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Review article

A Systematic Review of Analytical Methods for Quantification of Natural Indole Alkaloids from *Catharanthus* and *Rauvolfia* Species

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Abstract

Indole alkaloids are a class of alkaloids enclosing a structural moiety of indole; numerous indole alkaloids also comprise isoprene groups and are thus named terpene indole or secologanin tryptamine alkaloids and have more than 4100 identified diverse compounds. Indole alkaloids are one of the main classes of alkaloids identified in several significant plant groups, mainly *Catharanthus* and *Rauvolfia* plants from Apocynaceae family. The pharmacological actions of these plants have been studied scientifically, with some undergoing clinical trials while others were already approved for medicinal use. This review aimed at the fundamental objective of summarizing the developed analytical methods for quantification of indole alkaloids obtained from plants and complete examination of their present quantification method, which may result in the identification of alternative method developments for the effective and accurate quantification of indole alkaloids by considering the green method. This systematic review was conducted from January 2006 to June 2022, using electronic databases like PubMed, Web of Science and Embase. Several studies in the literature have been reported for quantitative estimation of indole alkaloids using different techniques including the spectrophotometric methods, high performance liquid chromatography (HPLC), and ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Most of the studies reported HPLC analysis for quantification. This review will offer a greater understanding of the available methods to develop a more precise and sensitive quantification method for indole alkaloids with the cost-effectiveness that is expected to emerge in line with clinical usage and to promote the development of the pharmaceutical industry for routine quality control analysis.

Keywords: Apocynaceae; gas chromatography; indole alkaloids; liquid chromatography; spectrum analysis

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Introduction

Plants are the well-established foundation of the traditional medical systems that have been used for many thousands of years to treat human diseases and improve health [1,2]. Plants are a wealthy reservoir of a huge assortment of active constituents that have substantial curative uses like antitubercular, anticancer, antiviral, and analgesic properties [3,4]. Among them, alkaloids are the imperative secondary metabolites that are originally exposed and used as early as four thousand years ago and are well documented for their rich remedial potential [5]. Based on their heterocyclic ring system and biosynthetic precursor, alkaloids are divided into

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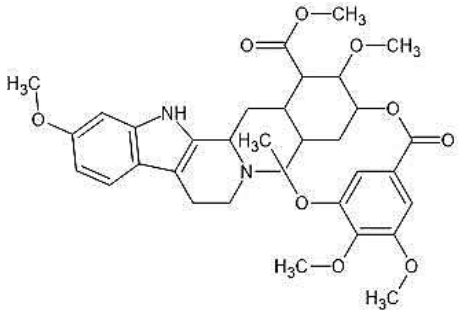
various categories, viz. indole, purine, quinoline, isoquinoline, tropane, and imidazole alkaloids [6,7].

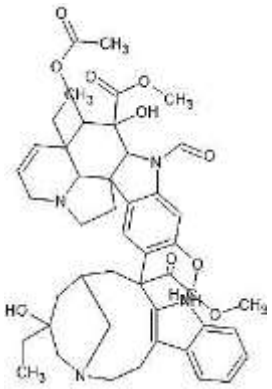
Indole alkaloids have a bicyclic structure, containing a 6-membered benzene ring fused to a 5-membered nitrogen-containing pyrrole ring. This pyrrole ring with nitrogen atom gives rise to the basic properties of indole alkaloids that make them particularly pharmacologically active [8]. Indole alkaloids are extensively distributed in plants belonging to the families Apocynaceae, Nyssaceae, Loganiaceae, and Rubiaceae. Important indole alkaloids which have been separated from plants comprise the anti-hypertensive drug, reserpine from *Rauvolfia serpentina* (L). Benth.ex Kurz [9], and the influential antitumor drugs, vinblastine, and vincristine from *Catharanthus* roseus (L.) G. Don [8] both belonging to the family Apocynaceae. The medicinal potential of indole alkaloids increases as their industrial application increases. Numerous studies have been performed on the pharmacological effects of various alkaloids derived from plants. The chemical structure of reserpine, vincristine, vinblastine is given in Figure 1.

