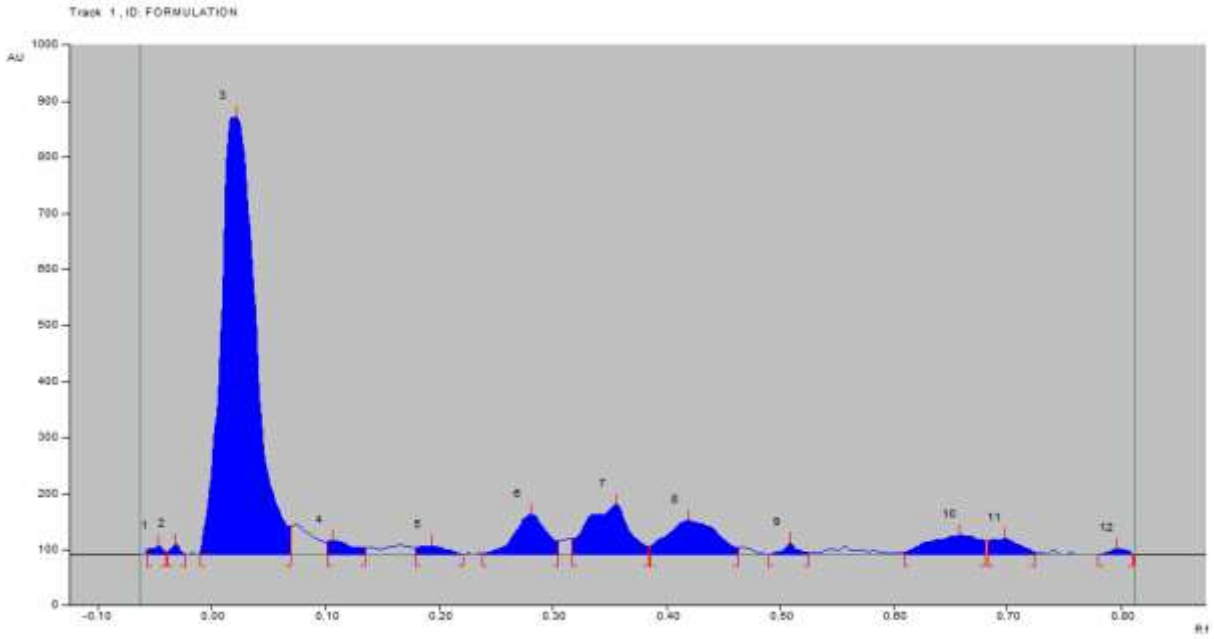

Figure 4: HPTLC chromatogram

Track 1, ID: FORMULATION

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.06 Rf	7.6 AU	-0.05 Rf	14.8 AU	1.27 %	-0.04 Rf	3.3 AU	140.5 AU	0.43 %	unknown *
2	-0.04 Rf	3.5 AU	-0.03 Rf	18.0 AU	1.54 %	-0.02 Rf	0.3 AU	122.5 AU	0.38 %	unknown *
3	-0.01 Rf	1.9 AU	0.02 Rf	780.7 AU	66.74 %	0.07 Rf	49.8 AU	21736.8 AU	66.87 %	unknown *
4	0.10 Rf	22.4 AU	0.11 Rf	24.2 AU	2.07 %	0.14 Rf	11.8 AU	499.5 AU	1.54 %	unknown *
5	0.18 Rf	11.2 AU	0.19 Rf	15.4 AU	1.32 %	0.22 Rf	0.5 AU	336.4 AU	1.04 %	unknown *
6	0.24 Rf	1.5 AU	0.26 Rf	71.1 AU	6.08 %	0.31 Rf	23.5 AU	1840.2 AU	5.67 %	unknown *
7	0.32 Rf	28.5 AU	0.36 Rf	90.0 AU	7.69 %	0.38 Rf	13.9 AU	2920.8 AU	9.00 %	unknown *
8	0.39 Rf	14.2 AU	0.42 Rf	60.3 AU	5.15 %	0.46 Rf	12.1 AU	2439.4 AU	7.51 %	unknown *
9	0.49 Rf	0.0 AU	0.51 Rf	21.3 AU	1.82 %	0.53 Rf	3.0 AU	216.8 AU	0.67 %	unknown *
10	0.61 Rf	4.4 AU	0.66 Rf	34.9 AU	2.99 %	0.68 Rf	25.0 AU	1401.4 AU	4.32 %	unknown *
11	0.68 Rf	24.6 AU	0.73 Rf	29.1 AU	2.49 %	0.73 Rf	4.6 AU	960.5 AU	2.96 %	unknown *
12	0.79 Rf	0.1 AU	0.80 Rf	10.1 AU	0.86 %	0.81 Rf	0.5 AU	134.2 AU	0.41 %	unknown *

Figure 5: Peak data of Gnaphalium polycephalum mother tincture

The HPTLC chromatogram of Gnaphalium polycephalum mother tincture exhibited a well-defined fingerprint profile with approximately 12 distinct peaks, indicating the presence of multiple phytoconstituents. A prominent major peak with high peak area was observed, suggesting the presence of a compound in higher concentration. Several minor peaks were also detected, representing compounds present in smaller quantities. The variation in retention times of the peaks indicates the presence of chemically diverse constituents. The peak area distribution reflects the relative abundance of these compounds, contributing to the overall pharmacological activity of the mother tincture.

CONCLUSION:

The present study evaluated the phytochemical composition and HPTLC fingerprint profile of Gnaphalium polycephalum mother tincture. Preliminary phytochemical screening revealed the presence of important bioactive constituents such as tannins, flavonoids, terpenoids, and volatile oils. These compounds are known for their pharmacological properties, including anti-inflammatory and analgesic effects.

The HPTLC fingerprinting analysis demonstrated a well-defined chromatographic profile with multiple peaks, indicating the presence of diverse phytoconstituents. The characteristic fingerprint obtained in this study can serve as a reliable tool for identification, standardization, and quality control of the mother tincture.

Overall, the findings of this study provide scientific support for the therapeutic potential of Gnaphalium polycephalum and establish a basis for further pharmacological and clinical investigations.

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