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Review article

Introduction to extraction and antioxidant activity of alkaloids

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ABSTRACT

Alkaloids are nitrogen-based cyclic compounds. In the recent past, the methods used to extract various types of alkaloids from various sources, and the methods utilised to extract them are all investigated. This review covers the basic synthesis process for alkaloids. Nicotine, cocaine, morphine, quinine, berberine, vinblastine, capsaicin, caffeine, and piperine are some of the alkaloids that have been researched for their biological actions and use. Alkaloids have a wide range of medical applications, making them valuable compounds in this review, the antioxidant activity of alkaloids from various sources found using different methods in the last 7-8 years is presented; however, common assays used to evaluate antioxidant activity are briefly discussed, including DPPH which stands for "2,2–diphenyl-1-picrylhydrazylhydrate free radical scavenger", Hydroxyl radical scavenging assay and Ferric reducing antioxidant power (FRAP) assay.

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1. Introduction

Traditional medicine has always been associated with plants, which have been utilized for thousands of years to cure human ailments and promote health. Plants include a wide variety of active chemicals with major medical uses such as antiviral, anticancer, analgesic, and antitubercular properties (Rupani et al., 2018). Alkaloids are natural chemical molecules that are mostly made up of nitrogen atoms. Many similar compounds with neutral or weak acidic properties are also included in this group. These are produced by a broad variety of species, including bacteria, fungi, plants, and animals. Alkaloids are in the category of the most varied, potent, and therapeutically significant plant chemicals. The word "alkaloids" comes from "alkaline" which is used to refer to any nitrogen-containing base. The heterocyclic ring structure and biosynthetic precursor of alkaloids like Indole, Purine, Quinoline, Iso-quinoline, Tropane, etc are used to categorize them (Roy et al., 2017).

An alkaloid is defined as "a cyclic compound containing nitrogen in a negative oxidation state with restricted biological availability." This definition comprises alkaloids that have nitrogen as a component of a heterocyclic system, as well as a few special cases that contain extra-cyclic nitrogen, such as colchicine or capsaicin. Biogenetically, alkaloids can be classified. For example, indole alkaloids are derived from tryptophan and can be classified as non-terpenoid or terpenoid indoles (Anaya et al., 2006).

2. General steps in the biosynthesis of alkaloids

The early phases of an alkaloid synthesis route are crucial because they serve as the entry point into a novel chemical environment. These are metabolically significant because reflux out from primary metabolism is initiated toward specialized metabolism. These are crucial chemically as well as enzymatically as these participate in the creation of a new molecular system. These are required to generate pathways. The very first four steps in complex alkaloid biosynthesis are: (I) amine precursor accumulation, (II) aldehyde precursor accumulation, (III) Iminium positively charged ion production, and (IV) a condensation reaction. The last stage is also known as the "scaffolding," the first committed step into a route (Lichman, 2021).

3. Extraction of alkaloids

3.1 Methods of extraction and isolation of Piperine alkaloids

Piperine alkaloids were separated and isolated from black pepper in this research. By combining 100 gm of black pepper powder with 300ml of petroleum ether, the Maceration Extract was produced. The extract solution was therefore concentrated to 30ml in a 600°C water bath. Yellow-brown needle-like crystals with a melting point of

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124°C were obtained. Piperine alkaloids isolated from black pepper seeds were identified using chemical tests such as Wagner's, Mayer's, melting point, and TLC (Thin Layer Chromatography). The bioactivity report showed that ethanol extraction of Piperine alkaloids is particularly efficient against *E.coli*, *Staphylococcus aureus, Pseudomonas, Streptococcus, Aeromonas, Klebsiella,* and *Acinetobacter* (Alyaseen et al., 2018).

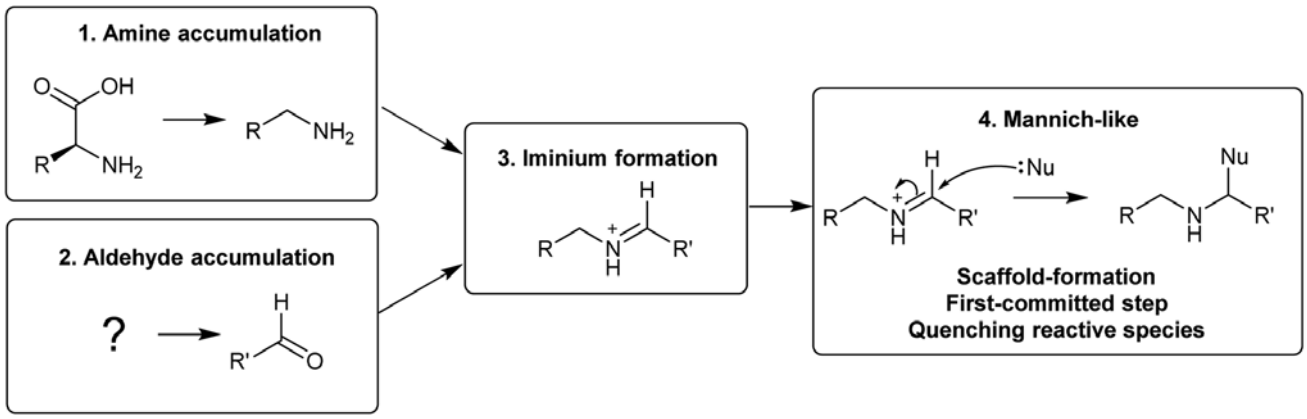

Figure 1: General steps in complex alkaloid biosynthesis (Adapted from Lichman, 2021 under CCBY Attribution, RSC 2021).

