COMPARATIVE PHYTOCHEMICAL AND BIOACTIVITY ASSESSMENT OF *CRINUM* SPECIES FROM VARIED ECOLOGICAL NICHES

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the Mahatma Ghandhi University in partial fulfilment of
the requirement for the degree of

MASTER OF SCIENCE IN BOTANY

By

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DECLARATION

I hereby declare that the work which is being presented in the dissertation, entitled **"Comparative Phytochemical and Bioactivity Assessment of *Crinum* Species from Varied Ecological Niches"** in fulfilment of the requirements for the award of the degree of **Master of Science in Botany and submitted to St. Teresa's College (Autonomous), Ernakulam** is an authentic record of my own work carried out during M.Sc. period under the supervision of Ms. I. K. Nishitha. The matter embodied in this dissertation has not been submitted by me for the award of any other degree of this or any other University/ Institute.

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Sameena V. S.

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1. INTRODUCTION

India's remarkable biodiversity plays a crucial role in sustaining ecosystems, supporting both life and livelihoods. Despite occupying only 2.4% of the world's land area, the country harbors 7-8% of the world's recorded species. This vast biodiversity includes approximately 45,000 plant species, which contribute significantly to ecological balance and provide essential resources for numerous life forms (Tiwari & Kumar, 2024; Basu *et al*., 2023). For centuries, native plant species have been integral to India's traditional medicinal systems, including Ayurveda, Siddha, and Unani. These practices have generated extensive knowledge about the therapeutic properties of plants, emphasizing the necessity of conserving these valuable resources for future generations (Dev, 1997).

India is home to over 8,000 species of medicinal plants, constituting nearly 50% of the country's higher flowering plants (Chandra, 2016). These medicinal plants are rich in diverse phytochemicals, including alkaloids, tannins, carotenoids, proteins, chlorophyll, phytosterols, glycosides, phenols, flavonoids, and diterpenes. These compounds exhibit various pharmacological activities such as antibacterial, antioxidant, anti-inflammatory, antidiabetic, anticancer, antiulcer, hepatoprotective, and cardioprotective properties (Harish *et al*., 2024). These plants have the potential to treat a wide range of human ailments including diabetes, migraine, eczema, cancer, cardiovascular disorders, jaundice, iron overload-related diseases, premenstrual syndrome, asthma, menopausal symptoms, chronic fatigue, irritable bowel syndrome, and rheumatoid arthritis (Kamboj, 2000). Given their bioactive potential, these plants have significantly contributed to drug discovery, particularly in plant families such as Amaryllidaceae, Asteraceae, Fabaceae, and Apocynaceae. Among these, members of the Amaryllidaceae family are well known for producing bioactive alkaloids with a broad spectrum of pharmacological activities, making them promising candidates for novel drug development (Danquah *et al*., 2022).

Amaryllidaceae Family

The Amaryllidaceae J. St.-Hil. is a family of monocotyledonous, bulbous flowering plants comprising over 1,600 species across 71 genera. Although the family is distributed pantropically, it is particularly abundant in three geographic regions: South

America, the Mediterranean, and southern Africa (Nair & Van Staden, 2013). These plants are primarily terrestrial (rarely aquatic), perennial, and bulbous, though some exhibit rhizomatous growth. The family derives its name from the genus *Amaryllis*, commonly known as the Amaryllis family. The leaves are often linear, while the flowers, typically bisexual and symmetrical, are arranged in umbels on a leafless stalk. The tepals undifferentiated petals and sepals form a floral tube at their base, sometimes featuring a corona. The characteristic scent of the onion subfamily (Allioideae) arises from compounds containing allyl sulfide. Amaryllidaceae species are widely recognized for their ornamental appeal as well as their unique alkaloid profiles, which contribute to various biological activities (POWO, 2025). Amaryllidaceae family is well known for its biochemical richness and aesthetic appeal (Zhong, 2016).

Crinum L.: An Important Genus of Amaryllidaceae

The genus *Crinum* derives its name from the Greek word "Krinon," meaning "white lily." It encompasses approximately 180 species of perennial plants, distinguished by their large, striking flowers borne on leafless stalks. *Crinum* species are typically found in seasonally moist habitats such as marshes, swamps, depressions, and riverbanks across tropical and subtropical regions. These plants hold substantial ornamental and medicinal value, as they are widely utilized in traditional medicine for their diverse pharmacological properties (Kwembeya *et al*., 2007; Afroz *et al*., 2018).

Plants in this genus are herbaceous plants that have large fleshy, tunicated bulbs which develop into a pseudostem formed by bases of old leaf sheathe, leaves are sword-linear in shape, they are arranged in a rosette or leaves may be arranged oppositely in rows of two, and the leaves have a sheathing base. There is one to several flowers in the inflorescence; the flowers have two spathe valves (bracts) that are spur-like from the side of the tall peduncle, the stamens can either be downwardly angled, curved or upright, the ovary which is either on the flower stalk or in the flower stalk tube, appears swollen. The berries are subglobose, sometimes beaked and the seeds are subglobose, they have a smooth, hairy or mostly impermeable seed coat (POWO, 2025).

The medicinal significance of *Crinum* stems from its rich phytochemical composition, particularly its alkaloid content. More than 170 distinct bioactive compounds have been identified within the genus, including galanthamine, haemanthamine, crinamine, and lycorine. These alkaloids have demonstrated therapeutic potential in treating conditions

such as Alzheimer's disease, inflammation, infections, and cancer (Mimrot *et al*., 2024; Chaichompoo *et al*., 2024). Additionally, *Crinum* species contain high levels of phenolic compounds, flavonoids, and antioxidants, which contribute to their anti-inflammatory, cardioprotective, and neuroprotective effects (Araújo *et al*., 2023; Masi *et al*., 2021). The antioxidant properties of *Crinum* species are often evaluated using assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), and reducing power assays. These methods help determine the plant's capacity to neutralize free radicals, thereby mitigating oxidative stress-related diseases such as anaemia and neurodegenerative disorders (Islam *et al*., 2024; Mehmood *et al*., 2023).

Distribution of *Crinum* L. in India

In India it is widely distributed throughout the country and represented by 15 different species viz; *C. asiaticum, C. amoenum* Roxb. ex Ker Gawl, *C. brachynema* Herb., *C. humile* Herb., *C. lorifolium* Roxb., *C. latifolium, C. malabaricum* Lekhak & S.R.Yadav, *C. reddyi* M. Patel & H. Patel, *C. pusillum* Herb., *C. solapurense* S.P.Gaikwad, K.U.Garad & R.D.Gore, *C. stenophyllum, C. stracheyi* Baker, *C. viviparum* R. Ansari & V.J. Nair, *C. woodrowii* Baker and *C. wattii* Baker (Lekhak and Yadav, 2012; Lekhak *et al*., 2015; Patel and Patel, 2019).

Crinum species thrive in wetland habitats, including marshes, riverbanks, and coastal regions, which provide the necessary moisture for their growth. Kerala, known for its rich biodiversity, hosts several species of *Crinum*. The state's unique climatic conditions, characterized by high humidity and abundant rainfall, create an ideal environment for these plants. *Crinum* species in Kerala are often found in paddy fields, along water bodies, and in gardens, where they are cultivated for ornamental purposes. *Crinum* species prefer well-drained soils that retain moisture (Bulletin of the Botanical Survey of India, 2024).

Among the notable Indian species, *Crinum malabaricum* Lekhak & S.R. Yadav is a newly described species found in a seasonal stream in Periya, Kerala. This discovery highlights the region's unique aquatic habitats and underscores the ecological diversity within the genus (Lekhak & Yadav, 2012). Other recently identified species include *Crinum andhricum,* discovered in the Northern Eastern Ghats of Andhra Pradesh, and *Crinum solapurense,* identified in Maharashtra's Solapur district near the Bhima River

(Rasingam *et al*., 2024; Gaikwad *et al*., 2014). These findings demonstrate the importance of systematic taxonomic studies and conservation efforts to protect these valuable species from habitat loss and ecological degradation.

Biochemical Importance of *Crinum* L.

The therapeutic properties of *Crinum* species have been extensively studied due to their diverse pharmacological activities and rich phytochemical composition. These plants are particularly known for their high concentrations of bioactive compounds, including flavonoids, alkaloids, phenols, and antioxidants. Thus, members of the genus *Crinum* are commonly used in treatment of various painful and inflammatory disorders such as rheumatism, earache, lumbago, edema, headache, worm infestation, vomiting, swelling, backache, wounds and haemorrhoids (Refaat *et al*., 2013).

Among the most significant bioactive compounds in *Crinum* species, alkaloids contribute substantially to their medicinal properties. Key alkaloids such as galanthamine, haemanthamine, crinamine, and lycorine have demonstrated potential in the treatment of Alzheimer's disease due to their neuroprotective effects (Mimrot *et al*., 2024; Chaichompoo *et al*., 2024). These compounds are widely recognized for their broad spectrum of biological activities and therapeutic potential.

