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PHYTOCHEMICAL ANALYSIS AND HPTLC FOR AZADIRACHTA INDICA AND RHIZOME EXTRACT OF CURCUMA LONGA

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Abstract
Phytochemical analysis and HPTLC were employed to investigate the bioactive compounds present in the extracts of Azadirachta indica (neem) and Curcuma longa (turmeric). During phytochemical analysis, the extracts were shown to include a range of bioactive substances, such as tannins, proteins, carbohydrates, terpenoids, steroids, and saponins. The HPTLC analysis of the extracts showed distinct chromatographic profiles, indicating the presence of different phytochemicals. The study highlights the importance of phytochemical analysis and HPTLC in understanding the bioactive compounds present in medicinal plants and their potential applications in disease prevention and treatment.
Keywords: *HPTLC, Azadirachta Indica, Curcuma Longa,* Phytochemical Analysis, Rhizome Extract

Introduction
Because of its antibacterial, anti-inflammatory, and antioxidant qualities, the Indian subcontinental tree Azadirachta indica, better known by its common name, neem, is extensively utilised in traditional medicine. The main active compound of neem is azadirachtin, which is responsible for its insecticidal and antifungal activities. Curcuma longa, or turmeric, is a plant belonging to the Zingiberaceae family and is widely used in traditional medicine and as a spice. The active compound curcumin, a polyphenolic curcuminoid, is responsible for its anti-inflammatory, antioxidant, and antimicrobial properties (Goyal P et al., 2024).

Materials and methods
PREPARATION OF EXTRACTS
To eliminate dust, the bark was washed with distilled water, and then dried in oven set at 40 °C ± 5 °C. The dried ethanol extract was obtained by macerating dried and powdered bark with ethanol and then vacuum drying the resulting mixture. A tiny amount of distilled water was used to dilute the dried crude ethanolic extract that was obtained. Following a sequence of increasing polarity, the resulting mother solution was partitioned by solvent-solvent using petroleum ether, benzene, chloroform, and acetone. Using a rotating evaporator and reducing the pressure, the fractions were concentrated.

Qualitative phytochemical analysis
To identify the presence or absence of metabolites such as tannins, saponins, flavanoids, steroids, terpenoids, alkaloids, and phenolic chemicals, the fractionated extracts were tested utilising a battery of qualitative chemical assays based on previously published methodologies.

Quantitative phytochemical analysis (Estimation of secondary metabolites)
Assessment of overall phenolic concentration Quantity of phenolic compounds in Curcuma longa's chloroform fraction, Azadirachta indica's acetone fraction, and ethyl acetate fraction.

Total phenolic content
A 25 ml volumetric flask was used to collect 1 ml of each sample. This was then mixed with 10 millilitres of water and 1.5 millilitres of folin ciocalteu reagent. After 5 minutes, add 4 millilitres of 20% sodium carbonate solution and mix with distilled water to make the volume 25 millilitres. The absorbance of the blue colour that emerged was measured at 765 nm after this mixture was maintained for 30 minutes.

Estimation of total flavonoid contents
Utilising an aluminium chloride colorimetric technique, the total flavonoid content was ascertained. This technique assessed the flavonoid concentration in five different extracts: acetone from Azadirachta indica, choloform and ethanol from Curcuma longa, dichloromethane, and the combined results. In order to estimate the total flavonoid concentration using the aluminium chloride colorimetric method, a sample solution containing 1.0 mg/ml was created for each fraction.

THIN LAYER CHROMATOGRAPHY
Confirmation of quercetin and ursolic acid content was achieved using thin layer chromatography examinations of Azadirachta indica acetone soluble fraction, Curcuma longa ethanolic extract, and Curcuma longa chloroform soluble fractions. Using thin layer chromatography, we checked each fraction for quercetin and ursolic acid. As an adsorbent, this method made use of Silica gel GF254 (designed for thin-layer chromatography; Shivarkar R et al., 2017). Plates were formed using the spreading technique, allowed to air dry overnight, and then activated at 110 °C for one hour.

