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Analysis of Phytochemicals and HPTLC fingerprints in leaf extracts of *Argyreia cuneata* (Willd) Ker Gawl.

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Abstract

Argyreia cuneata is a well-known traditional herb used to treat various ailments. A systematic phytochemical study is necessary to understand its chemical diversity for better understanding of the therapeutic efficiency. This study presents the results of phytochemical screening, HPTLC (high-performance thin-layer chromatography) fingerprinting analysis of various extracts of *A. cuneata* plant leaves. The phytochemical analysis revealed the presence of essential oils, saponins, triterpenoids, coumarins, bitter principles, tannins, and flavonoids in plant leaf extracts. HPTLC fingerprinting Rf (Retention factor) analysis further confirmed the presence of specific constituents in the five different solvents leaf extract of *A. cuneata*. These findings provide valuable information for understanding the chemical composition of *A. cuneata*, which may have potential applications in various fields, including medicine and natural product research.

Keywords *Argyreia cuneata,* Phytochemical screening, HPTLC, Bioactive compounds, Amrabad Tiger reserve

Introduction

Herbal drugs have been globally renowned for their traditional medicinal applications in treating various ailments. The rich plant biodiversity across the world serves as the primary source of herbal medicine, upon which 60% to 80% of the world's population relies for healthcare, tracing back to ancient times. India, recognized as the Botanical Garden of the World, stands as the leading producer of medicinal herbs. It is widely acknowledged that the medicinal efficacy of these plants resides in their bioactive phytochemical ingredients, which exert specific biological effects on the human body. These natural compounds form the foundation of modern pharmaceuticals (Edeoga 2005; Akinmo 2007; Rout 2009). Plants play a crucial role in sustaining life on Earth, with increasing global interest in natural colorant production. Plant pigments, unique chemicals found in plants, absorb different wavelengths of light, contributing to their vibrant hues. Essential to photosynthesis, plant growth, and development, these pigments, also known as biochromes, are metabolic by- products that define the characteristic colors of plants (Carvalho *et al*., 2011; Tanaka 2011). Plants possess the ability to produce free radical-scavenging molecules like vitamins, terpenoids, phenolic acids, lignins, tannins, flavonoids, quinones, coumarins, alkaloids and other metabolites with potent antioxidant activity (Cai *et al*., 2003). Numerous studies have demonstrated the anti-inflammatory, anti-atherosclerotic, anti-tumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral properties of these antioxidant compounds (Sahaa *et al*., 2008). Consumption of natural antioxidants has been linked to reduced risks of cancer, cardiovascular disease, diabetes, and age-related ailments (Ashok Kumar 2008; Veerapur 2009). Recent years have witnessed a global trend towards the consumption of natural phytochemicals found in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables (Aiyegoro *et al*., 2010). While medicinal plants have been utilized for centuries in human therapies, their application have significantly expanded beyond limited regions (Arokiyaraj *et al*., 2008). Plants boast a diverse array of secondary metabolites, including phenols, tannins, terpenoids, alkaloids and flavonoids, known for their antimicrobial properties. Plants exhibit an extensive capacity to synthesize aromatic substances, with phenols and their derivatives being among the most abundant (Elvin Lewis and M Lewis 1995). *A. cuneata* is a plant species known for its potential medicinal properties, with no scientific evidence for traditional claims. From the literature it is apparent that the phytochemical profile of *A. cuneata* is still elusive. To gain insights into its chemical composition, this study conducted a phytochemical screening to identify the presence of various chemical constituents in different solvent extracts of *A. cuneata* leaves. Additionally, HPTLC fingerprinting was performed for the first time for this plant to confirm the presence of diversity in the phytochemicals, and Rf (retention factor) analysis was used to determine the specific constituents present in the plant leaf extract.

Material and methods

Plant material collection and authentication

Argyreia cuneata plant leaves were collected from the Amrabad Tiger Reserve in Telangana were collected for the study and authenticated by the Central Council for Research in Ayurveda (AYUSH) Bangalore, Karnataka State, India. The collected leaf material underwent a series of preparatory steps, including washing, shade drying, and pulverization (Figure 1).

Figure 1. *Argyreia cuneata* plant and leaf powder

Preparation and extraction of leaf material

Twenty grams of dried leaf powder was subjected to successive extraction using Soxhlet apparatus starting from non-polar solvents to polar solvents viz., Petroleum ether (C_5H_{12}), Chloroform ($CHCl_3$), Ethyl acetate ($CH_3COOC_2H_5$), Acetone ($(CH_3)_2CO$), and Methanol (CH_3OH). The extraction process involved hot, continuous extraction for 24 hrs, at 60^0C. This successive extraction technique using different solvents facilitate the extraction of diversified phytochemicals in the plant material. The extract solution was collected, filtered, and the solvent was evaporated using water bath. The resulting crude extract was preserved in sealed airtight containers for subsequent analysis (Figure 2).