Catharanthus roseus (generally recognized as *Vinca rosea*, is a member of the Apocynaceae family and is well-known for pharmacological studies and as a decorative plant. There have been several identified *Catharanthus* species, seven of which are native to the Coast of South Africa, whereas *Catharanthus roseus* is dispersed globally. According to Indian traditional medicine (Ayurveda) and traditional Chinese medicine [TCM], *Catharanthus roseus* extract is used to treat a variety of illnesses, including diabetes, malaria, and Hodgkin's lymphoma [10-14].

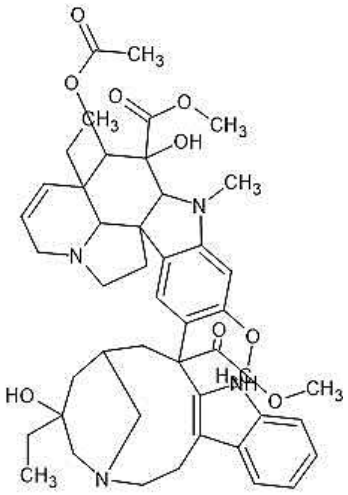
Rauvolfia a plant genus belonging to the Apocynaceae family, consists of one hundred ten species of trees and plants found across tropical regions, including India. It contains a large number of secondary metabolites like indole alkaloids [15]. *Rauvolfia serpentina* L. roots have been employed in Ayurveda to cure a variety of diseases [16] and are mostly used for the treatment of hypertension [17-19]. Reserpine, ajmaline, serpentine, serpentinine,ajmalicine, and yohimbine are primary alkaloids extracted from *R. serpentina* [20-22]. The essential components of *Rauvolfia* vary from species to species. [23] and alsoconstituents of plants [24]. The roots of *Rauvolfia serpentina* and *Rauvolfia vomitoria* are two significant shrubs that yield potentially helpful alkaloids. Reserpine is the most prominent indole alkaloid in several *Rauvolfia* species [17] and possesses both CNS depressive and hypotensive properties [16].



Reserpine



Vincristine



Vinblastine

Figure 1. Structure of indole alkaloids in *Catharanthus* and *Rauvolfia* species

Due to the commercialization of bioactive constituents from plants, numerous analytical techniques have been used for the identification and quantification of active moieties during the last three decades. Plant extracts obtained from indole alkaloids contain a vast variety of bioactive metabolites. Because of an extensive range of divergence indexes, separation techniques, as well as the identification and characterization of bioactive compounds are key subjects. For the isolation of active components, various analytical techniques including optimum performance laminar chromatography (OPLC), high performance thin layer chromatography (HPTLC), paper chromatography, column chromatography, gas chromatography, high performance liquid chromatography (HPLC), have been employed to separate active components.

HPLC is the most often used analytic technique for separation as well as quantification of organic and inorganic solutes in any sample, including biological, medicinal, food, ecological, commercial, etc. [25]. The key mechanism of this type of chromatography is adsorption. The separation of the compound is determined by the interaction between polar and nonpolar compounds closely packed inside the column and the running phase. In the pharmaceutical and food industries, the stationary phase is often nonpolar while the mobile phase is usually polar. Up to 400 bars of pressure are necessary to elute the molecules from the column until they pass through a diode array detector (DAD). The HPLC analysis is unable to detect vaporized molecules. Thermolabile substances cannot be analyzed by HPLC, but it is an excellent complement to GC for detecting alkaloid derivatives. For HPLC analysis, many types of analytical columns, such as C_{18} and C_8 columns, are used. There are various kinds of detectors, including UV, DAD, and Flame ionization detector (FID) [26].

