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OPTIMIZATION AND STANDARDIZATION OF ANDROGRAPHOLIDE YIELD IN *ANDROGRAPHIS PANICULATA*: IMPACT OF SOIL TYPES AND EXTRACTION CONDITIONS

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ABSTRACT

Andrographis paniculata has been recognized for centuries for its effectiveness in treating respiratory infections, fevers, herpes, sore throats, gastrointestinal issues, and various chronic infectious diseases. The main bioactive compounds found in this plant include diterpenoid andrographolides, neo-andrographolide, deoxy- andrographolide, and -19ß-D-glucoside, which are extracted from its aerial parts. The primary aim of the current study was to assess how cultural conditions affect the bioactive constituents in both sodic and normal soils. Additionally, the research sought to optimize extraction parameters (such as solvent type and duration) to enhance the yield of andrographolides and to evaluate the concentrations of key phytochemicals in different parts of the plant (leaves, stems, and roots). The highest levels of andrographolide, neo-andrographolide, and deoxy- andrographolide were found in 60-day-old plants cultivated in normal soil, with a noticeable decline as the plants matured under consistent cultural conditions. Methanol was identified as the most effective extraction solvent when using Soxhlet extraction for five hours. In plants grown in normal soil, andrographolide concentrations ranged from 30.41 mg/g to 22.53 mg/g; conversely, those in sodic soil exhibited lower levels and a narrower range (8.63–8.00 mg/g). The findings indicated that the concentration of andrographolide was highest in leaves, followed by stems and roots.

Keywords: *Andrographis paniculata,* Andrographolides, Extraction, Soil type, Liver protection

Introduction

An essential medicinal plant, *Andrographis paniculata* (King of Bitters, family Acanthaceae), was suggested in the 175 BC Charaka Samhita for the treatment of jaundice in multi-plant mixtures. It is included in the Indian Pharmacopoeia and is a key ingredient in at least 26 Ayurvedic formulations for liver problems, advanced stages of dysentery, general weakness, and fever recovery. *A. paniculata* is used in Chinese traditional medicine to remove toxins, treat fevers, and eliminate body heat. Although it is an annual plant that grows widely in Southeast Asia, India, Sri Lanka, Pakistan, and Indonesia, it is also widely grown from seeds in China, Thailand, Mauritius, and the East and West Indies, usually growing readily on a variety of soil types. Additionally, it thrives in soil that is nearly impossible for other plants to grow in, especially serpentine soil that has comparatively high levels of copper, zinc, and aluminium. Seasons, soil, geographic location, and climate all have a significant impact on the amount of bioactive diterpene lactone phytochemicals found in aerial portions. Diterpenoids, namely deoxyandrographolide, -19ß-D-glucoside, and neo-andrographolide, are the primary therapeutic compounds with bitter characteristics that have been extracted from the aerial portions (Joselin and Jeeva, 2014).