Figure 2. Soxhlet apparatus and crude extracts

HPTLC method

For the preliminary phytochemical screening, the HPTLC method was employed, utilizing instruments from the CAMAG HPTLC system (Switzerland). The system featured a Linomat V applicator for sample application, TLC scanner 3 for detection, Reprostar 3 for sample development, and WIN CATS -4 Software for data analysis.

Phytochemical screening

Phytochemical screening of the *A. cuneata* leaf extracts was conducted using increasing polarities of various solvents, including Petroleum ether (C5H12), Chloroform (CHCl3), Ethyl acetate (CH3COOC2H5), Acetone ((CH3)2CO), and Methanol (CH3OH). Qualitative analysis determined the presence or absence of specific chemical constituents.

HPTLC fingerprinting

HPTLC (High-performance thin-layer chromatography) is a valuable technique for separating and identifying components in complex mixtures. In this study, HPTLC was employed to profile the chemical composition of the petroleum ether, chloroform, ethyl acetate, acetone, and methanol leaf extract of *A. cuneata*. Different solvent systems and reagents were used for visualization, as outlined in (Table 1).

Table 1. Analysis of HPTLC fingerprinting profiles of different solvent leaf extracts of *A. cuneata*

Solvent system	Reagent	Constituents
n-Butanol:Methanol:Water	Anisaldehyde sulfuric acid	Steroids
Ethyl acetate:Methanol:Water	Anisaldehyde sulfuric acid	Glycosides
Toluene:Ethyl acetate	Anisaldehyde sulfuric acid	Essential Oils
Chloroform:Acetic acid:Methanol:Water	Anisaldehyde sulfuric acid	Saponins
n-Hexane:Ethyl acetate	Anisaldehyde sulfuric acid	Triterpenoids
Ethyl acetate:Formic acid:Glycial acetic acid	Anisaldehyde sulfuric acid	Coumarins
Toluene:Ethyl acetate:Methanol:Water	Anisaldehyde sulfuric acid	Bitter principles
Toluene:Ethyl acetate:Formic acid	Ferric chloride	Tannins
Ethyl acetate:Formic acid: Acetic acid	10% Methanol sulphuric acid	Flavonoids
Toluene:Ethyl acetate:Methanol:Ammonia	Dragendroff's	Alkaloids

Retention factor analysis

The Rf (retention factor) is a critical parameter in chromatography, aiding in the identification of specific compounds within a sample. The Rf values observed at both white and 366 nm wavelengths for the assorted leaf extracts of *A. cuneata* across various solvent systems (petroleum ether, chloroform, ethyl acetate, acetone, and methanol).

Results

Phytochemical screening

The analysis of *A. cuneata* leaf extracts in different solvents unveiled the presence of several chemical constituents. Essential oils, saponins, triterpenoids, coumarins, bitter principles, tannins, and flavonoids were consistently detected in the petroleum ether, chloroform, ethyl acetate, acetone, and methanol extracts. However, the quantities of essential oils and saponins varied among the different extracts (Table 2). These results indicate the potential presence of bioactive compounds in *A. cuneata* with possible health-promoting properties.

Table 2. Phytochemical screening of various solvent leaf extracts of the *A. cuneata*

S. No	**Chemical Constituents**	**Petroleum ether (C_5H_{12})**	**Chloroform ($CHCl_3$)**	**Ethyl acetate ($CH_3COOC_2H_5$)**	**Acetone ($(CH_3)_2CO$)**	**Methanol (CH_3OH)**
1	Steroids	- -	- -	- -	- -	- -
2	Glycosides	- -	- -	- -	- -	- -
3	Essential oils	- -	++	++	- -	++++
4	Saponins	- -	- -	++	--	++++
5	Triterpenoids	- -	- -	++	- -	++
6	Coumarins	++	- -	++	- -	+++
7	Bitter principles	- -	--	+++	++	+++
8	Tannins	--	++	++	++	++
9	Flavonoids	- -	- -	++++	++	+++
10	Alkaloids	- -	- -	- -	- -	- -

+: presence ; - : absence

HPTLC fingerprinting

HPTLC fingerprinting of the *A. cuneata* leaf extract in different solvents provided valuable insights into its chemical composition. Different solvent systems and reagents revealed the presence of essential oils, saponins, triterpenoids, coumarins, bitter principles, tannins, and

flavonoids (Figure 3). This comprehensive chemical profile is essential for understanding the potential pharmacological activities of *A. cuneata*.