Additionally, *Crinum* species are rich in phenolic compounds and flavonoids, which contribute to their strong antioxidant and anti-inflammatory properties. These compounds help combat oxidative stress which is induced by free radicals, a major factor in the development of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions (Araújo *et al*., 2023; Masi *et al*., 2021). Flavonoids, in particular, play a crucial role in scavenging free radicals, reducing oxidative damage, and modulating inflammatory responses. By neutralizing free radicals, antioxidants can help protect cells from damage and promote overall health. Beyond oxidative damage, there are neurodegenerative disorders like Alzheimer's disease that are intimately linked with disorders of enzyme regulation, particularly that of an overactive acetylcholinesterase (AChE), which is responsible for the breakdown of acetylcholine, a neurotransmitter of critical importance to cognition. This suggests that compounds with antioxidant properties along with AChE inhibitory agents could prove efficacious therapeutically (Zhan *et al*., 2017).

Antioxidant activity is a critical parameter when evaluating the medicinal potential of *Crinum* species. Commonly used assays for measuring antioxidant capacity include DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), and reducing power assays. These methods assess the ability of plant extracts to neutralize free radicals, thereby mitigating oxidative stress-related disorders such as anaemia and degenerative diseases. The high antioxidant potential of *Crinum* species underscores their pharmacological significance and justifies further investigation into their therapeutic applications.

Research Gap

Despite numerous studies on *Crinum* species, significant research gaps persist, particularly regarding their phytochemical profiles. Most studies have focused on the leaves, overlooking the potential of bulbs as reservoirs of bioactive compounds. Additionally, comprehensive comparative analyses of *Crinum* species from different habitats—aquatic, semi-aquatic, and terrestrial—remain largely unexplored. Understanding how environmental factors influence phytochemical diversity and antioxidant activity is crucial for optimizing the therapeutic applications of *Crinum* species.

The present study aims to conduct comparative analysis of methanolic extracts from the leaves and bulbs of three *Crinum* species from distinct ecological conditions: *Crinum asiaticum* (terrestrial), *Crinum viviparum* (semi-aquatic) *Crinum solapurense* (aquatic). The study will evaluate their phytochemical diversity (alkaloids, flavonoids, and phenols), antioxidant activity (DPPH, ABTS, and FRAP), and Acetylcholinesterase inhibition property. This research will contribute valuable insights into the pharmacological potential of *Crinum* species and support their conservation and sustainable utilization.

Objectives

- To perform a comparative examination on selected *Crinum* species from different ecological habitats (terrestrial, semi-aquatic and aquatic).
- To assess the phytochemical quantification (Total Alkaloid Content, Total Flavonoid Content and Total Phenolic Content) of the methanolic extract of the selected *Crinum* species.

- To evaluate the antioxidant activity (DPPH, ABTS and FRAP) of the selected *Crinum* species using *in vitro* antioxidant assays.
- To examine the Acetylcholine esterase inhibition property of selected *Crinum* species.

2. REVIEW OF LITERATURE

Amaryllidaceae; a biochemical rich family

The Amaryllidaceae family, belonging to the order Asparagales, is a large group of flowering plants, known for their extensive array of alkaloids with a range of interesting biological activities. It includes 85 or so genera and around 1600 species, mostly in the world's tropical and warm temperate regions, mainly rich in three regions of the world: South America, the Mediterranean, and southern Africa (Meerow & Snijman, 1998; Nair & Van Staden, 2013). This family is well known for their phytochemical diversity, particularly their alkaloids. More than 600 structurally diverse alkaloids have been fundamentally recognized from Amaryllidaceae plants. Biological activities are generally numerous for these alkaloids such as antitumor, antibacterial, antifungal, antimalarial, antiviral, analgesic, and cholinesterase inhibitory activities (Meerow & Snijman, 1998; Ding *et al*., 2017; Alcaraz *et al*., 2023). The Amaryllidaceae family consists of three subfamilies: Agapanthoideae, Allioideae, and Amaryllidoideae (Meerow, 2023).

The Amaryllidaceae members are likely to be terrestrial (though rarely aquatic) perennial bulbous plants, with some being rhizomatous. The family is named after the genus Amaryllis, or commonly known as the Amaryllis family. Leaves are usually shaped like blades, while flowers are mostly bisexual and symmetrical, and inflorescences are typically arranged in umbels attached to a leafless stalk. The tepals indistinguishable petals and sepals form a floral tube at their base, and occasionally include a corona. The characteristic smell of the onion subfamily (Allioideae), is derived from compounds containing allyl sulfide. Amaryllidaceae species are well recognized for their aesthetic appeal, along with their unique alkaloid composition that contributes to their varied biological activities (POWO, 2025). Amaryllidaceae family is recognized for their biochemical richness and aesthetic appeal (Zhong, 2016).

Earlier reports reported more than 600 alkaloids, that have been discovered from the Amaryllidaceae family, commonly referred to as Amaryllidaceae alkaloids, mostly from genera's like *Hymenocallis*, *Zephyranthes*, *Nerine*, *Crinum*, *Hippeastrum*, and *Lycoris* which demonstrates the ongoing researches and unveiling new compounds within this group (Jin, 2016; Evidente, 2023). The first Amaryllidaceae alkaloid that

was isolated was from *Narcissus pseudonarcissus*, in 1877, and named lycorine. However, the discovery and approval of the Amaryllidaceae alkaloid galantamine, for use in treating a neurodegenerative condition such as Alzheimer's disease, was likely the important outcome which initiated Chinese and Western scientific interests in the family (Heinrich *et al*., 2004). The key alkaloids of the family include galantamine, lycorine, norbelladine, hippeastrine, narwedine, haemanthamine, pancratistatin, pretazettine, montanine, galanthindole, cherylline and ismine. These secondary metabolites have extensive array of pharmacological properties such as antitumor, antifungal, antimalarial, antiviral, antibacterial, analgesic, cytotoxic activities and acetylcholinesterase (AChE) inhibitory (He *et al*., 2015).

Noteworthy genera within the family include Allium, Nerine, and *Crinum*; as the latter is the largest genus in a specific geographic area (Zimudzi *et al*., 2006). Within traditional medicine systems, Amaryllidaceae species have important analgesic, anti-inflammatory, antiviral, antimicrobial and anticancer activities (Ji *et al*., 2013). Amaryllidaceae plants have a rich history as medicinal plants to treat ailments such as fever, inflammation, and tumours. Previous research has also established anti-tumour, anti-inflammatory, antibacterial, antiviral, and antimalarial activities for these plants. The biological activities have been attributed to various alkaloids. Various studies had shown their alkaloids can influence multiple biological pathways (Jian-rong *et al*., 2011; Ji *et al.,* 2013; Gonzalez *et al*., 2020).

A review on genus *Crinum*

Crinum, an alkaloid-rich genus within the Amaryllidaceae family, is widely recognized for its therapeutic uses and ornamental value. The genus *Crinum* derives its name from the Greek word "Krinon," meaning "white lily." It encompasses approximately 180 species of perennial plants, distinguished by their large, striking flowers borne on leafless stalks. *Crinum* species are typically found in seasonally moist habitats such as marshes, swamps, depressions, and riverbanks across tropical and subtropical regions. These plants hold substantial ornamental and medicinal value, as they are widely utilized in traditional medicine for their diverse pharmacological properties (Kwembeya *et al*., 2007; Afroz *et al*., 2018).

Plants in this genus are herbaceous plants that have large fleshy, tunicated bulbs which develop into a pseudostem formed by bases of old leaf sheathe, leaves are sword-linear

in shape, they are arranged in a rosette or leaves may be arranged oppositely in rows of two, and the leaves have a sheathing base. There is one to several flowers in the inflorescence; the flowers have two spathe valves (bracts) that are spur-like from the side of the tall peduncle, the stamens can either be downwardly angled, curved or upright, the ovary which is either on the flower stalk or in the flower stalk tube, appears swollen. The berries are subglobose, sometimes beaked and the seeds are subglobose, they have a smooth, hairy or mostly impermeable seed coat (POWO, 2025).

Therapeutic potential of *Crinum*

The therapeutic properties of *Crinum* species have been extensively studied due to their diverse pharmacological activities and rich phytochemical composition. These plants are particularly known for their high concentrations of bioactive compounds, including flavonoids, alkaloids, phenols, and antioxidants. Thus, members of the genus *Crinum* are commonly used in treatment of various painful and inflammatory disorders such as rheumatism, earache, lumbago, edema, headache, worm infestation, vomiting, swelling, backache, wounds and haemorrhoids (Refaat *et al*., 2013). Traditional medicine has called upon *Crinum* species over several thousand years for treatment for many ailments, including rheumatism, headache, backache, edema, hemorrhoids, earache, piles, leprosy, cold, cough, vomiting, flatulence, carbuncles, swelling, intestinal complaints, and wounds (Cox, 2000; Refaat *et al*., 2013).

Crinum asiaticum is known for its extensive pharmacological activities, including anti-tumor and anti-inflammatory effects, it is widely used in traditional medicine (Patel et al., 2010). Crinum latifolium is recognized for its diverse therapeutic applications, including managing benign prostatic hyperplasia and cancer, supported by a rich phytochemical profile (Mimrot et al., 2024; Jenny et al., 2011). The medicinal significance of Crinum solapurense lies in its leaves, which exhibit notable antidiabetic activity through bioactive compounds like shikimic acid and gulonic acid, demonstrating potential pharmacological effects in managing diabetes in animal models (Mali et al., 2024). *Crinum viviparum* has demonstrated significant antibacterial effects, anti-inflammatory, thrombolytic, and anti-diarrheal activities, suggesting its potential in treating infections (Sikder et al., 2021).