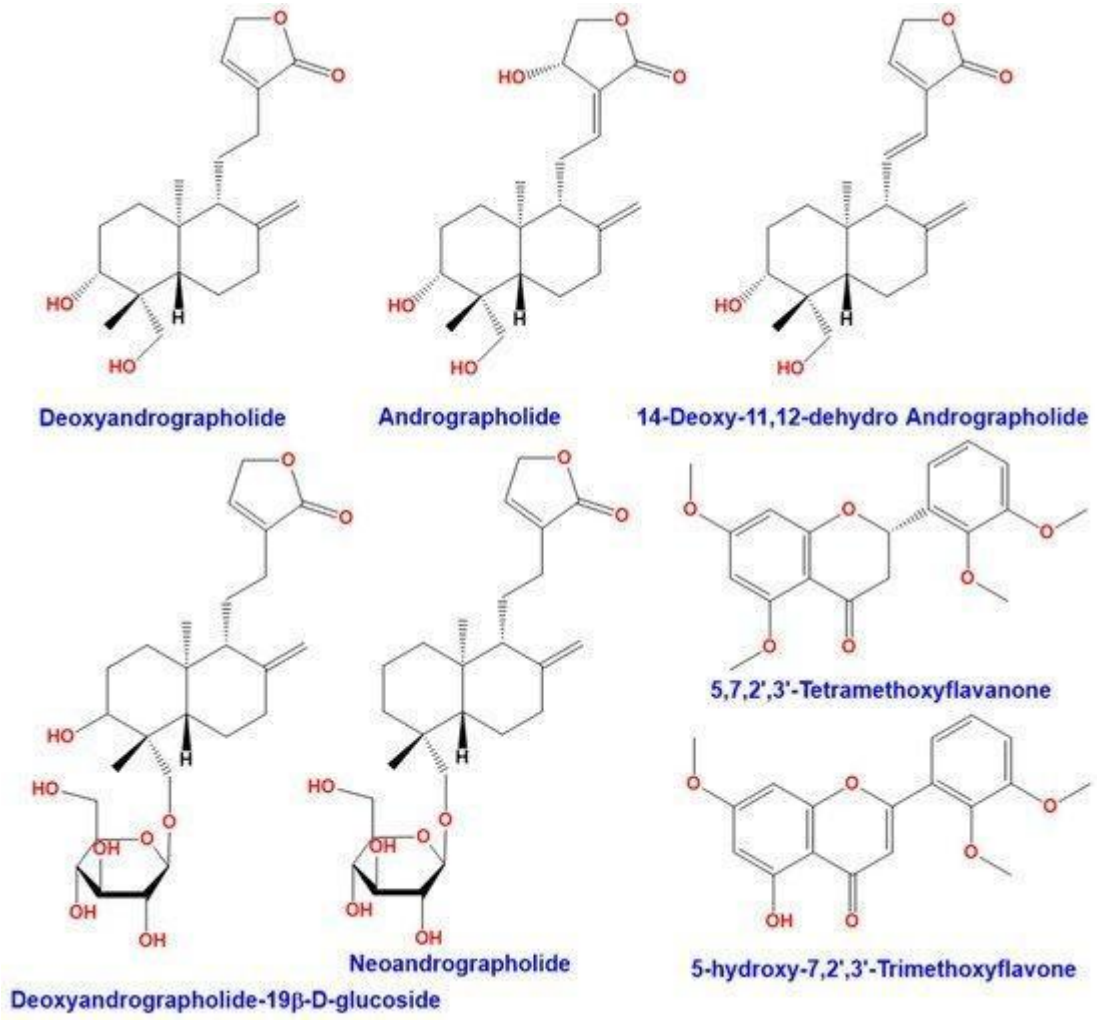


Figure 1: Chemical Structure of Andrographolide and its derivatives**.**

The structures of the major phytochemicals and their derivatives are depicted in Figure Known as andrographolides "diterpene lactone," the bioactive phytochemicals of Andrographis paniculata are colorless, crystalline, and have a very bitter taste. Other active ingredients include stigmasterol, andrographan, andrographon, andrographosterin, and 14-deoxy-11,12-didehydroandrographolide (andrographlide D), and homoandrographolide (3). Andrographolides are believed to improve the generation of white blood cells, the release of interferon, the activity of the lymphatic system, and the immune system's ability to scavenge germs and other foreign objects. Its excellent immune-boosting properties are heightened when paired with immune-stimulating substances like vitamin C, zinc, and the herb echinacea. According to Okhuarobo et al. (2014), andrographolides have been discovered to be helpful in cancer treatment. It is a prime option for the prevention and treatment of several illnesses, including cancer, liver, gallbladder, common cold, fever, and inflammation, due to its broad spectrum of immune-stimulating and regulating activities. It displayed strong cell differentiation-inducing action on leukaemia cells, according to the results of many investigations (Verma, 2018).

Plant leaf extracts have been shown to have a strong protective impact on the liver and to be cytotoxic (cell-killing) against cancer cells. According to Mannan (2017), this effect was ascribed to antioxidant capacity, which was just as potent as silymarin, another potent

antioxidant obtained from milk thistle. Furthermore, andrographolides increased bile flow, bile salts, and bile acids and were proven to be powerful stimulators of gallbladder function in animal tests. Furthermore, recent studies have shown that it has a significant deal of potential to disrupt HIV's capacity to replicate. These studies used signal transduction technology and discovered that these phytochemicals disrupted the virus's communications mechanism. Specifically, by altering cellular signal transduction, andrographolide stopped the virus from spreading to additional cells and stopped the disease's progression (Nuruzzaman and Kamrujjaman Das, 2017).

Andrographolide, neo-andrographolide, and dehydro-andrographolide have been demonstrated in many other Chinese studies to reduce fever caused by various fever-inducing agents, as well as the chemical 2, dinitrophenol,

Materials and Methods

Sigma-Aldrich USA provided the standard samples of diterpenoids, andrographolide, neo-andrographolide, and 14-deoxy-11, 12-didehydroandrographolide. We bought HPLC-grade solvents from Qualigens in India.