3.2 Palmatine isolation from *Tinospora cordifolia*

Methanolic extract was taken from *T. Cordifolia*, stem part was used to split into $CHCl_3$ (Chloroform) solution as well as aqueous solution. The $CHCl_3$ mixture vaporised leaving a viscous dark-brown remnant of 8 gm. The remains on eluted over silica gel column with $CHCl_3$ after which methanol was gradually added to produce 5 fractions. One of the fractions was eluted with a mixture of $CHCl_3$-MeOH in the ratio 10:1 and chromatography was done to get a yield of 2.8gm. The separated remains were then assessed with the use of ultraviolet, infrared, Gas chromatography, Mass spectrometry, and Nuclear Magnetic Resonance spectroscopy. Spectroscopical studies discovered existing alkaloid palmatine in significant concentration. Recrystallization with methanol purified the sample, giving palmatine (Ali et al., 2013).

3.3 Ultrasonic-assisted extraction (UAE) of bioactive alkaloids in *Rhizomacoptidis*

The response surface approach was used to boost the berberine and palmitine alkaloid extraction from *Rhizoma coptidis* utilizing UAE proved effective. The concentration of ethanol and extraction temperature were shown to be key factors in this technique. The best extraction parameters were calculated using an overlapping contour plot to be a 59 % ethanol concentration, a 47-minute extraction duration, and a temperature of 66°C. The presented approach is sensible and might be used to enhance alkaloid extraction from *Rhizomacoptidis* (Teng et al., 2014).

3.4 *Supercritical fluid extraction*

The alkaloid N - [7-(3',4'-methylenedioxyphenyl)-2(Z), 4(Z)-heptadienoyl] pyrrolidine was extracted from the leaves of *Piper amalago* L. Various extraction conditions were utilized to see how they influenced the alkaloid yield. All variables influenced the yield. Mainly Cosolvents make a significant improvement to the alkaloid extraction. The maximum alkaloid content Y_{Ap} = 3.80.8 mg g^{-1} was achieved by using the extract variables provided - a longer extraction period of 60 min, mild temperature and pressure conditions i.e. 40°C and 200 bar respectively, methanol is used as the cosolvent. This is the first study of the pyrrolidine alkaloid extraction conditions from this plant (Carrara et al., 2017).

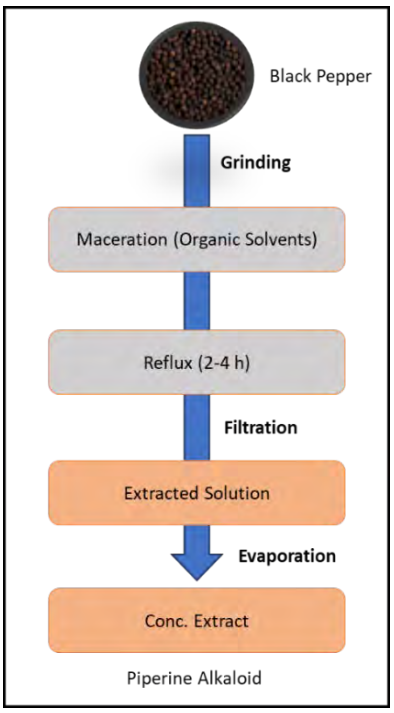

Figure 2: Extraction of piperine from black pepper.

3.5 *Ultrasound-mechanical stirrer assisted extraction (UMSAE) method.*

The dried powder of *Annona muricata* was weighed and added in a beaker to solvent ratios between 1:10 - 1:50 g/mL at different ethanol strengths ranging from 60–100 v/v %, sonicated at the suitable temperature of 30 - 70°C for variable power between 240 - 400 W and time duration between 30 - 60 min. The process was conducted on a 25 L electronic ultrasonic bath with a power of 400 W. After this, the solution was filtered. Then, the extract then

put on a 40°C temperature condition oven until it attained uniform weight. The extract contained the alkaloids Sinomenine, Codeine, N-Methylhernagine, Codeinone, Norcodeine, and Glaucine (Lee et al., 2021).

3.6 *Soxhlet extraction method*

The extraction efficiency of traditional Soxhlet extraction (SE) and UMSAE were compared. A dried powdered *A. muricata* sample weighing 20 gm was covered in a cellulose cover then it wasput in a Soxhlet apparatus. Thisprocess took 7 hours on80°C using 200 mL ethanol as a solvent, and the liquid used for extraction was removed and filtered under vacuum pressure (Dauda et al., 2015).