Bioactive compounds detected

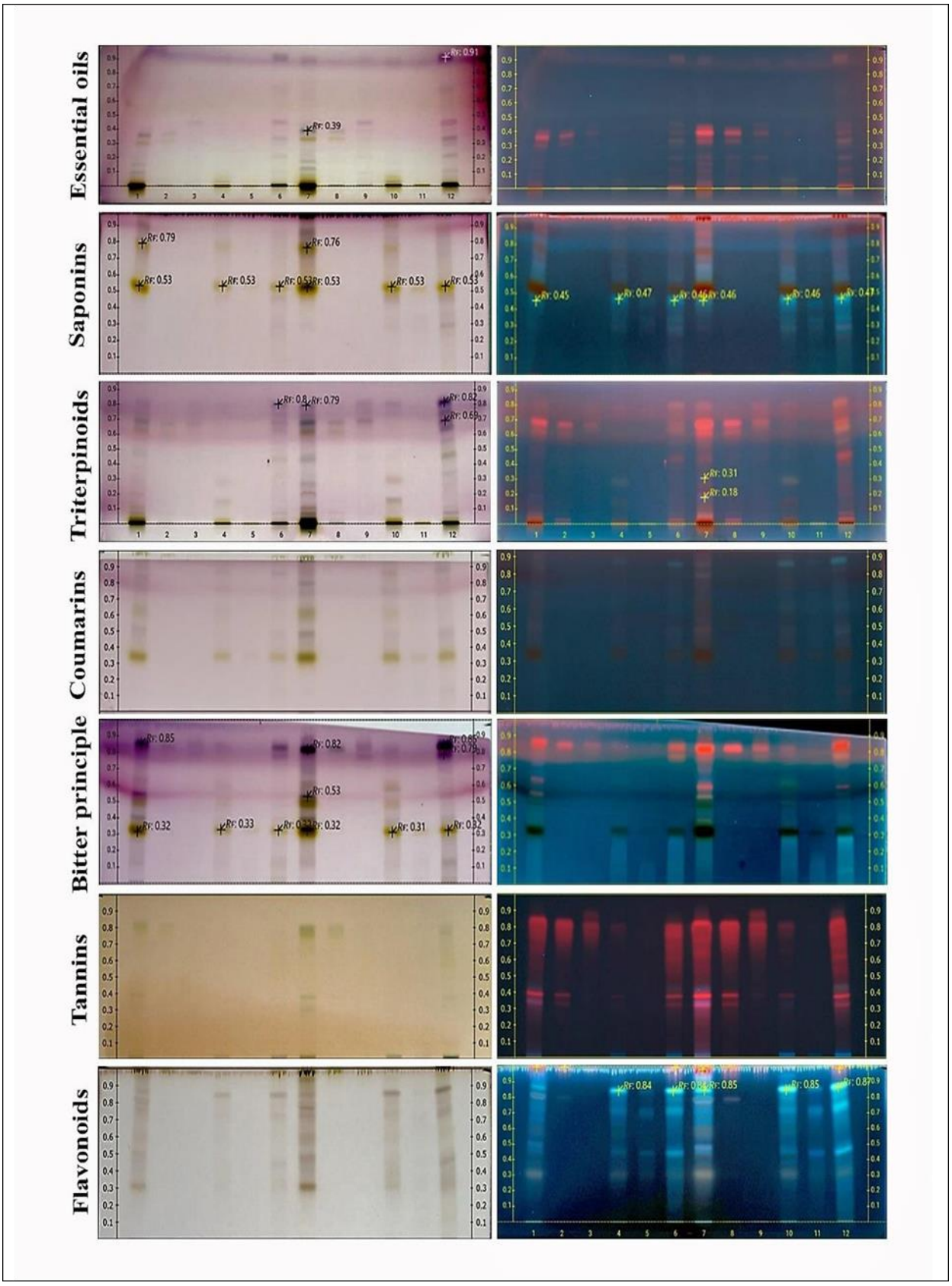

Figure 3. Phytochemicals of *A. cuneata* along with Rf values (HPTLC Analysis)

Rf analysis

The Rf analysis confirmed the presence of specific constituents in the leaf extract of *A. cuneata*. Following the HPTLC qualitative phytochemical screening of various solvent leaf extracts, specific components were identified. In the Toluene:Ethyl acetate (9.3:0.7) solvent system, two bands with Rf values of 0.39 and 0.91 suggested the presence of essential oil. The solvent system of Chloroform:Acetic acid:Methanol:Water (6.4:3.2:1.2:0.8) revealed six bands with Rf values ranging from 0.45 to 0.79, indicating the presence of saponins. The n- Hexane: Ethyl acetate (1:1) solvent system displayed three bands with Rf values of 0.69, 0.79, and 0.81, suggesting the presence of triterpenoids. Additionally, the Toluene:Ethyl acetate:Methanol:Water (7.7:1.5:0.8) solvent system exhibited seven bands with Rf values ranging from 0.31 to 0.85, suggesting the presence of bitter principles. Furthermore, in the Ethyl acetate:Formic acid:Acetic acid (100:11:11:26) solvent system, seven bands with Rf values ranging from 0.1 to 0.99 indicated the presence of flavonoids. These Rf values, detected across various wavelengths and solvent systems, can serve as reference data for future studies and quality control purposes (Table 3), enabling the identification and qualitative of compounds responsible for the plant's medicinal properties.