A variety of agroclimatic conditions are suitable for the cultivation of *Andrographis paniculata*. With a high production of bioactive elements tracked by extraction conditions, the studies were developed to maximize culture conditions. To assess and standardize the kind of solvent, extraction conditions (cold and hot (Soxhlet), and extraction time to maximize the production of andrographolide in medicinal plants cultivated in normal and sodic soil, whether fertilizers are applied. Assessing the crop's quality in terms of andrographolide production while growing it in sodic soil (pH 8–9) and regular unfertilized soil was another goal of the experiment. At Amity University's organic garden in Noida, 30-day-old seedlings were planted. In a medium-sized pot with 10 kg of soil, 10 g of salt per litre of water was added to create sodic soil conditions. Since there were no pests or diseases affecting the crop, no precautions were made to safeguard it. Early in

bacterial endotoxins, pneumococcus, hemolytic streptococcus, typhoid, and paratyphoid. Histamine, dimethyl benzene, croton oil (hemolytic necrosis), and acute pneumocystis brought on by adrenaline all had anti-inflammatory effects that were either much diminished or alleviated (Chao et al., 2010). All of the main andrographolides showed this effect, including deoxy-andrographolide, andrographolide with the strongest effect, neo-andrographolide, and dehydro-andrographolide. Finding the effects of various climatic, geographic, and agricultural circumstances on sodic and normal soils on bioactive contents was the main goal, given the relevance of andrographolides in the healthcare system. Additionally, the yield of andrographolide, neo-andrographolide, and deoxy-andrographolide was examined in relation to the standardization of extraction conditions (solvent, time)

September, the seeds were planted, and beginning a month following the plantation, the entire plant was harvested every 30 days.

Sample preparation

Following harvest, the various plant parts—leaves, stems, and roots—were separated and let to air dry. When necessary, hexane was used to de-fatten the samples. For extraction, four distinct solvents were selected: acetone, methanol, chloroform, and ethyl acetate. The extraction process was conducted in Soxhlet for five hours and under cold maceration conditions for varying durations. Following drying, the components were crushed to a 60-mesh powder and subjected to LC-MS/MS analysis for andrographolide, neoandrographolide, and 14-deoxy-11,12-didehydroandrographolide. The extract of *Andrographis paniculata* was initially standardized for extraction time, solvent, and conditions. Tables 1–3 present the findings.

Analysis by HPLC

The binary gradient HPLC apparatus LC-10ATVp (Shimadzu, Kyoto, Japan), which has

two LC-10ATVp pumps and is managed by SCL-10AT, was used for HPLC investigation. The UV detector SPD-10AVVp picked up the chromatogram at a wavelength of 232 nm. The Phenomenex Luna RP, C 18 column (4.6 x 250 mm) was the HPLC column utilized for the analysis. A Millipore filtering unit was used to filter the solvents prior to their use in the HPLC unit. Using an optimized binary gradient mobile phase apparatus, the extracts were dissolved to create a solution, filtered, and 20 µl of the solution was injected for analysis

Results and Discussion

Selection of extraction solvent

The solvent that maximizes extraction was chosen using the whole plant extract. Five different polarity solvents—hexane, chloroform, acetone, ethyl acetate, and methanol—were chosen to determine the best solvent. Five conical containers containing 1g of a pulverized sample of the *Andrographis paniculata* plant were filled with the appropriate solvents, and the containers were left overnight to extract the material. Following extraction, the solvent was extracted at low pressure and the extract was recovered. Twenty microliters of the dried extract were used for HPLC analysis after it had been redissolved in two milliliters' of HPLC-grade methanol. Methanol > Acetone > Chloroform > Ethyl-acetate > Hexane was the decreasing order of the quantity of andrographolide (Table 1) extracted in each solvent. The methanol extract was responsible for 1590.31 g/g, followed by acetone (1227.10 g/g), chloroform (996.05 g/g), and hexane extracts (97.62 g/g). Methanol was determined to be the most appropriate solvent based on these findings. Observing the effects of de-fatting plant material before extraction was another need of the study. Before extraction using the aforementioned solvents, the samples were de-fatted using hexane (Table 1). After de-fatting without any advantages, the data reveal a reduction of andrographolide contents (2.5 to 29.4%). However, the amount of loss varied per solvent, with methanol experiencing the least amount (2.5%), followed by acetone (17.5%) and chloroform (29.4%).