Ethnobotanical uses of *Crinum*

Crinum latifolium is used in ethnomedicine, to treat cases of benign prostatic hyperplasia as well as inflammation, and cancer treatment due to its anti-inflammatory and antitumor properties (Mimrot *et al*., 2024). *Crinum asiaticum* is reported to cure gastrointestinal problems, skin diseases, fever, and urinary problems, showing its general ability to treat common ailments (Haque *et al*., 2014; Sharma *et al*., 2020). *Crinum bulbispermum* used for joint problems, rheumatism, and infections, used as a remedy in southern Africa (Maroyi, 2016). *Crinum ornatum* is used in wound treatment, urinary tract infection and respiratory illness, demonstrating the great versatility (Riji *et al*., 2023). The bulbs of *C. asiaticum* is employed to alleviate pain from a multitude of health conditions such as abscess, bruises, ear infections, inflammation, fevers, fractures, intestinal problems, hernia, mumps, rheumatoid arthritis, tonsillitis, urinary problems and vomits etc (Mahmoodally *et al*., 2021).

Indian *Crinum* and its diversity

In India it is widely distributed throughout the country and represented by 15 different species viz; *C. asiaticum, C. amoenum* Roxb. ex Ker Gawl, *C. brachynema* Herb., *C. humile* Herb., *C. lorifolium* Roxb., *C. latifolium, C. malabaricum* Lekhak & S.R.Yadav, *C. reddyi* M. Patel & H. Patel, *C. pusillum* Herb., *C. solapurense* S.P.Gaikwad, K.U.Garad & R.D.Gore, *C. stenophyllum, C. stracheyi* Baker, *C. viviparum* R. Ansari & V.J. Nair, *C. woodrowii* Baker and *C. wattii* Baker (Lekhak and Yadav, 2012; Lekhak *et al*., 2015; Patel and Patel, 2019).

The diversity of Indian Crinum species is notable, with several new species identified across various regions, each exhibiting unique morphological and ecological characteristics. This diversity is further enriched by the medicinal properties attributed to these species, highlighting their significance in both traditional and modern medicine. Crinum reddyi is a new species found in Gujarat, consisting of round, creamish-white bulbs and fewer flowers compared to its relatives, with a focus on vegetative propagation (Patel & Patel, 2019). Crinum andhricum, another new species discovered in Andhra Pradesh, it is closely related to C. amoenum and C. stracheyi, differing in flower count and perianth shape (Rasingam et al., 2024). Crinum solapurense is also recently identified in Maharashtra, having distinct bulblets and a unique flower structure, setting it apart from similar species (Gaikwad et al., 2014).

Phytochemical constituents of *Crinum*

Crinum species is renowned for their phytochemical diversity. *Crinum* species are abundant in phenolic compounds and flavonoids that provide the species with strong antioxidant and anti-inflammatory activities. These compounds counteract oxidative stress generated by free radicals which are prime contributors to chronic diseases such as cancers, cardiovascular diseases, and diseases linked to neurodegeneration (Araújo *et al*., 2023; Masi *et al*., 2021). These plants are particularly known for their high therapeutic use which can be attributed to their secondary metabolites, including flavonoids, alkaloids, phenols, and antioxidants (Refaat *et al*., 2013). These bioactive compounds have several pharmacological activities, such as anti-inflammatory, anti-oxidative, and acetylcholinesterase inhibition. The genus is also well known for its varying alkaloid profiles, with the valued ones being lycorine, crinine, and galantamine. Alkaloids are nitrogenated secondary metabolites and exhibit a wide range of biological activities such as anticancer, neuroprotective, antimicrobial, and antioxidant (Anaele & Nwafor, 2025).

Crinum bulbispermum has a diverse set of phytochemicals, including isoquinoline alkaloids, flavonoids, sterols, aldehydes, acids, esters, alcohols, amines, amides, and fatty acids. Alkaloids are the key bioactive components that cause its pharmacological actions, which include antibacterial and neuroprotective properties (Maroyi, 2016). *Crinum asiaticum* leaves contained alkaloids, saponins, phenols, glycosides, flavonoids, steroids, terpenoids, and tannins, indicating their potential for therapeutic applications against selected ear pathogens such as Streptococcus pneumoniae, Staphylococcus epidermidis, Haemophilus influenzae, Aspergillus niger, Aspergillus fumigatus, and Aspergillus flavus (Kaushal *et al*., 2018). *Crinum defixum* Ker- Gawler leaves were studied with gas chromatography-mass spectrometry (GC-MS) to identify and compare distinct chemicals across different solvent extracts, indicating potential therapeutic capabilities and diverse phytochemical composition (Elaiyaraja & Chandramohan, 2018). *Crinum* species in Ethiopia contain alkaloids, flavonoids, saponins, tannins, and phenols. *Crinum bambusetum* contained the highest quantities of alkaloids (9.66%) and saponins (19.72%), whereas *Crinum ornatum* had the highest flavonoids (27.72%) (Senbeta *et al*., 2019). The presence of flavonoid and phenol in *C. latifolium* contribute to its antioxidant capacity (DPPH, superoxide radical scavenging, and reducing power determination) showing its therapeutic utility (Kumar *et al*., 2022).

Crinum jagus leaf extracts prevented toluene-induced liver and kidney damage in rats by containing considerable quantities of tannins (57.04 mg/g), flavonoids (35.43 mg/g), and polyphenols in the methanolic extract (Salihu *et al*., 2022). Terpenoids, saponins, alkaloids, phenols, steroids, and flavonoids were observed in *Crinum jagus* bulbs, indicating a diverse spectrum of bioactive chemicals that may contribute to analgesic, anti-inflammatory, and antipyretic activities (Julius, 2023). De Araújo *et al*. (2023) utilized ESI-FT-MS to detect 89 molecules in *Crinum americanum* L., including fatty acids, phenolic compounds, and alkaloids and this shows that the plant is nutritionally viable. Riji *et al*. (2023) revealed 45 components in the most active dichloromethane extract of *Crinum ornatum*, with eight key compounds discovered, including n-hexadecenoic acid and squalene, which contribute to its considerable antibacterial activity against diverse bacteria. Rakhi *et al*. (2024) identified hippeastrine, cycloneolitsol, and β-sitosterol in the methanolic leaf extract of *Crinum asiaticum*. Chandana *et al*. (2024) phytochemically screened *Crinum latifolium* leaf extract and found to be high in alkaloids, glycosides, tannins, flavonoids, gums, and mucilages, indicating possible antioxidant and antidiabetic properties.

Crinum; as a source of alkaloids

W. Meisner first introduced the term alkaloid at the beginning of nineteenth century to denote naturally occurring substance that behave like alkalis (Bruneton, 1999). Alkaloids are a varied group of naturally occurring nitrogen containing organic compounds often in heterocyclic structures. They are secondary metabolites derived from amino acids, displaying an extensive array of biological and physiological activities, contributing to their ecological and pharmacological significance (Yang *et al*., 2024; Dey *et al*., 2020; Talreja & Tiwari, 2024). Alkaloids are known to be present in most of the organisms including plants, animals, fungus and other microorganisms (Aniszewski 2015). Till date, six major classes of alkaloids have been identified, depending on their origin from amino acid, includes derivatives of L-ornithine, L-lysine, L-tyrosine/L-phenylalanine, L-histidine, L-tryptophan and Glycine/aspartic acid (Aniszewski, 2007; Kukula-Koch & Widelski, 2017).

Alkaloids have antiaging, antiviral and anticancer properties. In Indian medicine, alkaloid rich plants are used for bleeding disorders, eye diseases and as sedatives, stomatics, uterine muscle depressants, antiseptics, analgesics and sedatives (Da-Cunha

et al., 2005). It also possesses various properties like anti-fungal, anti-malarial, anti-inflammatory, antioxidant, anxiolytic, analgesic, Cerebro-protective, anti-HIV, immunoregulative, cardioprotective, anti-mutagenic and Vaso-relaxing (Zuo *et al*., 2006; Bribi, 2018). However, consumption of alkaloids also has several toxic effects on livestock, including endocrine disruption, developmental and reproductive malfunctions, and blood circulations (Poole & Poole, 2019; Adamski *et al*., 2020).

Alkaloids, particularly galantamine and other AChE inhibitors have the ability to inhibit the activity of acetylcholinesterase enzyme and hence maintain the level of acetylcholine in the brain. These inhibitors bind to the active site of AChE, and affect their functions (Elgorashi *et al*., 2004). Key alkaloids such as galanthamine, haemanthamine, crinamine, and lycorine have demonstrated potential in the treatment of Alzheimer's disease due to their neuroprotective effects (Mimrot *et al*., 2024; Chaichompoo *et al*., 2023). Elgorashi *et al*., (2004) evaluated the potential of twenty-three alkaloid for their AChE inhibitory activity, among which 1-O-acetyllycorine (IC_{50}: 0.96 ± 0.04) showed the highest AChE inhibition property.