Gas chromatography is only applicable for volatile substances. Both liquid and gaseous states are utilized. The gas phase serves as the mobile phase, whereas the liquid phase works as the stationary phase [27]. The moving rate is determined by the arrangement of chemical reactions in the gas phase. If the distribution is 100% then the liquid stationary phase, cannot flow at all. If a species' distribution is present in both phases, it will move at an optimum rate. Samples are first vaporized and inserted into the chromatographic column, and then the flow of the inert, gaseous mobile phase causes the samples to pass through the column. The stationary phase remains constant within the column [28].

This review summarized the developed analytical methods for the quantification of plant-derived indole alkaloids and gave a comprehensive evaluation of their known quantification method, which may help in new method development for effective and precise quantification of the indole alkaloids in the future from a pharmaceutical quality control perspective.

Methods

The current systematic review was prepared according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.

The literature search was conducted in an organized procedure The online literature search was made with PubMed, Scopus, Web of science, and Embase, using the MeSH (medical subject heading) combination terms "indole alkaloids," "*Rauvolfia*," "*Catharanthus*," "analytical method," "quantification," and "validation". The search consisted of studies listed from January 2006 up to June 2022. The articles were monitored for eligibility, using inclusion and exclusion criteria by reading the article title, abstract, and full-text.

Inclusion criteria

We included only the studies that reported indole alkaloids from *Rauvolfia* and *Catharanthus* species.

We included only the studies that reported analytical method development with validation report.

We included only the studies that reported the quantification method rather than qualitative analysis.

Exclusion criteria

We excluded the studies about indole alkaloids reported from species other than *Rauvolfia* and *Catharanthus*.

We excluded the studies that used other languages than English.

We excluded the summaries, systematic reviews, commentaries, letters, and conference articles.

We excluded the literature that was not available in full text.