Sl. No	Solvent	De-fatted (µg/g)	Without de-fatted (µg/g)
1.	Acetone	1011.94	1227.10
2.	Ethyl acetate	767.26	866.94
3.	Chloroform	703.33	996.05
4.	Methanol	1524.67	**1590.31**
5.	Hexane	--	97.64

Table 1. Andrographolide contents (µg/g dry wt.) in whole plant extracts in different solvents with and without de-fatting with hexane. The total aerial parts (with and without defatted) of the plant were extracted overnight with different solvents separately at room temperature.

Optimization of extraction procedure

Separate cold maceration and Soxhlet conditions were used to extract the powdered plant aerial components. In terms of the volume removed and the length of time needed for extraction, the results (Table 2) confirmed that Soxhlet extraction was superior to cold extraction. Even after being left overnight (12 hours), the quantity of andrographolide extracted using the cold technique was smaller than that obtained using the Soxhlet method. Its greatest percentage of the quantity extracted was 0.15%, which was lower than the maximum extraction by Soxhlet in 5 hours, which was 0.19%.

Sl. No.	Time	Andrographolide cold extraction*	Andrographolide Soxhlet extraction*
1.	1 Hr	437.3 (0.04)	1258.8 (0.12)
2.	2 Hrs	661.5 (0.06)	1377.4 (0.13)
3.	3 Hrs	940.4 (0.09)	1539.4 (0.15)
4.	4 Hrs	1099.2 (0.11)	1832.7 (0.18)
5.	5 Hrs	1231.9 (0.12)	1959.4 (0.19)
6.	6 Hrs	1362.4 (0.13)	1894.3 (0.18)
7.	12 Hrs	1503.9 (0.15)	1914.3 (0.19)

Table 2. Effect of temperature and duration of Soxhlet extraction (time) on the quantity (µg/g) of andrographolide contents in the aerial parts of *Andrographis paniculata.*
* The total aerial parts (without defatted) of the plant were extracted with methanol for the period as indicated at room temperature and another sample(s) at extracted under reflux.

The impact of a 5-hour extraction period on the concentrations of three diterpene lactones—andrographolide, neo-andrographolide, and 14-deoxy-11,12-didehydroandrographolide—was also examined in a more thorough investigation using samples extracted for varying lengths of time in Soxhlet (Table 3). All three lactones needed roughly the same amount of time to extract to their full potential. After five hours of extraction, they achieved their maximum extraction (%). To maximize the conditions for the extraction of all three diterpenoid lactones, these findings were crucial.

Sl. No.	Time	Andro (%)	Neo-Andro (%)	Deoxy-Andro (%)
1.	1 Hr	0.129	0.013	0.035
2.	2 Hrs	0.131	0.015	0.037
3.	3 Hrs	0.153	0.017	0.049
4.	4 Hrs	0.183	0.028	0.097
5.	5 Hrs	0.195	0.046	0.105
6.	6 Hrs	0.189	0.047	0.105

Andro = andrographolide; Neo-Andro = Neo- andrographolide; Deoxy-Andro = 14-deoxy-11,12-didehydro- andrographolide

Table 3. Composition of andrographolides (%) (Andro = andrographolide, Neo-Andro = neo andrographolide and Deoxy-Andro = 14-deoxy-11,12- didehydro-andrographolide) in aerial parts of *Andrographis paniculata* extracted with methanol through Soxhlet for different duration.

Variation of andrographolides

The andrographolide concentrations of plants cultivated in normal soil and sodic soil were studied to determine the more optimal cultivation conditions. Although it is more concentrated in the leaves and stems, andrographolide is essentially present throughout the plant. In general, each condition had a low andrographolide concentration in the aerial sections of plants growing in sodic soil (Table 4). The goal of the study was to examine how the three diterpenoid lactones—andrographolide, neo-andrographolide, and 14-deoxy-11,12-didehydroandrographolide—changed with plant age and with the sodic and normal soil conditions. HPLC analysis was performed on the samples that were obtained at various stages of development. Table 4 presents the findings about the variance of andrographolide in mature leaves and soil type.