Duc *et al*., (2024) isolated seven novel alkaloids from *C. asiaticum* var. *sinicum* including a new alkaloid named bis-(-)-8-demethylmaritidine. From the bulbs of *C. latifolium*, fifty-one Amaryllidaceae alkaloids have been isolated, out of which eleven were previously undescribed (Chaichompoo *et al*., 2024). A phytochemical investigation of chloroform bulb extracts of *C. bulbispermum* contributed to the isolation of eight crystalline alkaloids, along with lycorine (Abou-Donia *et al*., 2012). *C. macowanii* used for the isolation of several alkaloids, such as lycorine, 1-O-acetyllycorine, crinine,powelline, cherylline, crinamine, crinamidine, 3-O-acetylhamayne and 1-epideacetylbowdensine, documented significant organ-to-organ and seasonal variation in alkaloids. Lekhak *et al*., (2022) recorded the highest Total Alkaloid Content (TAC) in the methanolic root extracts of *C. moorei* and in the aqueous root extracts of *C. lorifolium*. Bulbs of *C. asiaticum* is reported to have high TAC value in several earlier studies which contributed to their antibacterial activity, making it a candidate for development of potential drugs for several diseases (Rahman *et al*., 2011; Refaat *et al*., 2013). Oloyede *et al*. (2010) also reported that the presence of alkaloids are responsible for the effective free radical scavenging activities in *C. ornatum*. Methanolic leaf extracts of *C. x powelii* and C. solapurense exhibited highest TAC content, which makes it a promising drug for cancer and Alzheimer's disease. Cytotoxic

and antiviral properties of *C. jagus*, determined using a reporter-encoding dengue virus (DENV) vector and the β-coronavirus HCoV-OC43, attributed to its high alkaloid content, particularly lycorine and cherylline. The acid-base fractions exhibited higher cytotoxicity than their methanolic extracts of the plant material (Jayawardena *et al*., 2025). *C. erubescens* reported for the isolation of foue alkaloids with potential anti-plasmodial, pesticidal and anti-proliferative activity from its antimalarial bioassay-guided fractionation of its plant material (Presley *et al*., 2016). The acid organic extracts of the bulbs of *C. jagus* used for the isolation of three undescribed alkaloids, namely gigantelline, gigantellinine, and gigancrinine along with the already known alkaloids such as lycorine, cherylline, crinine, flexinine, sanguinin and isoquinolinone (Ka *et al*., 2020).

Antioxidant potential of *Crinum*

Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) and the body's ability to detoxify them or repair the harm they cause in our body. In other words, oxidative stress occurs when there are more reactive radical species in the environment than the body's natural antioxidants can handle (Barnham *et al*., 2004; Sayre *et al*., 2008). The high level of ROS in the body which usually causes oxidative stress can come from many things, including typical metabolic processes, environmental conditions, and exposure to toxicants. Oxidative stress can oxidatively damage essential macromolecules like lipids, proteins, and nucleic acids (Barber *et al*., 2006; Ames *et al*., 1993). Such damage can disrupt cellular function, potentially leading to cell death. The aging process may be associated with an accumulation of oxidative damage over time. Such accumulation may contribute to the decline of cellular activity and the biological aging of tissues. Oxidative stress has been associated with many neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS), Parkinson's disease (PD), and Alzheimer's disease (AD) (Barnham *et al*., 2004; Ames *et al*., 1993). Cognitive decline and motor impairment can be a result of oxidative damage of neurons. Elevated oxidative stress comes into play in many chronic diseases like cancer, diabetes, and cardiovascular disease, to name a few. Oxidative damage has the ability to increase inflammation, and speed up the progression of disease progression (Lleó *et al.,* 2006; Moosmann *et al.,* 2004; Troadec *et al.,* 2001)

The radical scavenging properties of several synthetic and natural antioxidants are based on their ability to donate a hydrogen atom (A-OH) to a free radical. When a radical scavenger meets up and donates a hydrogen atom to a free radical, they form a new radical. The new radical is less reactive, and more stable than the previous radical. Free radicals can also donate electrons and have antioxidant properties through hydrogen atom transfer (HAT), proton coupled electron transfer (PCET), single electron transfer (SET) and proton transfer (HAT → PCET), sequential proton loss electron transfer (SPLET), radical adduct formation (RAF), and sequential proton loss hydrogen atom transfer (SPL-HAT) (Klein *et al*., 2007; Litwinienko and Ingold, 2007; Galano, 2015; Galano *et al*., 2016; Mazzone *et al*., 2016) (Table 1).

Table 1: Different antioxidant mechanisms.

Different antioxidant mechanism	**Mechanism of action**	**Chemical equation**	**Reference**
Hydrogen Atom Transfer (HAT)	Transfer a hydrogen atom from an antioxidant to a free radical, resulting in charge dispersion into a new, less reactive and more stable radical	$AH+\cdot R \rightarrow A+\cdot RH$	Rose & Bode, (1993)
Proton-Coupled Electron Transfer (PCET)	Transfers an electron and proton simultaneously and generally stabilizes the resultant radical	$AH \rightarrow A+H^{+}+e^{-}$	Rose & Bode, (1993)
Single Electron Transfer-Proton Transfer (SET-PT)	Transfer of single electron followed by single proton, resulting in formation of stable product.	$AH \rightarrow A^{+}+e^{-}$ followed by $A^{+}+H^{+} \rightarrow A$	Marković, (2016)

Sequential Proton Loss Electron Transfer (SPLET)	proton lost one by one prior to electron transfer, leading to antioxidant activity.	$AH \rightarrow A^+ + H^+$ followed by $A^+ + e^- \rightarrow A$	Litwinienko & Ingold, (2003)
Radical Adduct Formation (RAF)	The reaction of an antioxidant and radical creating a stable adduct that reduces the reactivity of the radical.	$AH + \cdot R \rightarrow A\text{-}R$	Marković, (2016).
Sequential Proton Loss Hydrogen Atom Transfer (SPLHAT)	The stability of the final products can be enhanced by combining hydrogen atom transfer and sequential proton loss	$AH \rightarrow A^+ + H^+$ followed by $A^+ + \cdot R \rightarrow A\text{-}R$	Marković, (2016)

Antioxidant activity of a biological material can be monitored by many methods. The most commonly used are radical chromogen reagents that induce the formation of reductive oxygen species. They are so widely used because they are easy, quick, and sensitive. Antioxidants will react with these radical chromogens and eliminate them. Common techniques include free radical scavenging screening assays including DPPH (1,1-diphenyl 2picryl hydrazyl) assays, ABTS (2,2'-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid) assay, FRAP (Ferric reducing antioxidant property activity), Super oxide anion (O_2^-) radical scavenging, Peroxide (H_2O_2) radical scavenging, Nitric oxide (NO) radical scavenging, Hydroxyl radical (OH^-) scavenging assays, Malondialdehyde (MDA) or Thiobarbituric acid-reactive substances (TBARS) assays, total antioxidant capacity, total phenolic contents, etc (Kaur & Geetha, 2006).

In the present study, in vitro antioxidant activity was assessed using standardised methods like DPPH (1,1-diphenyl 2picryl hydrazyl) assays, ABTS (2,2'-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid) assay and FRAP (Ferric reducing antioxidant property activity).

Brand Williams *et al*. (1995) employed 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay method to measure the free radical scavenging activity. The assay method is based on

the reduction of a methanolic solution of the coloured free radical DPPH by free radical scavenger. DPPH is a stable free radical having purple colour. The decrease in absorbance of DPPH at its absorbance maximum of 517nm is proportional to the concentration of free radical scavenger added to the DPPH reagent solution. Ascorbic acid can be used as standard.

The radical scavenging activity of plant extracts is assayed using 2,2'-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay according to the protocol of Re *et al.* (1990). ABTS produces blue/green $ABTS^{\bullet+}$ chromophore when reacted with Potassium persulfate, which has an absorption maximum at 645 nm, 734 nm and 815 nm. The addition of antioxidant can reduce $ABTS^{\bullet+}$ to ABTS, depending on its ability, concentration and duration. This reduction leads to decrease in absorption of light, which is proportional to the antioxidant scavenging activity. BHT can be used as standard.

Reducing power of FRAP is determined by the method given by Oyaizu (1986). The reducing capacity of a compound Fe/ferricyanide complex to the ferrous form occurred due to the presence of reductants in the solution, which serve as a significant indicator of its antioxidant capacity. The key to reducing power is the presence of reductones, which demonstrate their antioxidant properties by donating a hydrogen atom to break the chain of free radicals. The absorbance is measured at 700 nm.