3.7 *Acid/basic extraction*

The alkaloid extract was yielded by an acid/basic extraction technique. 500g of dry powder of *Prosopis juliflora* was extracted using ethanol while petroleum ether and water were used to fractionate the ethanol extract. The aqua layer containing alkaloids was removed/extracted three times using petroleum ether. Then stirred with 0.2N HCl for 16 hours before being filtered. To eliminate the non-basic ingredient, the solution was shaken with chloroform. Ammonium hydroxide was mixed into the aqueous layer to basify it, then chloroform was used to extract it. The chloroform phase was evaporated, yielding the Alkaloid-rich part. It was maintained at a concentration of 10 mg/ml. Bismuth tetraiodide which serves as an alkaloid detecting reagent was utilised for testing the occurrence of alkaloids (Singh et al., 2011).

3.8 *Bioactive alkaloids extraction using Deep Eutectic Solvents (DES)ws2*

Tailor-made deep eutectic solvents (DESs) have been identified as sustainable and highly effective diluents for the extraction of alkaloids, including Morphine, Berberine, Benzopyrrole, and Norlupinane. While demonstrating commendable success in yielding these alkaloids, it is noteworthy that the DESs exhibited a comparatively lower extraction yield for Bisbenzylisoquinoline. The investigation also highlighted the critical role of water content as a significant factor influencing the extraction process. This research marks a pioneering effort to assess the efficacy of DESs in extracting a diverse range of bioactive alkaloids from herbal medicines. Leveraging their structural flexibility, cost-effectiveness, and minimal environmental impact, tailor-made DESs emerge as promising candidates for sustainable and efficient alkaloid extraction, paving the way for advancements in herbal medicine research and extraction methodologies (Jiang et al., 2019).

3.9 *Tropane alkaloids (TAs) extraction*

The Supported Liquid Extraction method (SLE) was employed for extracting TAs mainly from herbal tea using acidic solution of organic and aqueous solvents like MeOH: AcOH in the ratio of 2:1, ACN: H_2O as 3:2 v/v, 0.2% FA, and MeOH: H_2O as 75:25 v/v, 0.4% FA ([Rivera-Pérez et al., 2023).The herbal SLE extracts were typically filtered and pre-concentrated utilizing the Solid-phase extraction technique (SPE). Thirteen tropane alkaloids were extracted from tea (herbal) using SLE, following a Solid phase extraction clean-up and pre-concentration stage yield in the range of 75 -128% (Romera-Torreset al., 2018). Four Tropane alkaloids:7β-hydroxy hyoscyamine, atropine, homatropine, and hyoscine isolated from tea then diluted to 1:5 with the extractant. However, the recovery rates were considerable (86–103 %), and the limit of quantification (LOQs) of 25.0 g/kg were higher than those reported in the previous study, i.e. 5.0–20.0 g/kg. This illustrates how including concentrating the mixture before analysis and clean-upusing the solid phase extraction step permits reduced LOQs of propane alkaloids in herbal mediums (Cirlini et al., 2019).

Table 1 provides a comprehensive overview of diverse extraction methods utilized in the process of obtaining alkaloids. These methods encompass a range of techniques employed across scientific research and industrial applications to effectively isolate alkaloid compounds from various sources. The table details the methodologies employed, reflecting the versatility and evolving nature of extraction processes in the quest for isolating alkaloids with precision and efficiency. This compilation serves as a valuable reference for researchers, offering insights into the diverse strategies utilized in alkaloid extraction and contributing to the broader understanding of methodologies employed in natural product extraction.

Table 1: Different extraction methods used to extract alkaloids.

Alkaloids	Source	Method of extraction	Ref.
Piperine	Black Pepper	Maceration extract	(Alyaseen et al., 2018)
Palmitine	*Tinospora cordifolia*	Silica gel column chromatography	(Ali et al., 2013)
Berberine and palmitine	*Rhizoma coptidis*	Ultrasonic assisted extraction (UAE)	(Teng et al., 2014)
N-[7-(3',4'-methylenedioxyphenyl)-2(Z),4(Z)-heptadienoyl] pyrrolidine	*Piper amalago*L.	Supercritical fluid extraction (SFE)	(Carrara et al., 2017)
Sinomenine, Codeine, Hernagine, N-Methylhernagine, Codeinone, Norcodeine and Glaucine	*Annona muricata*	Ultrasound-mechanical stirrer assisted extraction (UMSAE)	(Lee et al., 2021)
Tropane	Herbal tea	Supported Liquid Extraction (SLE)	(Rivera-Pérez et al., 2023)

4. Biological activities of alkaloids and their potential applications

4.1 Nicotine (Pyridine group)

Nicotine (Fig. 3) is a naturally found alkaloid present primarily in the dry leaves of the Solanaceae plant Nicotiana tabacum. Nicotine binds with and activates nicotinic acetylcholine receptors (nAChRs). It stimulates chemical transmitter release and alters the conduct of the body. Nicotine activation of central nAChRs causes the discharge of a type of neurotransmitter in the human brain (Benowitz et al., 2009, Shah 2009). Its lot of derivatives have been found to increase dopaminergic neuronal survival and synaptic plasticity. Nicotine also lowers neuroinflammation and oxidative stress, minimising the risk of pathogenic disorders (Kharkwal et al., 2016). Apart from its potential neuroprotective potential in neurodegenerative disorders, nicotine has a variety of negative side effects. Nicotine's toxicity is caused due to the breakdown of mitochondrial membranes, also the generation of reactive oxygen species (ROS). Furthermore, it causes Endoplasmic reticulum stress, which causes protein disintegration with activation of caspase-3 and then ultimately apoptosis (Mayer, 2014).