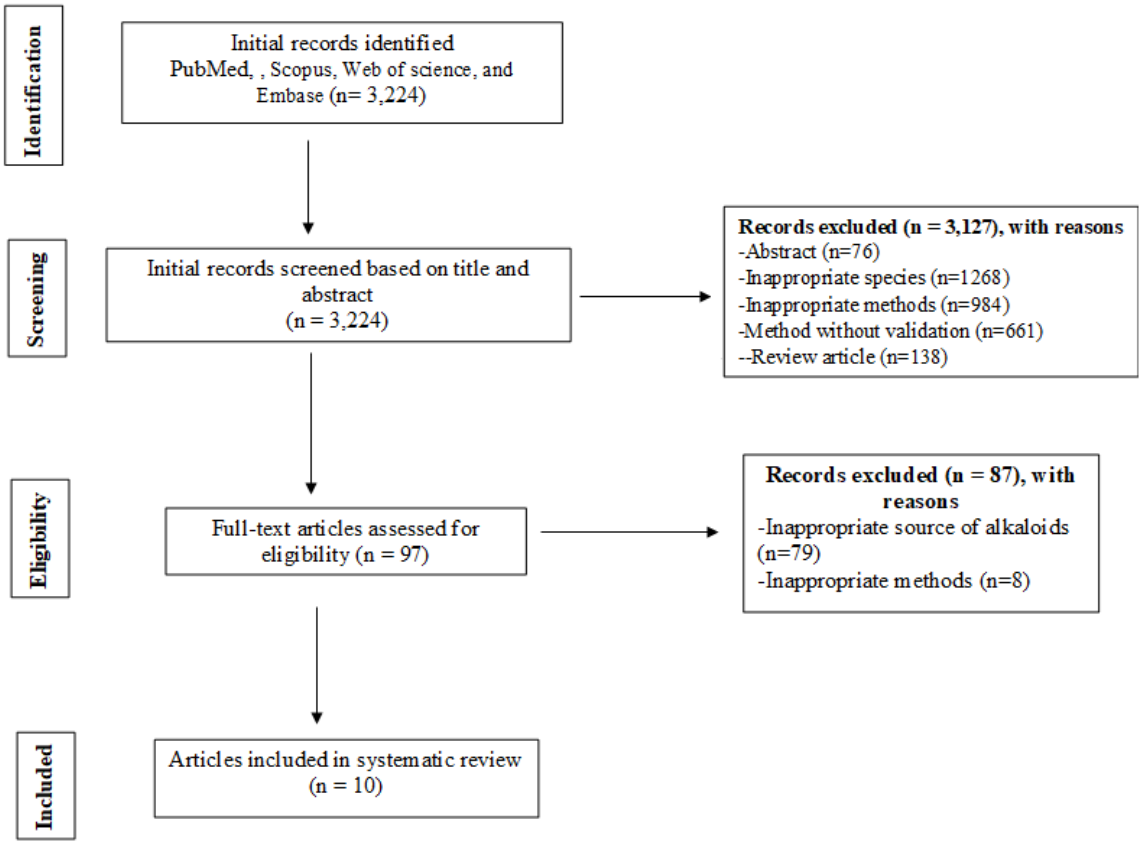


Figure 2. Flow diagram for the selection of studies for analytical method of indole alkaloids from *Catharanthus* and *Rauvolfia* species

Results and Discussion

The combined keywords search on databases identified 3,224 articles for the Indole alkaloids from *Rauvolfia* and *Catharanthus* species, of which 3,127 were excluded because of duplication or that the studies were conducted outside the region of interest, described biosynthesis of indole alkaloids, purification process, pharmacological activity, or were review articles. Of the remaining 97 articles, 10 papers met the inclusion criteria and were included for data in this review; they are presented in Figure 2. The analytical technique used for the quantification of indole alkaloids from natural sources by HPLC and ultra-high performance liquid chromatography (UHPLC) coupled with MS are presented in detail in Table 1. Out of the ten included studies, five were for *Catharanthus roseus* and four studies were for *Rauvolfia serpentina*, and one study was for *Rauvolfia verticillate*. Totally, 13 indole alkaloids were reported including ajmalicine, ajmaline, catharanthine, reserpine, sarpagine, serpentine, serpentinine, vinblastine, vincristine, vindesine, vindoline, vinleurosine, and yohimbine. Most of the studies used MeOH extract from plants. HPLC and UPLC-MS were the most common methods in the selected studies. PDA and UV detectors were used for HPLC and UHPLC analyses. Electrospray ionization detector was used for mass spectroscopy detection. Most of the studies applied gradient elution program. Water and acetonitrile were the common mobile phase for elution.

Shrivastava et al., developed a UV spectroscopy method for reserpine from *Rauvolfia serpentina* using UV-Visible double beam spectrophotometer-1800 (Shimadzu, North America) with a 1 cm cell pathway. They used MeOH for the extraction of alkaloids and selected 217 nm from the spectrum analysis for the quantification of reserpine. They successfully validated the developed method with a good degree of linearity (R^2=0.9972). The LOQ value was 1.05 mcg/mL, LOD value was 3.19 mcg/mL. The %RSD for intraday and inter-day precision

was 1.4. The recovery rate was 99.41± 0.14% which shows a highly cost-effective method [43]. The validation parameter for the included studies by HPLC and UHPLC with MS is presented in Table 2. Almost all studies reported a good degree of linearity, an R^2 above 0.99.

Table 1. The analytical techniques used for the quantification of indole alkaloid from natural sources by HPLC and UHPLC with MS