Islam *et al*., (2024) reported *C. asiaticum* as a vital source of bioactive compounds with antioxidant and thrombolytic potential. The ethyl acetate of *Crinum* asiaticum leaves determined using DPPH radical scavenging method recorded strong antioxidant activity and is higher than the hexane fractions and aqueous extracts. This strong antioxidant potential is due to its total phenolic and flavonoid contents due to their reported scavenging of reactive oxygen species and free radicals in biological systems. Araújo *et al*., (2023) documented high antioxidant activity in the hexane and methanolic extracts of *C. bulbispermum*, particularly in their leaves than their bulbs and roots using DPPH and FRAP assays. The nutritional elements within the plants are determined to be a safe alternative for consuming directly at a certain dosage. DPPH method revealed the high antioxidant activity of ethanolic leaf extracts of *C. asiaticum* which emphasizes its ability to neutralise free radicals and also for the treatment of Carrageenin-induced paw edema due to their high anti-inflammatory activity (Uddin *et al*., 2012). *C. latifolium* aerial parts are reported to have high antioxidant activity in the

hydroethanolic extracts, using DPPH method, which attributes to their high phenolic and flavonoid contents (Kumar *et al*., 2022). Ghane *et al*. (2018) investigated antioxidant property of methanolic and aqueous leaf extracts of 12 *Crinum* genotypes, out of which methanolic extracts of *C. asiaticum* exhibited highest DPPH and FRAP activity, whereas the aqueous extracts of *C. latifolium* showed the highest ABTS activity. Antioxidant activity of ultrasonic-assisted methanolic and aqueous leaf extracts of 16 *Crinum* genotypes were explored by Lekhak *et al*. (2022) and reported the highest DPPH and ABTS activities in the methanolic and water extracts of *C. moorei* and *C. lorifolium* respectively. Methanolic leaf extracts of *C. asiaticum* exhibited the maximum FRAP activity. *C. asiaticum* bulb noted considerable DPPH scavenging activity from different solvent extracts of *C. asiaticum* bulb, indicating the high free radical scavenging activity of *C. asiaticum* in various extraction solvents (Soni *et al*., 2015). *C. bulbispermum* exhibit significant DPPH and ABTS free radical scavenging activity, particularly in their ethyl acetate root extracts (Adewusi and Steenkamp, 2011). Alawode *et al*. (2019) documented remarkable DPPH and FRAP activity in the methanolic extracts of leaf and ethyl acetate extracts of bulbs of *C. jagus*. Antioxidant assessment on the ethanolic bulb extracts of *C. latifolium* revealed that several alkaloids (4,8-dimethoxy-cripowellin, 9- methoxy-cripowellin, 4-methoxy-8-hydroxy-cripowellin, cripowellin) exhibited remarkable DPPH and ABTS free radical scavenging activities (Chen *et al*., 2018).

Acetylcholinesterase and human nervous system

Alzheimer's disease is one of the most common neurodegenerative diseases within the elderly population (usually above 65 years old) and is defined by slow, progressive, and irreversible loss of cognitive and mental functioning. Furthermore, it usually results in extreme memory loss and diminished ability to encode new memories resulting in disorders of behaviour (Price *et al*., 2015). The major clinical manifestations are memory impairment, cognitive dysfunction, mental symptoms, and behavioural abnormalities (Yun *et al*., 2021).

ACh is amassed in the structure called vesicles in the terminal ends of neuron cells (Sahani, 2020). When the action potential goes down the nerve cells and reaches the axon terminals then ACh diffuses from vesicles into the synaptic cleft and binds to the nicotinic or muscarinic receptor. Thus, the electrical impulses are passed through the

cholinergic system. The released ACh, however, has a very short-half life because of the presence of large quantities of a hydrolase enzyme, acetylcholinesterase (AChE) in the synaptic cleft of both CNS and PNS which catabolizes ACh into inactive choline and acetate metabolites (Figure 1) (Taqui *et al*., 2022).

An essential enzyme called acetylcholinesterase (AChE) is in charge of hydrolysing the neurotransmitter acetylcholine, which stops cholinergic signalling. Acetylcholine levels can rise as a result of AChE inhibition, which is important in a number of therapeutic settings, especially in neurodegenerative illnesses like Alzheimer's. Determining the effects of certain pesticides and nerve agents as well as creating efficient remedies requires an understanding of the mechanisms underlying AChE inhibition (Thapa & Xu, 2017). Galantamine (GAL) is potential plant growth-inhibitor (Iqbal *et al*., 2006) and exhibits anti-malarial, anti-viral, anti-bacterial, and acetylcholinesterase inhibition properties (López *et al*.,2002).

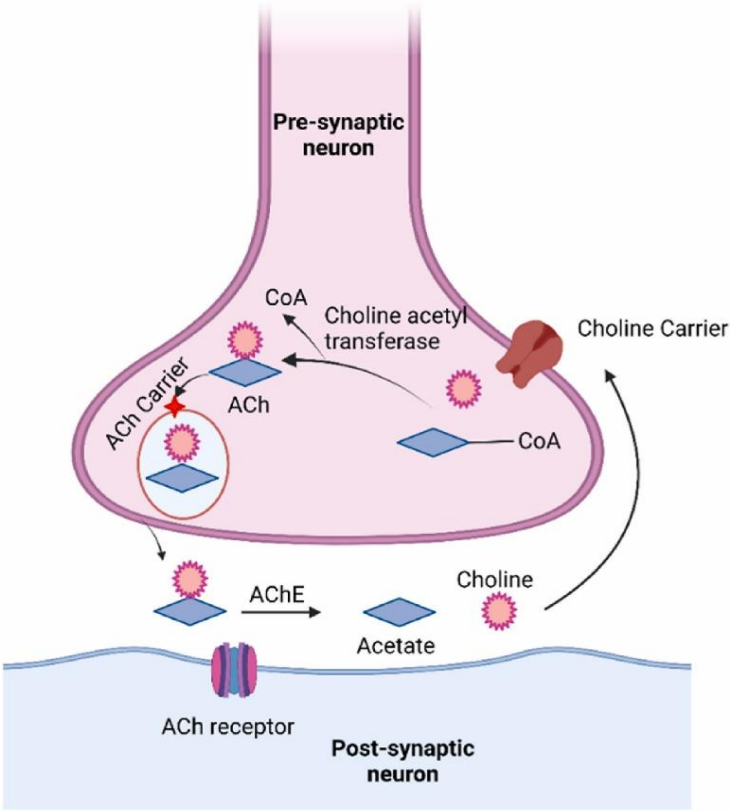

Figure 1: Mechanism of AChE inhibition (Sobha *et al*., 2024).

Crinum as a source of neuroprotectant

Galantamine has been approved as an acetylcholinesterase inhibitor, which is important for the treatment of Alzheimer's disease. Alkaloids like galanthamine have been the

subject of much research, but the non-alkaloidal components of *Crinum* have gotten relatively less attention from scientists (Refaat *et al*., 2013). Jagtap *et al*. (2014) examined the galanthamine content of five Indian *Crinum* species and discovered that the bulbs of *C. malabaricum* had the highest concentration. The lowest and equal galanthamine content, on the other hand, was found in *C. brachynema* and *C. pratense*. A novel galanthamine-type alkaloid, crijaponine B (2), was discovered from *C. asiaticum*. The study also assessed the isolated compounds acetylcholinesterase (AChE) inhibitory activity, with crijaponine B helping to clarify the galanthamine-type alkaloids in this plant, which is known for its therapeutic qualities (Endo *et al*., 2019). *Crinum* contains the alkaloid galantamine, which has been linked to potential therapeutic advantages, particularly in the treatment of Alzheimer's disease. It is also found in a variety of *Crinum* species, indicating its role in boosting cognitive function via acetylcholinesterase inhibition (Nair & Staden, 2021). The critically endangered medicinal plant *Crinum malabaricum*, contains high concentration of galanthamine, which is valued for its therapeutic properties, particularly in the treatment of cognitive illnesses such as Alzheimer's (Chahal *et al*., 2022). *C. brachynema* in therapeutic applications and highlights the necessity of conservation efforts since meta-Topolin, which increases antioxidant activity (Kaur *et al*., 2022). Among the bioactive substances found in the ultrasonic-assisted extracts of *Crinum* roots is galanthamine. Galanthamine, demonstrating its coexistence with other phenolics in *Crinum* roots, which enhances the plant's total bioactive capacity (Lekhak *et al*., 2022). A well-known alkaloid called galantamine improves cholinergic neurotransmission by acting as a reversible inhibitor of acetylcholinesterase. Because *Crinum* × *amabile* contains cholinesterase inhibitors, its bulbs may be useful in creating therapies for diseases like Alzheimer's, where cholinesterase inhibition is helpful (Chaichompoo *et al*., 2022). According to Duc *et al*. (2024) Alkaloids from *C. asiaticum*, especially the novel compound bis-(-)-8-demethylmaritidine, demonstrated a strong inhibition of acetylcholinesterase in vitro, indicating that it may be used to treat Alzheimer's disease by modulating AChE activity.