Days after plantation	Season	Andrographolide		Neo-Andrographolide		Deoxy-andro grapholide	
		Normal Soil	Sodic Soil	Normal Soil	Sodic Soil	Normal Soil	Sodic Soil
30	Oct	30286.3	8657.3	2368.81	1137.84	2246.18	**978.37**
60	Nov	30411.4	8630.4	2399.28	1203.43	2301.49	**956.32**
90	Dec	25793.3	8396.9	2203.01	1011.27	2132.63	**901.96**
120	Jan	24932.1	8204.5	2019.26	992.55	1933.01	**788.20**
150	**Feb**	**22537.2**	**8002.9**	**1967.31**	**823.41**	**1900.76**	**765.92**

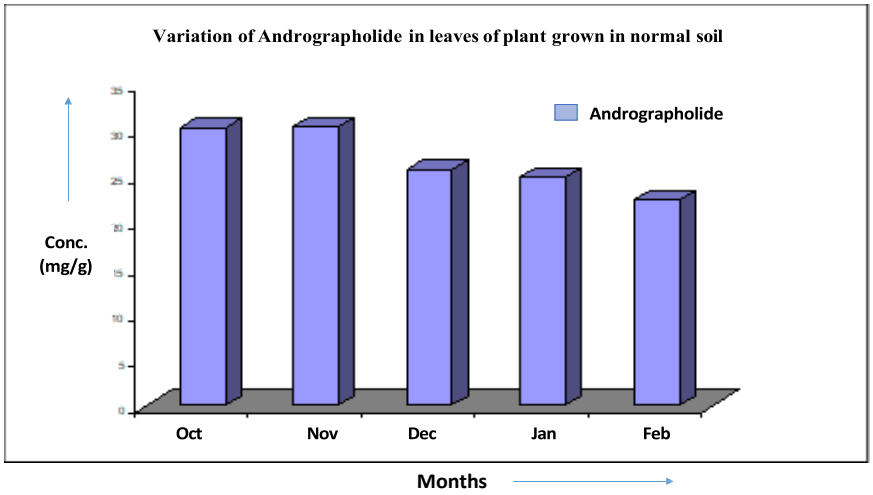
Deoxy- andrographolide = 14-deoxy-11,12- didehydro-andrographolide

Table 4. Variation of andrographolides (µg/g) with maturity and composition (andrographolide, neo andrographolide and 14-deoxy-11, 12- didehydro-andrographolide) in leaves of *Andrographis paniculata* extracted with methanol in Soxhlet 5h.

Compared to plants cultivated in sodic soil, plants grown in normal soil had much higher levels of andrographolides. The plants that were 60 days old had the greatest concentrations of all three andrographolides that were examined. In plants cultivated in normal soil, the quantity of andrographolide ranged from 30.41 mg/g to 22.53 mg/g, but in plants grown in sodic soil, the amount was lower, and the range was narrower (8.63 -8.00 mg/g). As a result, plants cultivated in regular soil had higher levels of andrographolide, which decreased as they grew older (Figure 4). These findings imply that 60 days is the ideal harvest period for plans with the maximum concentration of bioactive components. Additionally, plants cultivated in normal soil have greater levels of neo andrographolide in their leaves than those grown in sodic soil. Neo andrographolide levels ranged from 2.39 to 1.96 mg/g in plants grownin regular soil and from 1.20 to 0.82 mg/g in plants grown in sodic soil. The highest levels were found in November in plants that were 60 days old, and the lowest levels were found in February in plants that were 150 days old. As a result, neo-andrographolide decreased with maturity, and 14-deoxy-11,12-didehydro- andrographolide had a comparable impact in mature normal and sodic soil. In sodic soil, the range was 0.97 to 0.76 mg/g, but in normal soil, it was 2.30 to 1.90 mg/g. At around two months of age, the concentration of all three of the andrographolides under study peaked, and as plants matured, the andrographolides decreased. Therefore, November appears to be the ideal month for gathering plant leaves. However, the output of leaves per plant per hectare is another element that should be taken into account. The quantity of andrographolide extracted per plant may be lower since the plant is not yet completely mature at 60 days, which will result in a lower dry matter yield per plant.

Conclusion

According to the discussion above, methanol was the ideal solvent to use with Soxhlet (hot) for five hours to extract the highest amount of andrographolide from Andrographis paniculata. The three diterpenoid lactones—andrographolide, neo-andrographolide, and 14-deoxy-11,12-didehydroandrographolide—were also found to have different concentrations depending on the plant's age and the soil's quality. Plants that were 60 days old and cultivated in regular soil had the highest concentration of andrographolides. After 60 days of maturity, the phytochemicals in plants grown on sodic soil decreased correspondingly. The current study's findings may be applied to the quick screening of A. paniculata plants for drug analysis, culture, and genotype quality evaluation



Conflict of Interest

The author(s) confirm that this article's content has no conflict of interest.

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Ethical Issues

There is none to be declared.

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