No	Plant name	Extraction solvent	Technique	Instrumentation	Column	Condition	Mobile phase	MS	Ref.
1	*Catharanthus roseus*	70% MeOH	UPLC-MS	Waters Acquity UPLC system, with PDA detector	C_{18} column (2.1×50mm, 1.7 µm)	Column temperature: 45°C; flow rate: 0.4 mL/min; injection volume: 1.5 µL	A: water containing 0.1% CH_2O_2 and 10 mM NH_4HCO_2 B: ACN	ESI	[33]
2	*Rauvolfia serpentina*	EtOH	UHPLC-MS	Acquity UPLC BEH coupled with API 4000 QTRAP MS/MS system from AB Sciex	C_{18} column (2.1×50 mm, 1.7 µm)	Column temperature: 25°C; flow rate: 0.3 mL/min; injection volume: 2.0 µL	A: 0.1% CH_2O_2 in Water B: ACN	Positive ESI	[34]
3	*Rauvolfia verticillate*	MeOH	HPLC-UV	Agilent/HP 1100 series HPLC with VWD-UV detector	Diamonsil C_{18} (250× 4.6 mm, 5 µm)	Column temperature:30° C; flow rate: 1.0 mL/min; injection volume: 5 µL; UV: 280 nm	A: Water B: MeOH	---	[35]
4	*Catharanthus roseus*	MeOH	HPLC-MS	Agilent 1050 HPLC system with UV detector	Phenomenex Gemini C_{18} column (150 ×2.0 mm, 5µm).	Flow rate: 0.3 mL/min; injection volume: 5 µL; UV: 214nm	A:10 mM $C_2H_7NO_2$ buffer (pH 5.0) B: ACN C: MeOH	ESI	[36]
5	*Catharanthus roseus*	Aqueous Extract	HPLC	HPLC System (Varians) with PDA detector	C18, Microsorb - MV column (250 mm x 4.6 mm, 5 µm)	Column temperature: 25°C; flow rate: 2.0 mL/min; UV: 254nm	A: phosphate buffer (5 mM, pH 6.0) B: ACN	---	[37]
6	*Catharanthus roseus*	EtOH	UHPLC-MS	Waters Acquity UPLC system with API 4000 QTRAP MS/MS system	C18 column (1.7 µm, 2.1 × 50 mm)	Column temperature: 35°C; flow rate: 0.3 mL/min; injection volume: 4 µL	A: CH_2O_2 (0.1%) in water B: ACN	Positive ESI	[38]
7	*Catharanthus roseus*	$C_4H_8O_2$	HPLC-MS	Agilent 1100 series HPLC	C_{18} column (Agilent Eclipse C_{18}, 4.6 × 150 mm, 5 µm).	Flow rate: 1.0 mL/min; injection volume: 5 µL	MeOH: 15 mmol/L $C_2H_7NO_2$ containing 0.02% CH_2O_2 (65:35, V/V).	ESI	[39]
8	*Rauvolfia serpentina*	MeOH	HPLC	Shimadzu LC-8A HPLC	C_{18} column (100 ×4.6mm)	Column temperature: 26 °C; flow rate: 1mL/min	A: ACN B: 0.01M phosphate buffer containing 0.5% glacial acetic acid; pH 3.5	---	[40]
9	*Rauvolfia serpentina*	MeOH	UV	UV-visible double Beam spectrophotometer-1800 (Shimadzu)	---	217 nm, 1 cm path length cell	---	---	[41]
10	*Rauvolfia serpentina*	Acidic MeOH	UHPLC-PDA	Waters Acquity UPLC system with PDA detector	UPLC BEH Shield RP_{18} (1.7µm, 50×2.1 mm)	Column temperature: 40 °C; Sampler temperature: 15 °C; flow rate: 0.2 mL/min; injection volume: 2µL	A: Water (0.05 % CH_2O_2) B: ACN (0.05 % CH_2O_2)	---	[42]

MeOH: methanol; mm: millimeter; µm: micrometer; mL/min: milliliter per minute; µL: microliter; CH_2O_2: formic acid; NH_4HCO_2: ammonium formate; ACN: acetonitrile; EtOH: ethanol; $C_2H_7NO_2$: ammonium acetate

Table 2. Validation parameters for the analytical methods for quantification of indole alkaloids from natural sources by HPLC and UHPLC with MS