4.2 Cocaine (Tropane group)

Cocaine (Fig. 3) is obtained from dry leaves of *Erythroxylon coca* and *E. truxillense*, two plants of family, *Erythroxylaceae*. The pure type is a white, crystalline powder. Cocaine has a sympathetic central function and serves as a stimulant to the Central Nervous System (Chen et al., 2019).

4.3 Quinine (Example of quinoline group)

Quinine (Fig. 3) is obtained from *Cinchona officinalis* bark, a *Rubiaceae* family member. It aids in the treatment of malaria. It was the earliest anti-malarial medicine to be utilized. On intra-erythrocytic malaria parasites, it exerts a rapid schizonticidal impact. *Plasmodium vivax* and *P. malariae* are gameto-cytocidal but *P. falciparum* is not (Achan et al., 2011, Manna et al., 2020).

4.4 Berberine (Example of isoquinoline group)

Berberine (Fig. 3) is a type of is quinoline alkaloid found in plants like Berberis vulgaris, Coptis chinensis, and B. aristata. It possesses a variety of medical properties, such as antibacterial, glycemicor cholesterol-reducing, anti-cancer, also immune-modulatory properties. Various findings implied Berberine to have cardiovascular advantages due to its vasodilator and hypotensive qualities, as well as its ability to avoid heart disease, cardiac hypertrophy, arrhythmia, etc (Pirillo et. al., 2015).

4.5 Morphine (Example of phenanthrene group)

Morphine (Fig. 3) is an isoquinoline alkaloid that is used to alleviate pain and has significant narcotic and analgesic qualities. The analgesic action of morphine is mediated by the μ-opioid receptor (Pang et al., 2015, Kaur et al., 2015). Several experimental studies have shown that morphine can be helpful in the therapy of nervous system injury (Ye et al., 2014).

4.6 Vinblastine (Example of indole group)

Vinblastine (Fig. 3) is obtained by the Apocynaceae family's Catharanthus roseus. It is an anticancer medication that stops mitosis during metaphase through binding to microtubule resulting in microtubule crystallization and mitotic arrest/apoptosis. It possesses an immunosuppressive effect. The most frequent side effects include coughing, fever, and uncomfortable urination (Moudi et al., 2013).

4.7 Capsaicin (Terpenoid group)

It can be seen in the fruits of various Solanaceae pepper types, genus Capsicum. Capsaicin (Fig. 3) can attach to the Transient receptor potential cation channel (TrpV1), which is found primarily in sensory neurons. It has been proven to have irritative and analgesic characteristics, as well as to affect thermoregulation and adipose tissue metabolism, antioxidative, hypotensive, and antibacterial potentials. The most frequent harmful reactions include Dysesthesia, dehydration, discomfort, inflammation, or pain (Adaszek et al., 2019).

4.8 Caffeine (Example of purine group)

Caffeine (Fig. 3) is a methylxanthine component extracted from the Coffea arabica, and it is extensively used as a psychoactive stimulant, mostly in beverages (Choudhury et al., 2014). Caffeine has been shown to have a particularly beneficial pharmacological influence on the prevention of neurodegenerative disorders (Faudone et al., 2021).

4.9 Piperine

It is the principal alkaloid present in Piper longum and P. nigrum, both members of the Piperaceae family (Ghavami et al., 2014). Piperine (Fig. 3) is found to have a wide spectrum of pharmacological effects, including anti-inflammatory, anti-fungicidal, insecticidal, anti-hypertensive, anti-pyretical, anti-cancerous, painkilling potential, and various neuroprotective qualities like antioxidant, antidepressant, and antiepileptic (Yeggoni et al., 2015, Damanhouri et al., 2014).

4.10 Hordenine

It is a naturally occurring phenethylamine substance found Hordeum vulgare, a cereal belonging to the Poaceae family. Hordenine (Fig. 3) has a similar structure as tyramine, that generated from tyrosine. It is a natural cerebral stimulant chemical which improves cerebral function. Hordenine works as an MAO-B (monoamine oxidase-B) suppressor. Classified as a noradrenaline uptake restrictor as it aids in enhancing the level of nor-epinephrine (Debnath et al., 2018).