Results and discussion
Phytochemical analysis – Qualitative method
Qualitative analysis of the alcoholic extracts of Azadirachta indica and Curcuma longa fractions of each plants indicates the presence of various phytoconstituents i.e. tannins, flavonoids, steroids, terpenoids, alkaloids, and phenolic compounds

Table 1: Qualitative analysis of ethanolic extract and its fractions of Azadirachta indica

Constituents Results	Ethanolic extract	Petroleum Ether fraction	Benzene fraction	Chloroform fraction	Acetone fraction
Tannins	+	-	-	+	-
Flavonoids	+	-	-	-	+
Steroids	+	+	-	-	+
Terpenoids	+	+	-	-	+
Alkaloids	+	-	-	+	+
Phenolic compounds	+	-	-	-	-

Alkaloids, phenolic chemicals, tannins, flavonoids, steroids, terpenoids, and an ethanolic Azadirachta indica extract were detected. The presence of terpenoids and steroids is indicated by the petroleum ether fraction of the ethanolic extract of Azadirachta indica. There are tannins and

alkaloids in the chloroform fraction of the ethanolic Azadirachta indica extract. Azadirachta indica contains phenolic chemicals, steroids, terpenoids, and flavonoids, as shown by the acetone fraction of the ethanolic extract.

Table 2: Curcuma longa methanolic extract and its fractionated extracts analysed qualitatively for phytochemicals

Constituents Results	Ethanolic extract	Petroleum Ether fraction	Benzene fraction	Chloroform fraction	Acetone fraction
Tannins	+	-	-	+	-
Flavonoids	+	-	-	-	+
Steroids	+	-	-	+	+
Terpenoids	+	-	-	-	+
Alkaloids	+	-	-	+	+
Phenolic compounds	+	-	-	-	-

Curcuma longa contains tannins, saponins, flavonoids, steroids, terpenoids, and phenolic components, as seen in its ethanolic extract. Curcuma longa contains tannins, flavonoids, steroids, terpenoids, and phenolic chemicals, as shown by the Chloroform fraction of the methanolic extract. Curcuma longa contains tannins, saponins, flavonoids, steroids, terpenoids, and phenolic chemicals, as shown by the Acetone fraction of the methanolic extract.

Quantitative Phytochemical Analysis

In the acetone fraction of Azadirachta indica, the amount of gallic acid was determined to be 450 ± 1.6 mg per gramme of fraction. There was found to be 712.4 ± 2.6 mg gallic acid/1 gramme in the chloroform fraction and 432.5 ± 1.5 mg in the ethanol fraction of Curcuma longa, respectively, in terms of phenolic content. The chloroform fraction of Azadirachta indica has a considerably greater total phenolic content compared to the ethanol fraction of Curcuma longa.

Table 3: Total phenolic content of Azadirachta indica and Curcuma longa fractionated extracts

Alcoholic extracts of plants	Fractionated extracts	TPC (mg gallic acid/1 gm fraction ± S.E.M.)
Azadirachta indica	Acetone	450 ± 1.6
Curcuma longa	Chloroform	712.4 ± 2.6
	ethanol	432.5 ± 1.5

Table 4: Total flavonoid content of fractionated extracts of Azadirachta indica and Curcuma longa

Alcoholic extracts of plants	Fractionated extracts	TFC (mg Quercetin /1g fraction ± S.E.M.)
Azadirachta indica	Acetone	256 ± 1.5
Curcuma longa	Chloroform	413 ± 2.0
	ethanol	217 ± 3.0

The amount of quercetin (256 ± 1.5 mg/g) in the acetone fraction of Azadirachta indica was determined to be the flavonoid content. The chloroform fraction of Curcuma longa had 413 ± 2.0 mg quercetin/1 gm, while the ethanol fraction contained 217 ± 3.0 mg quercetin/1 gm.