No	Plant Name	Technique Used	Alkaloids	Linearity R^2	LOD (ng/mL)	LOQ (ng/mL)	Precision		Accuracy/ recovery	Ref.
							Intraday %RSD	Inter day %RSD		
1	*Catharanthus roseus*	UPLC-MS	Ajmalicine	0.9991	5 ng/mL	15 ng/mL	1.7	5.7	104.1 %	[33]
			Catharanthine	0.9996	1 ng/mL	3 ng/mL	0.4	1.0	101.8 %	
			Serpentine	0.9995	3 ng/mL	9 ng/mL	1.0	1.0	100.5 %	
			Vincristine	0.9990	10 ng/mL	30 ng/mL	2.1	2.6	101.4 %	
			Vindoline	0.9997	1 ng/mL	3 ng/mL	0.9	1.6	100.9 %	
			Vinblastine	0.9998	10 ng/mL	20 ng/mL	2.3	3.1	92.8 %	
2	*Rauvolfia serpentina*	UHPLC-MS	Ajmaline	0.9996	0.07 ng/mL	0.22 ng/mL	1.60	1.38	1.82%	[34]
			Yohimbine	1.0000	0.15 ng/mL	0.44 ng/mL	0.53	0.83	2.23%	
			Ajmalicine	0.9999	0.06 ng/mL	0.19 ng/mL	1.61	2.74	1.75%	
			Serpentine	0.9990	0.13 ng/mL	0.41 ng/mL	0.92	1.42	0.12%	
			Reserpine	0.9997	0.06 ng/mL	0.18 ng/mL	2.24	0.76	0.45%	
3	*Rauvolfia verticillate*	HPLC-UV	Sarpagine	0.9989	NA	0.10 mcg/mL	1.3	1.2	NA*	[35]
			Yohimbine	0.9990		0.20 mcg/mL	1.2	0.9		
			Ajmaline	0.9996		0.78 mcg/mL	1.5	1.5		
			Ajmalicine	0.9988		0.05 mcg/mL	0.9	1.5		
			Reserpine	0.9993		0.39 mcg/mL	0.7	0.6		
4	*Catharanthus roseus*	HPLC-MS	Catharanthine	≥0.9990	0.2 mcg/mL	2.7 mcg/mL	NA	NA	NA	[36]
			Vindoline		0.15 mcg/mL	2.0 mcg/mL				
			Vinblastine		0.1 mcg/mL	1.3 mcg/mL				
			Vincristine		0.08 mcg/mL	1.1 mcg/mL				
5	*Catharanthus Roseus*	HPLC	Vinblastine	0.9990	0.0230 µg/mL	0.0698µg/mL	0.41	2.22	95.0 ±1.28	[37]
6	*Catharanthus Roseus*	UHPLC-MS	Ajmaline	0.9998	0.039 ng/mL	0.118 ng/mL	1.45	2.87	100.64±0.38%	[38]
			Yohimbine	0.9999	0.294 ng/mL	0.892 ng/mL	2,58	2.72	101.26±0.46%	
			Vindesine	0.9998	0.044 ng/mL	0.134 ng/mL	2.37	1.83	101.42±1.53%	
			Ajmalicine	0.9998	0.550 ng/mL	1.666 ng/mL	2.59	2.52	102.02±3.09%	
			Serpentine	0.9931	0.437 ng/mL	1.326 ng/mL	0.23	0.40	101.71±0.80%	
			Vincristine	0.9988	0.048 ng/mL	0.147 ng/mL	0.74	0.58	99.70±1.86%	
			Vinblastine	0.9993	0.388 ng/mL	1.178 ng/mL	1.73	1.39	101.24±0.68%	
			Vindoline	0.9998	0.254 ng/mL	0.771 ng/mL	0.51	0.41	99.63±0.22%	
7	*Catharanthus Roseus*	HPLC-MS	Vinblastine	0.999 5	0.75 ng/mL	1.5 ng/mL	4.5	4.5	± 10.9%	[39]
			Vincristine	0.9980	0.75 ng/mL	1.5 ng/mL	3.3	5.1	± 10.9%	
			Vinleurosine	0.9979	0.75 ng/mL	1.5 ng/mL	1.5	1.4	± 10.9%	
			Vindoline	0.9953	1.5 ng/mL	3.1 ng/mL	8.4	5.2	± 10.9%	
			Catharanthine	0.9989	1.5 ng/mL	3.1 ng/mL	5.5	6.2	± 10.9%	
8	*Rauvolfia serpentina*	HPLC	Ajmaline	1.0000	6 mcg/mL	19 mcg/mL	2.77	NA	98.27±2.14%	[40]
			Ajmalicine	1.0000	4 mcg/mL	12 mcg/mL	2.51		97.03±2.05%	
			Reserpine	1.0000	8 mcg/mL	23 mcg/mL	2.38		98.38±1.20%	
9	*Rauvolfia serpentina*	UV	Reserpine	0.9972	1.05mcg/mL	3.19mcg/mL	0.14	0.14	99.41± 0.14%	[41]
10	*Rauvolfia serpentina*	UHPLC-PDA	Ajmaline	0.9993	0.5 mcg/mL	1.0 mcg/mL	3.75	2.83	93.71%	[42]
			Yohimbine	0.9993	0.5 mcg/mL	1.0 mcg/mL	2.90	2.87	96.94%	
			Corynanthine	0.9994	0.5 mcg/mL	1.0 mcg/mL	3.39	3.08	95.83%	
			Ajmalicine	0.9994	0.5 mcg/mL	1.0 mcg/mL	0.68	3.42	99.05%	
			Serpentine	0.9993	0.1 mcg/mL	0.5 mcg/mL	1.89	3.04	95.57%	
			Serpentinine	0.9992	0.5 mcg/mL	1.0 mcg/mL	2.75	3.32	85.90%	
			Reserpine	0.9993	0.1 mcg/mL	0.5 mcg/mL	3.45	2.89	95.25%	