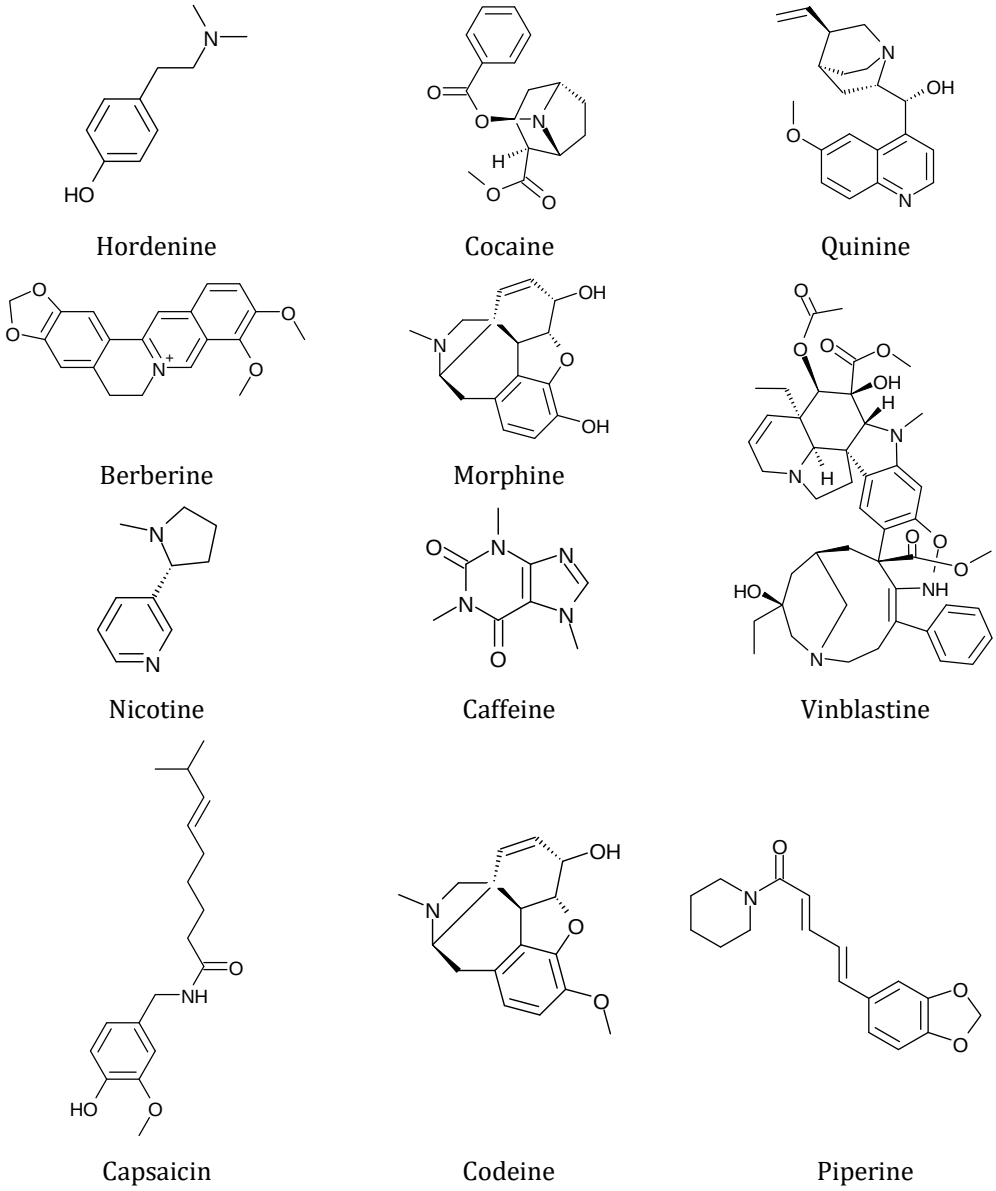

Figure 3: Chemical structures of some alkaloids.

4.11 Codeine

Codeine (Fig. 3), derived from the opium poppy (Papaver somniferum L.), is a versatile pharmaceutical compound renowned for its dual roles as an antitussive and analgesic agent. Beyond its well-established applications, codeine demonstrates intriguing properties, including potent antioxidant capabilities, making it valuable in combating oxidative stress. This aspect positions codeine as a multifunctional remedy with potential benefits in reducing the impact of free radicals and supporting overall cellular health. Additionally, its antimutagenic and anticarcinogenic effects suggest a broader role in genetic stability and cancer prevention, elevating codeine's significance in the evolving landscape of therapeutic interventions.

5. Different types of antioxidant assays

Prevalent assays utilized for evaluating radical scavenging activity like a source of new antioxidants, a plant's antioxidant capacity should first be assessed. Evaluating the radical scavenging activity is a common way of testing antioxidant activity. The most frequent assays used to evaluate radical scavenging activities are listed here (Adegbola et al, 2020).

5.1 DPPH free-radical scavenging activity

The 2, 2-diphenyl—picrylhydrazyl radical scavenging activity of extract containing abundant alkaloids was accomplished through combining 1 mL alkaloid plenty extract of different doses by 1 mL 0.1 mM DPPH mixture. As a reference, the same quantity of methanol and DPPH was used. The optical density was found out at 517 nm after 20-minute incubation in the dark. The following formula was used to compute the % Scavenging = [(AC – AS)/AC] 100%, as AC - the control transmittance, AS - the sample transmittance (Gurudeeban et al., 2015).