Table 3. Quantitative phytochemical screening of *A. cuneata* leaf extract by HPTLC

Solvent system	Rf values	No. of Bands	Constituents
Toluene:Ethyl acetate (9.3:0.7)	0.39, 0.91	2	Essential Oil
Chloroform:Acetic acid:Methanol:Water (6.4:3.2:1.2:0.8)	0.45, 0.46, 0.470.53, 0.76, 0.79	6	Saponins
n-Hexane:Ethyl acetate(1:1)	0.69, 0.79, 0.81	3	Triterpenoids
Toluene:Ethyl acetate:Methanol:Water (7.7:1.5:0.8)	0.31, 0.32, 0.33, 0.53, 0.79, 0.82, 0.85	7	Bitter principles
Ethyl acetate:Formic acid: Acetic acid (100:11:11:26)	0.1, 0.84, 0.85, 0.87, 0.89, 0.98, 0.99	7	Flavonoids

Discussion

Standardization of herbal medicine is essential to ensure the safety and therapeutic efficacy. Various analytical, biological techniques have been adopted in recent times and help in the maintain the quality standards of herbal based products. HPTLC is playing a significant role in ensuring quality control of herbs and herbal medicine (Ahmad *et al*., 2014). These medicinal compounds derive their therapeutic efficacy from secondary metabolites (Ahmad 2015). Identification is indispensable to distinguish an organism from other populations and plant species. In the systematic examination of plants, both molecular weight and morphological characteristics serve as invaluable tools, playing pivotal roles in taxonomic categorization. Furthermore, a range of markers, such as morphological, cytological, biochemical, and molecular markers, have been employed for organism classification (Sampath Kumar *et al*., 2011).

The extraction of phytochemical substances from medicinal herbs can be a difficult and laborious activity. Thus, phytochemical screening serves to find and remove unnecessary chemicals. This technique facilitates the extraction of novel targets, the highlighting of significant structures with potential activities, and the identification of non-producing molecules (Nonita *et al*., 2010).

In this research, the initial phytochemical analysis confirmed the existence of essential oils, saponins, triterpenoids, coumarins, bitter principles, tannins, and flavonoids. These findings support the presence of pharmacologically active constituents in *A. cuneata*. The identified phytochemicals, including essential oils, saponins, triterpenoids, coumarins, bitter compounds, tannins, and flavonoids, are recognized for their role in contributing to the antioxidant properties of the plant.

The study investigated the phytochemical composition of *A. cuneata* plant leaves through phytochemical screening and HPTLC fingerprinting. The results revealed the presence of essential oils, saponins, triterpenoids, coumarins, bitter principles, tannins, and flavonoids in various leaf extracts. HPTLC fingerprinting Rf analysis confirmed specific constituents in the leaf extract of *A. cuneata*. These findings provide valuable insights into *A. cuneata* chemical composition, which could have significant implications in medicinal and natural product research. Furthermore, the study successfully reported a range of bioactive compounds in *A. cuneata* leaf extracts, which hold promise for isolation and drug discovery. These bioactive compounds present in the plant extracts offer potential avenues for pharmacological investigation and the development of novel drugs. Phytochemicals such as triterpenoids, saponins are known to have anti-inflammatory properties. Whereas, essentials are reported to possess antimicrobial, insect repellent, insecticidal, and mood elevating properties. Polyphenols like tannins and flavonoids are renowned for their antioxidant and protective role in the cellular stress. Bitters are pharmacologically proved to ameliorate dyspepsia. Similarly, coumarins are

famous in skin care.

The HPTLC fingerprinting analysis provided conclusive evidence of specific constituents in the leaf extract, which could serve as leads for further isolation and drug development processes. The presence of diverse bioactive compounds suggests that *A. cuneata* holds considerable potential for medicinal applications and natural product development.

Conclusion

This study provides valuable insights into the chemical composition of *A. cuneata* leaf extracts, highlighting the presence of various phytochemicals, including essential oils, saponins, triterpenoids, bitter principles, and flavonoids. HPTLC fingerprinting analysis further confirm the specific constituents present in the leaf extract. Further research and exploration of the bioactive compounds identified in *A. cuneata* could lead to the discovery of new therapeutic agents and contribute to advancements in drug discovery and plant-based medicine.

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Competing interests

The authors declare that they have no competing interests

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Availability of data

All the data produced presented in this paper

Reference

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