Ecological diversity of *Crinum* species

The ecological diversity of *Crinum* species is extremely broad, reflecting their adaptation to multiple habitats and geographic areas. *Crinum* species are primarily bulbous geophytes and are able to thrive in areas with strong seasonality but some have

adapted to special ecological niches, such as riverine environments. The morphological variation among *Crinum* species, their geographical distributions, and near affinity for specific ecological adaptations is fascinating. *Crinum* species exhibit amazing morphological diversity, specifically in flower shape, including both star-shaped and bell-shaped flowers, which does not organize themselves neatly into phylogenetic groupings, indicating ecological importance over taxonomic importance (Kwembeya *et al*., 2007; Bjorå *et al*., 2009). In mainland Africa, for example, about 85% of the *Crinum* species have zygomorphic flowers, while 15% have radially symmetrical flowers illustrating a wide array of floral adaptations (Nordal & Kwembeya, 2004). Eight species have been recorded in Bangladesh, each with their own habitat preference and economic potential (Afroz *et al*., 2018). While *Crinum* species can be found in a wide array of environmental circumstances in Africa, including the Guineo-Congolean rainforests to the Zambesian region, it illustrates the ecological adaptation to different climates and ecological conditions (Nordal & Kwembeya, 2004). Some *Crinum* species have adapted to grow in riverine environments while also abandoning the geophyte habit. Species such as *Crinum* natans (L.) Herb, have adapted special morphological characteristics, such as leaves without stomata, along with seeds that do not buoyant but still germinate in inundated conditions (Bjorå *et al*., 2009). Evidence from the ecological trends in *Crinum* phylogeny imply that geographical and environmental factors may have a more significant influence in their evolution as opposed to taxonomic classification based on flower morphology (Kwembeya *et al*., 2007).

Ecological niche effect in phytochemicals

Plants can apply their unique phytochemical profiles, as aspects of their niche, to choose or to prosper in environments. Phytochemical traits can assist plants in adjusting to conditions consistent with the local environment which supports survival and fitness. When plants produce certain chemical compounds, they can modify the environment to some degree, impacting soil chemistry, neighbouring plants, and some aspects of the ecosystem. In plant ecology, morphology and physiology are widely considered niche dimensions, while phytochemical traits, although there is acknowledgement of their important roles in ecological response and mediation, are generally ignored (Bakhtiari *et al*., 2021; Müller & Junker, 2022; Walker *et al*., 2022).

3. MATERIALS AND METHODS

3.1. Plant species studied

3.1.1. *Crinum asiaticum* L. (POWO, 2025)

Synonym : *Crinum toxicarium* var. *asiaticum* (L.) Herb.

Common Name : Poison Bulb, Crinum Lily, Asiatic Poison Lily, Spider Lily.

IUCN Status : Not Threatened

Taxonomical Classification

Kingdom	:	Plantae
Phylum	:	Angiosperms
Class	:	Monocots
Order	:	Asparagales
Family	:	Amaryllidaceae
Genus	:	*Crinum*
Species	:	*Crinum asiaticum*

Geographical Distribution

It is a terrestrial plant, distributed in South Asia, South east Asia and Pacific South. In India it is distributed throughout the country.

Morphological Features

Crinum asiaticum possess fluffy green leaves that can grow up to 2 m tall. This plant has huge, spectacular blooms with white corollas. Leaves clustered from the apex of bulbs, oblong, lanceolate, flat, coriaceous, shiny, narrow at base, margin entire, apex gradually tapering. It can reach a height of 1.5 m. The anthers are 2 cm long. The stamens are in groups of 6 and have a single stigma. The fruits have a sub-globose form and contain a big seed (Patel, 2017; Win, 2011).

Figure 2: Habit of *C. asiaticum*.

3.1.2. ***Crinum viviparum R.Ansari & V.J.Nair*** (POWO, 2025)

Synonym	:	*Crinum bulbispermum*
Common Name	:	River Crinum lily, Visha moongil
IUCN Status	:	Least Concern

Taxonomical Classification

Kingdom	:	Plantae
Phylum	:	Angiosperms
Class	:	Monocots
Order	:	Asparagales
Family	:	Amaryllidaceae
Genus	:	*Crinum*
Species	:	*Crinum viviparum*

Geographical Distribution

Crinum viviparum is an semi-aquatic plant that thrives in stony, shallow riverbeds, canals, ponds, lakes, and marshlands. Native to Bangladesh, Bhutan, India, Indonesia, Myanmar, Nepal, Sri Lanka, Thailand and Viet Nam.

Morphological Features

Crinum viviparum is an upright herb with huge globose or oblong bulbs that range in diameter from 7.5 to 10 cm. It can bloom easily when submerged in water. The leaves are straight, concave, or channeled, and measure roughly 60 to 90 cm in length and 2-4 cm in width. The white to reddish blooms are produced in 6–15 flowering umbels on a cylindrical stalk that is 45–75 cm long. The fruits have short stalks, are subglobose, and have a diameter of 2.5 cm. The seeds are rugose (Sharma *et al*., 2013; Bhandary and Chandrashekhar, 2011).

Figure 3. Habit of *C. viviparum*.

3.1.3. *Crinum solapurense S.P.Gaikwad, Garad & Gore* (POWO, 2025)

Common Name : River Crinum lily, Visha moongil

IUCN Status : Threatened

Taxonomical Classification

Kingdom	:	Plantae
Phylum	:	Angiosperms
Class	:	Monocots
Order	:	Asparagales
Family	:	Amaryllidaceae
Genus	:	*Crinum*
Species	:	*Crinum solapurense*

Geographical Distribution

Crinum solapurense is an aquatic herb having underground bulbs. Its native to Maharashtra, India.

Morphological Features

Leaves are radical and lanceolate. Lateral growth of bulbs into scapes. Flowers are white and grow in umbels of 10-30 flowers each. Perianth segments 6 are lanceolate. There are six stamens. Style purple towards apex, white below. Capsule 3-22 per inflorescence. The prolonged perianth tube beaks the irregularly globose seeds, which are chlorophyllous and dark purple when mature (Garad *et al*., 2014; Nagathan *et al*., 2023; Mali *et al*., 2024).

Figure 4. Habit of *C. solapurense*.

3.2. Chemicals and Reagents

All the chemicals used in the analytical methods and reagent preparation were of analytical grade with maximum available purity purchased from the following manufacturers.

Table 2: Reagents and standard chemicals used for this study.

Sl. No.	Chemicals & reagents used	brand
1.	2,2' azinobis (3-ethyl-benzothiozoline-6-sulfonic acid (ABTS)	Hi-media
2.	Acetyl thio-choline iodide (AChI)	Merck
3.	Acetylcholinesterase (AChE) type VI-S	Merck
4.	Ammonium molybdate	Hi-media
5.	Ascorbic acid	Hi-media
6.	Butylated hydroxytoluene (BHT)	Hi-media
7.	Bromocresol Green	Hi-media
8.	Chloroform	Hi-media
9.	Diethylamine	Merck
10.	1,1-diphenyl -2- picrylhydrazyl (DPPH)	Hi-media
11.	5,5' –di thio bis [2- nitro benzoic acid] (DTNB)	Hi-media
12.	Ferric chloride hexahydrate	Hi-media
13.	Ferrous sulphate	Hi-media
14.	Folin-Ciocalteu reagent	Hi-media
15.	Galantamine	Sigma-Aldrich
16.	Gallic acid	Sigma-Aldrich
17.	Hydrochloric acid	Hi-media
18.	Lycorine	Sigma-Aldrich
19.	Methanol	Hi-media
20.	Potassium persulphate	Hi-media
21.	Quercetin	Hi-media
22.	Sodium acetate	Hi-media
23.	Sodium hydroxide	Hi-media
24.	Sodium phosphate	Hi-media
25.	Trifluoroacetic acid	Sigma-Aldrich

3.3. Instruments

Table 3: Instruments used for this study.

Sl. No.	Instruments	Model
1.	UV –Visible Spectrophotometer	**Jasco, Japan**
2.	Weighing balance	Sartorius, Germany
3.	pH meter	LAQUA pH-1100, Horiba, Japan
4.	Magnetic stirrer with hot plate	iStirrer
5.	Centrifuge	Eppendorf, Germany
6.	Water Bath	Julabo, SW 22

3.4. Collection and Authentication of Plant Materials

Collection

Plant specimens were collected from diverse geographical regions of India, including Kerala (*C. asiaticum, C. viviparum*) and Maharashtra (*C. solapurense*) (Table 4). Field notes such as date of collection, locality, altitude, habit and habitat, soil conditions, colour of leaves, flowers, and fruits and other key morphological traits were recorded in a field book. The collected specimens were photographed subsequently transferred to KSCSTE- Malabar Botanical Garden and Institute of Plant Sciences, Kozhikode, Kerala, India for conservation and research purposes.

Table 4: Details of the voucher specimens of *Crinum* species used for the study.

Species	Location	Latitude	Longitude
C. asiaticum L.	Olavanna, Kozhikode, Kerala	11°14'36.1"N	75°49'37.4"E
C. viviparum R.Ansari & V.J.Nair	MBGIPS, Kozhikode, Kerala	11°14'09.8"N	75°49'46.3"E
C. solapurense S.P.Gaikwad,Garad&Gore	Machnur, Maharashtra	17°33'54.4"N	75°33'33.8"E

Authentication

The three species of *Crinum* selected for the study were made into herbarium specimens for future reference. The collected specimens were tagged properly by

giving field numbers. The herbarium was made according to the method suggested by Jain and Rao (1977). The herbarium sheets of all the plants under study were deposited at KSCSTE- Malabar Botanical Garden and Institute of Plant Sciences, Kozhikode, Kerala, India. The collected plant materials were identified on the basis of taxonomical characters with the help of Flora of the Presidency of Madras (Gamble, 1956) and plants of world online (POWO, 2025) and authenticated by the experts at KSCSTE-MBGIPS.