5.2 FRAP Assay (Ferric Reducing Antioxidant Power Assay)

Reduction potential evaluated utilising the Oyaizu approach. Examined substances in quantity of 0.5 mL combined using phosphate buffer of 0.5 mL, 0.2 M, pH 6.6 and potassium ferricyanide [K3Fe(CN)6] at various strengths 0, 50, 100, 150, 200 mg/mL(0.5 mL, 1%). The solution was kept at 50 °C about 20 minutes. After that, mixture was centrifuged about 10 min on 3,000 rpm using 0.5 mL 10% trichloroacetic acid (1 kg). The mixture's top layer (0.5 mL) was mix up in distilled water (0.5 mL) and FeCl3 (0.1 mL, 0.1 percent) for 10 minutes before determining absorbance at 700 nm with a UV-Vis spectrometer.The blank reference was ethylene diamine tetra acetic acid (EDTA) (Zahari et al., 2016).

5.3 Hydroxyl radical scavenging

The method is measured by theamount of 2-deoxyribose breakdown product by concentrating usingThio-barbituric acid (TBA). The Fe3+-ascorbate-EDTA-H2O2 system produced hydroxyl radicals through the Fenton reaction.The reaction mixture comprises 2-deoxy-2-ribose (2.8 mM), KH2PO4-KOH buffer (20 mM, pH 7.4), FeCl3 100 M, EDTA 100 M, H2O2 (1.0 mM), ascorbic acid 100 M, and different strengths of the test sample or reference component in a final volume of 1 ml. After 1 hour at 37°C, 0.5 ml of the reaction media was added to 1 ml of 2.8% TCA, followed by 1 ml of 1% aqueous TBA, and the mixture was incubated at 90°C for 15 minutes to generate the colour. The absorbance at 532 nm was measured after cooling in comparison to blank sample. All tests were carried out 6 times. A well-known OH scavenger, mannitol, has been used as a blank solution. In comparison of the test and blank mixtures, the percentage inhibition was calculated (Chaudhuri et al., 2012).

6. Recent aspects of antioxidant activity of alkaloids

6.1 Antioxidant effects of the highly substituted carbazole alkaloids

The 3,8-dihydroxycarbazoles carbzomadurins and its synthesized precursors outperformed 3-monohydroxycarbazoles carazostatin and the synthetic precursors of carquinostatin A in DPPH-radical and ABTS+ (2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) assays. In the PAO-SO (Potential Antioxidant in Oil Solution) analysis, 3,8-dihydroxycarbazoles carbazomadurin and synthesized precursors outperformed all 3-monohydroxycarbazoles in terms of total potential capacity. The overall potential antioxidant potential of 3,4-Dioxocarbazole carquinostatin was the lowest of all. The radical scavenging actions of 3-hydroxy- and 3,8-dihydroxycarbazoles may attribute upon participating N atom positioned on the pyrrole structure in the formation of an iminoquinone structure by contributing anH radical or an electron to the free radical (Hieda et al., 2014).

6.2 Antioxidant Activities of Compounds from Nelumbo nuciferaGaertn. cv. Rosa-plena

In the ABTS and DPPH tests, O-Nornuciferineandlysicamine show radical scavenging activity equivalent to vit C on similardosageie 100µM. The Fe2+ ion chelating actions of studied samplesare described in comparison to Butylated hydroxyanisole (BHA) - standard solution. In the chelating assay, only (-)-nuciferine and pheophytin-A had limited impact. Lysicamine has a low ferric reducing power. Furthermore, the ABTS and DPPH tests show that the Methanol extract of leaves has 35.6 % and 40.2 % radical scavenging activity, respectively. The methanolicleaves extract has a ferrous ion chelating activity of 13.3%. According to these findings, the radical scavenging activity of Methanol extract obtained is mostly due to the occurence of (-)-N-methylasimilobine and lysicamine (Liu et al., 2014).

6.3 Antioxidative properties of Delphinium linearilobum

Norditerpene alkaloids isolated from the roots of *D. linearilobum*, particularly linearilobin, linearilin, and lycotoninebrowniine, were shown to have antioxidant action for DPPH and metal chelating assays. El-Desouky et al. discovered the strong DPPH activity of pyrrole alkaloid isolated, effect of alkaloids like berberine, canadine, anonaine, and antioquine in the same way as α-tocopherol and trolox were discovered (Kaur et al., 2015).

6.4 Antioxidant activity of cinchona alkaloids

Cinchona has strong antioxidant activities, as reported by Gurung et al., (2017). The study focused primarily on three radicals: superoxide, hydroxyl, and nitric oxide since these are the primary radicals accounting for the oxidative destruction of the human body cellular components. By suppressing DPPH, methanolic and water extracts demonstrated concentration-dependent antiradical effects. Cinchona extract's potential to scavenge free radicals was confirmed via methanolic extract.

6.5 Mahanimbineas an antioxidant

Aegle marmelos and *Murrayakoenigii*, *Rutaceae* plants displayed fine antioxidant abilities. The antioxidant activities of *Murrayakoenigii*extractis greater as compared to*Aegle marmelos*extract in free radical scavenging activities and metal ion reducing ability. In this study it was concluded that Mahanimbineis the most potentially biologically active compound according to the results of FRAP and ABTS, thus it may act as an antioxidant (Ng et al., 2018).