In comparison to the chloroform fraction of Curcuma longa, the ethyl acetate fraction of Azadirachta indica had a considerably greater total flavonoid concentration.

THIN LAYER CHROMATOGRAPHY (TLC)

Improvements to the mobile phase After trying several different mobile phase ratios, the best one was found to be (5:4:0.1 v/v/v) of toluene, ethyl acetate, and formic acid in the case of quercetin, and (8.2:1.8:0.1 v/v/v) of petroleum ether, ethyl acetate, and acetone in the case of ursolic acid. Azadirachta indica and Curcuma longa fractionated extracts were found to contain quercetin using TLC figure printing. An Rf value of 0.38 was used to distinguish quercetin.

Table 5: TLC fingerprinting of quercetin and fractionated Azadirachta indica and Curcuma longa extracts

S.no	Sample	Rf values
A]	Quercetin (std.)	**0.38**
B]		**0.39**
C]		**0.38**

The presence of ursolic acid was revealed by the TLC figure printing of Curcuma longa fractions prepared using dichloromethane and ethyl acetate. An Rf value of 0.10 was used for the separation of ursolic acid.

Table 6: Two-dimensional liquid chromatography fingerprinting of curcuma longa fractionated extracts and ursolic acid

S.no	Sample	Rf values
1.	Quercetin (std.)	**0.38**
2.		**0.39**
3.		**0.38**

Conclusion

The alcoholic extracts of Curcuma longa and Azadirachta indica, along with their fractions, were subjected to a qualitative phytochemical examination, which revealed the presence of many phytoconstituents, including tannins, flavonoids, steroids, terpenoids, alkaloids, and phenolic compounds. Kumar A et al. (2017) used folin ciocalteu and aluminium chloride colorimetric techniques, respectively, to measure the total phenolic and flavonoid levels. The n-butanol fraction of Azadirachta indica had the lowest concentration of phenolic and flavonoid compounds, whereas the ethyl acetate fraction of Curcuma longa had the greatest. The plants were analysed for their phytochemical content using TLC/HPTLC. All plants had quercetin, but Curcuma longa contained ursolic acid, according to the TLC study. According to the data from the HPTLC analysis, the herbal hepatoprotective formulation included 0.113 µg/10 mg of quercetin and 344.53 ng/10 mg of ursolic acid (Brahmbhatt H et al., 2023).

Reference

1. Chew YL, Khor MA, Xu Z, Lee SK, Keng JW, Sang SH, Akowuah GA, Goh KW, Liew KB, Ming LC. *Cassia alata, Coriandrum sativum, Curcuma longa* and *Azadirachta indica*: Food Ingredients as Complementary and Alternative Therapies for Atopic Dermatitis-A Comprehensive Review. Molecules. 2022 Aug 26;27(17):5475.
2. Goyal P, Kumar S, Das AM. FORMULATION AND EVALUATION OF HERBAL OINTMENT CONTAINING AZADIRACHTA INDICA AND CURCUMA LONGA. Journal of Higher Education Theory and Practice. 2024;24(1):176.
3. Shivarkar R, Bhise S, Rama V, Gupta M. Standardization and quantification of curcumin present in Curcuma Longa extract using HPLC. International Journal of Phytopharmacology. 2017;8(3):117-22.
4. Brahmbhatt H. Formulation And Evaluation of an Herbal Ointment containing Neem and Turmeric Extracts. Rivista Italiana di Filosofia Analitica Junior. 2023 Aug 31;14(2):1674-84.
5. Kumar A, Thakur A, Maji JK, Bedarkar P, CR H. DISCRIMINANT ANALYSIS OF PURIFICATION ON TURMERIC (CURCUMA LONGA LINN) SAMPLES BASED ON PHYTO-PHARMACOGNOSTICAL AND MULTIVARIATE CHEMOMETRIC TECHNIQUE.