*: not applicable

All the included studies reported acceptable LOD, LOQ, Intraday, inter-day precision, and accuracy levels.

Alkaloids are usually low-molecular-weight molecules that constitute almost 20% of plant-based secondary metabolites.

Approximately 12,000 alkaloids have so far been identified from various plant genera [6]. The plants of the Apocynaceae family contain a wide range of alkaloids. The most important indole alkaloids include reserpine, an antihypertensive agent from *Rauvolfia serpentina*, vinblastine, an anticancer agent, and vincristine from *Catharanthus roseus*. [8,9]. For the routine analysis of the quality and quantity of natural alkaloids, a scientifically proven and validated quantification method must be available to help the pharmaceutical industry. In this systematic review, we evaluated the available quantification methods for the natural alkaloids from *Catharanthus* and *Rauvolfia* species.In this current review,four studies were reported with UHPLC, five studies were reported with the HPLC method, and One study from the UV spectroscopy method [33-42]. UVand PDA detectors were commonly used in HPLC and UHPLC methods. While alkaloids have an aromatic ring structure, UV absorbance is a preferred method for identification. Due to the presence of pi-delocalized electrons in the structure of alkaloids, autofluorescence phenomena are feasible, allowing for their quick identification with a reduced baseline background, particularly for the highly luminous alkaloids serpentine and ajmalicine.

Among the various detectors used in high-performance liquid chromatographyanalysis, ultraviolet and fluorescence are chiefly competent in the quantification of alkaloids, andthese detectors are simply accessible to the huge scientific society. A photodiode array detector with multiple ultraviolet channels seems ideal for the quantitative approach to alkaloid combinations because the wavelengths causing maximal absorbance do not exactly match for all alkaloids. Modern improvements in photodiode array identification and separationcolumn methods have contributed to drastically decreasing the limit of detection. However,the limit of detection under ultraviolet can be extensively pretentious by the existence of othercompounds in the immediacy of the peaks of attention [43,46].