6.6 Antioxidant property relation with a hydroxyl group

The novel compounds, as well as the existing alkaloids, were examined for antioxidative action in vitro with the help of the DPPH test. All of the products exhibited antioxidant activity against ascorbic acid, which served as the control sample (Aldulaimi et al., 2019). The existence of a hydroxyl group may explain why iraqiine, muniranine, and kinabaline have greater activity than the other substances (Othman et al., 2017). The isoquinolineoxoaporphine alkaloids which don'tcomprise an OH group have a low DPPH activity.

6.7 Superoxide dismutase (SOD) and ascorbate peroxidase (APX) analysis

Rauvolfia serpentina dry samples were made in powder form with liquid nitrogen, and several assays were performed using the powder after dissolving in the required solvents/buffers. With slight adjustments, superoxide dismutase (SOD) and ascorbate peroxidase (APX) were studied. SOD activity (one unit) was equivalent to 50% colour removal and was stated in enzyme unit (EU)/mg protein/h which tells about the minimum amount of enzyme required to break down ascorbate (1.0 mM) for each min. The coefficient of absorption (2.8 mM/cm) was used to assess APX enzyme activity (Dey et al., 2020).

6.8 The antioxidant property of imidazole alkaloids

The "density functional theory" (DFT) and the "Marcus theory" were used to investigate the redox reactions of 5 alkaloids in aq.This showed that the mono-anion is the most active species, however, this is dependent on their molar fraction. The imidazole group of the neutral class, as per the reactivity criteria, plays a vital role if an electron transfer reaction occurs; although, it is not thermodynamically desirable. Considering their thermal and kinetic information, 3 lepidines were discovered to be considerably effective as hydroperoxyl free radical scavengers. The rate constants for mono-anion species were found to be comparable and about 10^6 $M^{-1}s^{-1}$. It possesses crucial suggestions about the free radical scavenging ability for this class of chemicals as mono-anion species must be prevalent in given physiological circumstances based on their pKa values. As a result, the occurrence of at least one active species under such circumstances is confirmed by this dispersion. As a result, under physiological conditions, lepidines are projected to be capable of deactivating hydroperoxyl free radicals in aqueous medium. Acid-base equilibrium was discovered to be essential in their activity (Pérez-González et al., 2020).

6.9 Quinoline and Isoquinolineas antioxidants

The computational DFT methodology was utilised for successfully examining antioxidant activity in alkaloidal constituent-type quinolone as well asisoquinolone. The projected results imply that the SPLET (sequential proton loss electron transfer) route was beneficial for antioxidative effect in two investigated alkaloids in solvents, particularly Dimethyl sulfoxide (DMSO). Chemical parameters like ionisation energy, spin density, and proton affinity deliver important supporting data to confirm that radical quenching processes occur primarily through the rupture of O–H and N–H bonds. The 5-OH group of compounds has the lowermost proton affinity value in DMSO of 24.84 kcal/mol (Dung et al., 2020).

6.10 AntioxidantProperties of Resperine and Barettin

The DPPH assay revealed that reserpine inhibits the DPPH radical by 42%. 2 distinct biochemical tests were used - FRAP and ORAC "Oxygen Radical Absorbance Capacity", for determining barettin's antioxidant activity. Barettin was found to have a possible antioxidant characteristic that is dose-dependent. Barettin showed values of FRAP and ORAC came to be 77 and 5.5 µM Trolox equivalents (TE) on a dosage of 30 µg/mL, respectively (Dey et al., 2020).

6.11 FRAP of different varieties of cocoa

The ferric-reducing activities of cocoa soluble partsvaried from 350 to 1,483 µmol Fe^{2+} g^{-1}. These findings are consistent with the reporting (Di Mattia et al., 2013) of the discoverer of FRAP values ranging from 713 to 930 µmol Fe^{2+} g^{-1} in raw Costa Rican nibs. 141 mol TE was discovered for nibs (Vertuani et al., 2014). These findings support the notion that the source of cocoa employed in premium chocolates has stronger antioxidant activity compared to unidentified cheap beans allocated for customary items. Commonly, antiradical activity like DPPH, ABTS, and ORAC assays carried to soluble fraction displayed close similarity to FRAP, with the soluble fractions exhibiting stronger radical scavenging activity in comparison to that of the insoluble fractions.