3.5. Preparation of plant extract

The robust and fresh matured leaves and bulbs of *Crinum sp.* were harvested. The leaves and bulbs were separated, washed and cleaned thoroughly with tap water to remove soil and other extraneous matter and then using distilled water thrice. The plant materials were finely shredded and shade-dried on blotting paper inside the laboratory for two weeks until completely dried. The dried plant materials were further ground into a fine powder in a mixer grinder. Using the hot plate magnetic stirrer extraction method, the coarsely powdered samples were macerated in methanol to obtain the crude extract using Erlenmeyer flask for 3 hours at 500 rpm below the boiling point of methanol (64.7°C). The resulting mixture was then filtered using Whatman No. 1 filter paper and transferred to a beaker. The filtrates were then allowed to evaporate at room temperature for 3-4 days and the crude methanolic extracts were stored in labelled air tight vials. These extracts were then stored in refrigerator for further analysis.

3.6. Phytochemical analysis

3.6.1. Total Phenolic Content (TPC)

Total Phenolic Content (TPC) was assessed by Folin-Ciocalteu method (Singleton and Rossi, 1965) with some minor modifications.

Reagents

- 10% Folin-Ciocalteu's reagent
- 7% Sodium carbonate solution
- Gallic acid

Procedure

0.5 mL of plant extract (1 mg/mL) was mixed with 0.5 mL of 10% Folin-Ciocalteu's reagent in a clean dry test tube. This reaction mixture was dissolved in 6.5 mL of

distilled water and added 5 mL of 7% Sodium carbonate solution. Reaction mixture was mixed thoroughly and incubated in a dark room for 2h followed by measuring the absorbance at 760 nm using UV/VIS spectrophotometer. Increasing concentrations (10, 20, 40, 60, 80 and 90 µg/ mL) of Gallic acid was used as standard to obtain the calibration curve. The % absorbance was plotted against concentration, and the linear regression equation was obtained as: $y = mx + c$

Where, y = Absorbance at 765 nm

x = Gallic acid concentration (µg/mL)

m = Slope and c = Intercept

Using this equation, the gallic acid equivalent (GAE) concentration x for the sample was calculated, and the total phenolic content was expressed as mg Gallic Acid Equivalent per gram of extract (mg GAE/g E) using the formula:

$$\text{mg GAE per g Extract} = \frac{x \text{ X V}}{M}$$

Where, x= equivalent concentration of Gallic acid (mg/mL)

V= total extract volume used (mL)

M= mass of extract used per mL (in g)

3.6.2. Total Flavonoid Content (TFC)

Total Flavonoid Content was determined using Aluminium chloride method (Sakanaka *et al*., 2005) with some minor modifications.

Reagents

- 10% Aluminium chloride
- 1 M Sodium acetate
- Quercetin

Procedure

250 µL of extract was mixed with 750 µL Methanol and 100 µL of 10% Aluminium chloride. Followed by the addition of 100 µL Sodium acetate and 800 µL distilled water. This reaction mixture was incubated at dark for 30 min at room temperature. The reaction mixture was thoroughly mixed and absorbance was noted immediately at 415 nm using UV/VIS Spectrophotometer. For preparation of blank, water was used instead of plant extract. Different concentrations of 200 mg/mL Quercetin (10, 20, 40, 60, 80 and 90 µg/mL) were used as standard to obtain the calibration curve. The absorbance

values were plotted against the concentration, and the linear regression equation was obtained as: $y = mx + c$

Where, y = Absorbance at 765 nm

x = Quercetin concentration (µg/mL)

m = Slope and c = Intercept

Using this equation, the quercetin equivalent (QE) concentration x for the sample was calculated, and the total phenolic content was expressed as mg Quercetin Equivalent per gram of extract (mg QE/g Extract) using the formula:

$$\text{mg QE per g Extract} = \frac{x \text{ X V}}{M}$$

Where, x= equivalent concentration of Quercetin (mg/mL)

V= total extract volume used (mL)

M= mass of extract used per mL (in g)

3.6.3. Total Alkaloid Content (TAC)

Alkaloid content was determined by the method given by Ghane *et al*. (2018) with some minor modifications.

Reagents

- Bromocresol green
- 2N NaOH
- 2N HCL
- Chloroform
- Sodium phosphate buffer (pH 4.7)
- 0.2 M Citric acid

Procedure

Bromocresol green (69.8 mg) was mixed with 5 mL double distilled water and 3 mL of 2N NaOH, heated for some period followed by dilution about 1000 mL using distilled water. Plant extract (250 µL) was oven-dried at 45°C until crystallization occurred. The dried crystals were dissolved in 5 mL of 2N HCL and then filtered. The filtrate was extracted using 10 mL of chloroform in a separating funnel. Separation process was repeated for 5 times and the upper layer was collected and pH was adjusted to 7. To this add 5 mL Bromocresol green and 5 mL sodium phosphate buffer (Sodium phosphate

buffer was mixed with 0.2 M Citric acid and pH was altered to 4.7). By sequential chloroform extraction using 1 mL, 2 mL, 3 mL, 4 mL, resulting in 10 mL of reaction mixture. Absorbance of organic (chloroform) layer was measured on UV VIS Spectrophotometer at 470 nm wavelength. Different concentrations of Lycorine (20, 40, 60, 80 and 100 µg/mL) were used as standard to obtain the calibration curve. The absorbance values were plotted against the concentration, and the linear regression equation was obtained as: $\boldsymbol{y = mx + c}$

Where, y = Absorbance at 248 nm

x = Lycorine concentration (µg/mL)

m = Slope and c = Intercept

Using this equation, the Lycorine equivalent (LE) concentration x for the sample was calculated, and the total phenolic content was expressed as mg Quercetin Equivalent per gram of extract (mg LE/g Extract) using the formula:

$$\text{mg CE per g E} = \frac{x \text{ X V}}{M}$$

Where, x= equivalent concentration of Lycorine(mg/mL)

V= total extract volume used (mL)

M= mass of extract used per mL (in g)

3.7. *In vitro* Antioxidant activity

3.7.1. DPPH free radical scavenging activity

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay method (Brand Williams *et al*., 1995) was employed to measure the free radical scavenging activity. The assay method is based on the reduction of a methanolic solution of the coloured free radical DPPH by free radical scavenger. DPPH is a stable free radical having purple colour. The decrease in absorbance of DPPH at its absorbance maximum of 517nm is proportional to the concentration of free radical scavenger added to the DPPH reagent solution.

Reagents

- 0.25 mmol/L DPPH in methanol
- Ascorbic acid

Procedure

DPPH reagent was prepared in methanol (0.25 mmol/L). To clean, dry Eppendorf tubes, 1.9 mL of 0.25 mmol/L DPPH in methanol was mixed with 100 µL of leaf and bulb

extracts (200 mg) of *C. asiaticum, C. viviparum* and *C. solapurense.* The tubes were then incubated at room temperature for 20 minutes in dark, and the absorbance was measured at 517nm with UV/VIS spectrophotometer (Jasco, Japan). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. All measurements were performed in triplicate and reported as the average value. 100 µL of Methanol with 1.9 mL DPPH is used as blank.

$$\text{Radical scavenging capacity (\%)} = (A_{blank} - A_{sample})/A_{blank} \times 100,$$

Where A_{sample} is the absorbance of DPPH mixed with plant extract and A_{blank} is the absorbance of DPPH in which sample has been replaced with methanol.

Different concentrations of ascorbic acid (10, 20, 50 and 100µg/mL) were used as standard to obtain the calibration curve.

From the calibration curve obtain the linear equation: **y= mx + c**

Where, **y** = % scavenging activity

x = ascorbic acid concentration (µg/mL)

m = slope and **c** = intercept.

Using the equation, the **ascorbic acid equivalent concentration (x)** for the sample's scavenging activity was calculated, and results were expressed as milligrams of ascorbic acid equivalent per gram of extract (mg AAE/g Extract) using the formula.

$$\text{mg AAE per g Extract} = \frac{x \text{ X V}}{M}$$

Where, x= equivalent concentration of ascorbic acid (mg/mL)

V= total extract volume used (mL)

M= mass of extract used per mL (in g)

3.7.2. ABTS scavenging activity

The radical scavenging activity of plant extracts were assayed using 2,2'-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay according to the protocol of Re *et al.* (1990) with some modifications. ABTS produces blue/green $ABTS^{\bullet+}$ chromophore when reacted with Potassium persulfate, which has an absorption maximum at 645 nm, 734 nm and 815 nm. The addition of antioxidant can reduce $ABTS^{\bullet+}$ to ABTS, depending on its ability, concentration and duration. This reduction leads to decrease in absorption of light, which is proportional to the antioxidant scavenging activity.

Reagents

- 7 mmol/L ABTS in methanol
- 2.45 mmol/L potassium persulfate
- Butylated hydroxytoluene (BHT)

Procedure

The ABTS radical solution was prepared mixing 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate in an Amber bottle and kept at refrigerator overnight (12-16h). 200 mg samples were subsequently mixed with 1900 µL ABTS radical solution. The mixture was incubated in dark for 7 min and the absorbance was measured at 737 nm with UV/VIS spectrophotometer. Methanol with reagent was used as blank.

$$\text{Radical scavenging (\%)} = ([A_{control} - A_{sample}]/A_{control} \times 100),$$

Where A_{sample} is the absorbance of the ABTS mixed with the sample, $A_{control}$ is the absorbance of the ABTS mixed with deionized water.