The studies conducted by HPLC or UHPLC coupled with MS reported that the electrospray ionization technique was used to ionize the alkaloids [33,34,36,38,39]. Alkaloids are easily ionized by electrospray in positive mode; hence ESI (+) MS is preferred for their detection.

The extraction solvents applied for the selected studies were EtOH, MeOH, water, acidic EtOH, and ethyl acetate [33-42]. The solubility of the alkaloids plays a crucial role in the choice of the extraction phase. It appears that using extracting solutions in an acidic environment increases the alkaloids' stability and solubility. The solvent phase range is also contingent on the nature of the study to be executed. For example, when methanol extract of the plant is examined by the high-performance liquid chromatography method, phosphate buffer as mobile phase should be avoided since methanol can impulsively settle phosphate salts in diverse parts of the high-performance liquid chromatography system and create performance issues of the system. In addition, while advanced molecular categorization has to be achieved using a mass spectrometer detector, non-volatile solvents particularly phosphoric acid must be forbidden to avoid any harm to the mass spectrometer detector. For mass spectrometer analysis, trifluroacetic acid (TFA) must be used with safeguard as high trifluroacetic acid concentration will cause ion-suppression in the mass spectrometer, and peak tailing can afterward be observed [45,46]. Almost all the reported studies used organic solvents for the mobile phase preparation.

All the reported studies with HPLC/UPLC, used C_{18} columns with lengths varying from 50 to 250 mm; internal diameter varied from 2.0 to 4.6 mm [35-44]. The column selection is important because a precise improvement may be accomplished more easily using a column with a greater diameter and similar dimensions to the one used at the analytical range.

The validation parameters reported from the selected studies were linearity, LOD, LOQ, precision by intraday and inter-day precision, and accuracy/recovery. UHPLC method has a low limit of detection values when compared to the HPLC method. All the reported methods showed good agreement with validation parameters [33-42]. The purpose of analytical procedure validation is to determine the performance characteristics of analytical applications through

experimental testing, resulting in an analytical technique that is suitable for its intended use [47].

Conclusion

Alkaloids hold an amazingly remedial and community attention as a resource of the most new important compounds for drug research against different lethal diseases. Indole alkaloids are well-known for their therapeutic properties against numerous diseases including hypertension, cancer, and neurological problems. They are configurationally exclusive biologically active constituents with efficient remedial capability. Adequate technical support is collected in this review concerning the available analytical method for quantification of *Catharanthus* and *Rauvolfia* species based on major types of alkaloids; however, none of the studies reported green methods for the estimation of the selected alkaloids. The use of green methods reduces the hazardous reagents or the production of hazardous waste. The organic solvent waste from the reported methods will definitely affect human health and the environment. Hence in the near future, the green method must be developed without affecting the method's precision, and make use of less hazardous chemicals or solvents to protect human health and the environment.

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Author contributions

Lakshmipriya Murugesan contributed in conceptualization, data curation, investigation and drafted the manuscript; Kokilambigai Karumandampalayam Shanmugaramasamy designed the study, supervised and drafted the manuscript; Ilango Kaliappan contributed in revising, editing, and all the authors have approved the final draft of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

HPLC: high performance liquid chromatography; TCM: traditional Chinese medicine; CNS: central nervous system; OPLC: optimum performance laminar chromatography; HPTLC: high-performance thin layer chromatography; GC: Gas chromatography; LC-MS: liquid chromatography mass spectrometry; GC-MS: gas chromatography mass spectroscopy; UHPLC: ultra-high performance liquid chromatography; FID: flame ionization detector; DAD: diode array detector; ACN: acetonitrile; MeOH: methanol; LOD: limit of detection; LOQ: limit of quantitation; RI: refractive index; PDA: photo diode array; NPD: nitrogen phosphorous detector