6.12 Supramolecular Nano-capsules of harmala alkaloids

Peganum harmala seeds were used to extract the alkaloid-rich portion of harmala. Using a thin-film hydration method, this portion then encapsulating into amphiphilic p-SC6 self-build nanovesicles. The nanocapsules were developed using the three alkaloids namelyVasicine, harmmol, and Banisterine. The antioxidative property of H/p-SC6 nanocapsules using DPPH reported to be 5 times as much as the harmala alkaloid-rich portion. When the nanocapsules were tested on human skin fibroblasts, they were seen to be safe and nontoxic. Thus, encapsulating *P. harmala* alkaloid-plenty portions in self-accumulated supramolecular nanocapsules significantly boosts the natural antioxidant properties of harmala alkaloids (de Rezende-Mudenuti et al., 2021; Fahmy et al., 2021; Beemkumar et al., 2023)

6.13 Role of antioxidants in diabetes

Hyperglycemia generates oxidative stress, which leads to micro- and macrovascular problems. Diabetes issues can be addressed with antioxidant treatment. Antioxidants response to reactive radicals by taking or giving electrons, by reducing the production of free radicals due tohindering the free radical-producing enzymes, else via improving the activities and exhibition of enzymes accountable for antioxidant synthesis. Alkaloids likeoriciacridone demonstrated antioxidant action in contrast to α-glucosidase inhibitory activity (Adhikari et al., 2021, Yusuf et al., 2023).

6.14 Antioxidant Activity Against Breast Cancer Cells

Antioxidant chemicals were extracted and analyzed from the root of *Berberis aristata* - BA (*Berberidaceae*). To test the bioactivity of the compounds, antioxidant assays were performed. Berberine, Jateorrhizine, and Thallifendine were discovered, and found to have antioxidant activity over breast cancer cells. According to this research, the isolated chemicals target EGFR (epidermal growth factor receptor), which is highly expressed in breast cancers (Kumari et al., 2022).

6.15 In Vitro Antioxidant activity

This research looked at the chemicals produced by plants and in-vitro antioxidative properties in *Papaver decaisnei*, indigenous species in the plant kingdom of Kurdish-Iraqi flora. Plant extract's antioxidant activity were between 39.1-143.5 µg/ml for DPPH and 123.12-276.4 µg/ml for ABTS tests, whereas FRAP results were between 12.4-34.3 and 42.6-75.8 µg/ml for CUPRAC test (Jabbar et al., 2022).

Table 2 outlines and extensively explores the antioxidant activities exhibited by various classes of alkaloids. The table serves as a comprehensive compilation, detailing the diverse antioxidant properties inherent in alkaloids of different types. It delves into the specific antioxidant mechanisms associated with each class of alkaloids, shedding light on their potential applications in mitigating oxidative stress and promoting cellular health. This comprehensive analysis offers valuable insights into the multifaceted roles played by alkaloids in antioxidant activities, providing researchers and practitioners with a nuanced understanding of the potential therapeutic benefits associated with different types of alkaloids in combating oxidative damage.

Table 2: Antioxidant activities of different types of alkaloids.

Alkaloid studied	Antioxidant assay used	Result and Discussion	Ref.
3,8-dihydroxycarbazoles carbzomadurins	DPPH, ABTS+ & PAO-SO	Radical scavenging activity by the involvement of N atom at pyrrole ring in forming an iminoquinone structure through the donation of H^+or a free radical provided with an electron	(Hieda et al., 2014)
(−)-N-methylasimilobine and lysicamine (*N. nucifera*)	DPPH & ABTS	Radical scavenging activity of the methanolic leaves extract is35.6% by ABTS and 40.2% by DPPH assays. The Fe^{2+} ion chelating activities is 13.3%.	(Liu et al., 2014)
Linearilobin, Linearilin, Lycotonine&Browniine (*Delphinium linearilobum*)	DPPH and metal chelating assays	Strong radical scavenging ability found	(Kaur et al., 2015)
Quinine (Cinchona)	DPPH (free radicals present superoxide, hydroxyl and nitric oxide radicals)	Methanolic and water extract displayed a concentration reliant antiradical activity by inhibiting DPPH.	(Gurung et al., 2017)
Mahanimbine (*Aegle marmelos* and *Murrayakoenigii*)	FRAP & ABTS	The antioxidant activity of *Murrayakoenigii* extract is greater than *Aegle marmelos* extract	(Ng et al., 2018)
Iraqiine, Muniranine and Kinabaline	DPPH	Higher activity due to the presence of hydroxyl group	(Othman et al., 2017)
Vasicine, Harmmol, and Banisterine	DPPH	Encapsulating harmala alkaloid-rich portion to self-build supramolecular nanocapsules improves innate antioxidative properties	(Fahmy et al., 2021)

7. Conclusion

Alkaloids are naturally occurring chemical molecules that are primarily composed of basic nitrogen atoms. They are a diverse and important group of plant compounds that possess therapeutically beneficial properties. The alkaloids are classified into several categories, including indole, purine, quinoline, isoquinoline, tropane, imidazole, and more. Several extraction methods for alkaloids have been studied, such as the extraction of piperine alkaloids, which can be identified using tests like Wagner's, Mayer's, melting point, and TLC. Other extraction techniques, such as the isolation of Palmatine from *Tinosporacordifolia*, UMSAE method, and Acid/basic extraction, among others, have also been discussed. Researchers have discovered various conditions that improve the alkaloid extraction process. The text also summarizes the biological activities and applications of specific alkaloids. Alkaloids containing antioxidants can be useful for diabetic patients. Recent studies on the antioxidant activity of alkaloids have been studied and tabulated. Various procedures have been done over the alkaloids to increase their antioxidant properties. The article also includes a comparison of different alkaloids obtained from various sources.

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