Different concentrations of BHT (20, 40, 60, 80, 100, 120 µg/mL) were used as standard to obtain the calibration curve. The free radical-scavenging capacity was calculated by the following equation.

Measure their % ABTS scavenging activity and plot a graph. From the calibration curve obtain the linear equation: $y = mx + c$

Where, **y** = % scavenging activity

x = BHT concentration (µg/mL)

m = slope and c = intercept.

Using this equation, the BHT-equivalent concentration (xxx) for the sample's scavenging activity was determined. The results were expressed as milligrams of BHT equivalent per gram of dried extract (mg BHTE/g Extract) using the formula:

$$\text{mg AAE per g Extract} = \frac{x \text{ X V}}{M}$$

Where, x= equivalent concentration of BHT (mg/mL)

V= total extract volume used (mL)

M= mass of extract used per mL (in g)

3.7.3. Ferric Reducing Antioxidant Activity (FRAP)

FRAP was determined by the method given by Oyaizu (1986) with some minor modifications. The reducing capacity of a compound Fe/ferricyanide complex to the ferrous form occurred due to the presence of reductants in the solution, which serve as a significant indicator of its antioxidant capacity. The key to reducing power is the presence of reductones, which demonstrate their antioxidant properties by donating a hydrogen atom to break the chain of free radicals. The absorbance was measured at 700 nm.

Reagents

- 0.2M Phosphate buffer (pH 6.6)
- 1% Potassium hexacyanoferrate II
- 10% Trichloro acetic acid (TCA)
- 0.1% $FeCl_3$
- Butylated hydroxyl toluene (BHT)

Procedure

The FRAP reagent was freshly prepared by combining 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$, and 300 mM acetate buffer (pH 3.6) in a 1:1:10 ratio. Before use, the reagent was incubated at 37 °C. For the assay, 200 µL of sample extract (at concentrations of 0.1, 0.2, 0.5, 1, and 2 mg/mL) was added to 2.8 mL of the FRAP reagent and incubated at 37 °C in a water bath for 30 minutes. Following incubation, the absorbance Fe^{2+} complex is recorded at 593 nm. The antioxidant activity of the sample is quantified as Fe(II)-equivalent concentration using the $FeSO_4$ calibration curve equation.

$$\mathbf{Fe(II)eqyuivalent}(\mu\mathbf{M}) = \frac{\text{Absorbance of sample} - \text{c}}{\text{m}}$$

Where, m = Slope of the standard curve and c = Intercept.

To express the antioxidant activity as mg Fe (II) equivalent per gram extract (mg Fe (II)/g Extract), the following formula was used:

$$\mathbf{mg\ Fe(II)\ per\ g\ DE} = \frac{x\ \text{X V}}{\text{M}}$$

where: x = Fe(II) equivalent concentration from the calibration curve (mg/mL)

V = Total extract volume used (mL)

M = Mass of extract per mL (g)

3.8. Acetylcholinesterase (AChE) inhibitory activity

AChE inhibitory activity of all the extracts was performed according to the method of Ellman *et al*. (1961) with few modifications.

Reagents

- 50 Mm Tris HCL (pH 8.0)
- AChE solution
- 5,5-dithiobis- (2-nitrobenzoic acid) (DTNB)
- Acetylcholine iodide
- Galantamine

Procedure

The organic (Chloroform) layer obtained during TAC estimation was used for AChE inhibition analysis. The reaction mixture was prepared by combining 500 µL of 3 mM DTNB, 100 µL of 15 mM AChI, 275 µL of 50 mM Tris–HCl buffer (pH 8), and 100 µL of ethanolic extract dissolved in ethanol, water, or DMSO. This mixture was placed in a 1 mL cuvette and used as the blank.

For the experimental reaction, 25 µL of buffer in the blank was replaced with 25 µL of enzyme solution containing 0.28 U mL^{-1}. The enzymatic reaction was monitored at 405 nm for 5 minutes, and reaction velocities were determined. Enzyme activity was expressed as a percentage of the reaction velocity compared to the control assay, where the buffer was used instead of the inhibitor (extract). The percentage inhibition was calculated as:

$$\textbf{Inhibition}\ (\%) = \textbf{100} - \textbf{Enzyme activity}\ \%$$

Each experiment was performed in triplicate, and results represent the mean values. Ethanolic extracts were reconstituted in the minimum necessary volume of DMSO or ethanol. The final concentrations of extracts in the reaction cuvettes corresponded to 0.5 mg/mL and 1 mg/mL.

3.9. Statistical Analysis

All values were expressed as mean ± standard error (SE). Data were analyzed using a one-way analysis of variance (ANOVA), and significant differences between mean values were determined using Duncan's multiple range test (DMRT) at a significance level of $p < 0.05$. Statistical analyses were performed using SPSS version 25.0.

4.1. Phytochemical profiling

The phytochemical quantification of methanolic extracts of selected *Crinum* species were performed to examine the total alkaloid, phenol and flavonoid contents.

Table 5: Phytochemical analysis (TPC, TFC and TAC) of methanolic extracts of *Crinum* species.

S. No.	Genotype	Plant part	TPC mg GAE/g E (mean ± SEM)	TFC mg QE/g E (mean ± SEM)	TAC mg LE/g extract (mean ± SEM)
1	*C. asiaticum*	Leaf	110.97 ± 0.10	61.08 ± 0.11	6.74 ± 0.01
		Bulb	**85.93 ± 0.06**	68.56 ± 0.12	**12.22 ± 0.02**
2	*C. viviparum*	Leaf	**119.4 ± 0.17**	**70.47 ± 0.22**	7.56 ± 0.02
		Bulb	78.79 ± 0.09	**77.36 ± 0.17**	7.66 ± 0.02
3	*C. solapurense*	Leaf	118.82 ± 0.15	70.12 ± 0.13	**7.71 ± 0.10**
		Bulb	78.56 ± 0.07	76.15 ± 0.08	8.16 ± 0.06

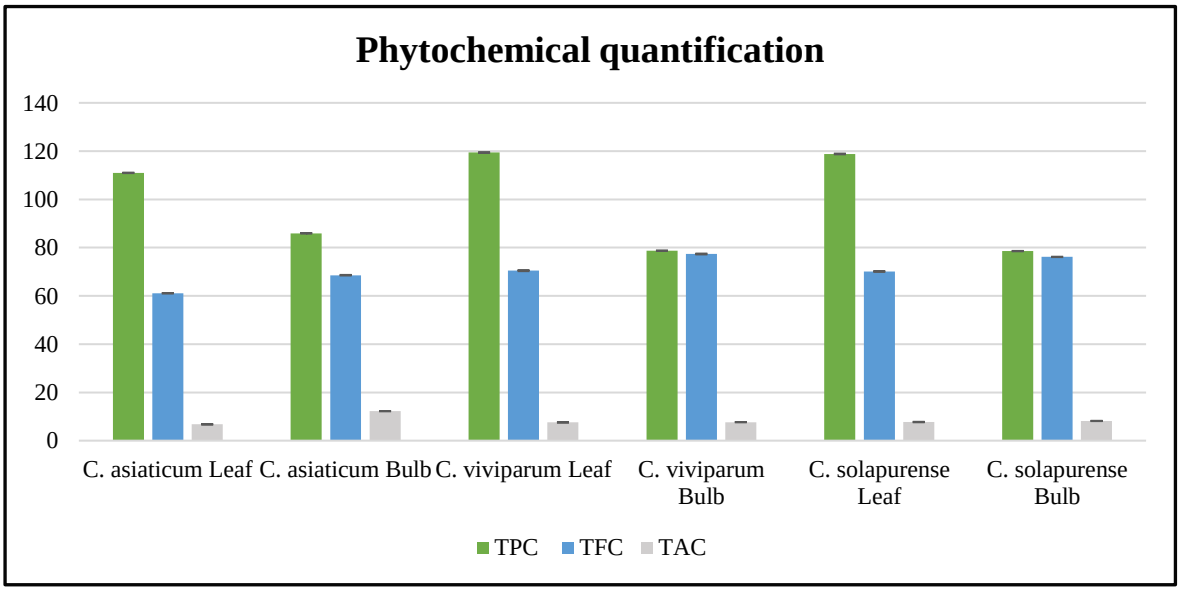

Figure 5: Phytochemical analysis (TPC, TFC and TAC) of methanolic extracts leaves and bulbs of *Crinum* genotypes. Error bars represent standard error.

4.1.1. Total Phenolic Content (TPC)

The Total Phenolic Content is measured as equivalents of gallic acid using the Folin-Ciocalteu method. It was expressed as mg GAE/g extract using the equation obtained from the calibration curve (Figure 6): y=0.0107x+0.0119, R^2=0.9992, where X is the Gallic acid equivalent (GAE) and Y is the absorbance at 760 nm. The absorbance values for different concentrations of standard gallic acid were plotted in the standard graph (Figure 6).

Calibration Curve of Gallic acid

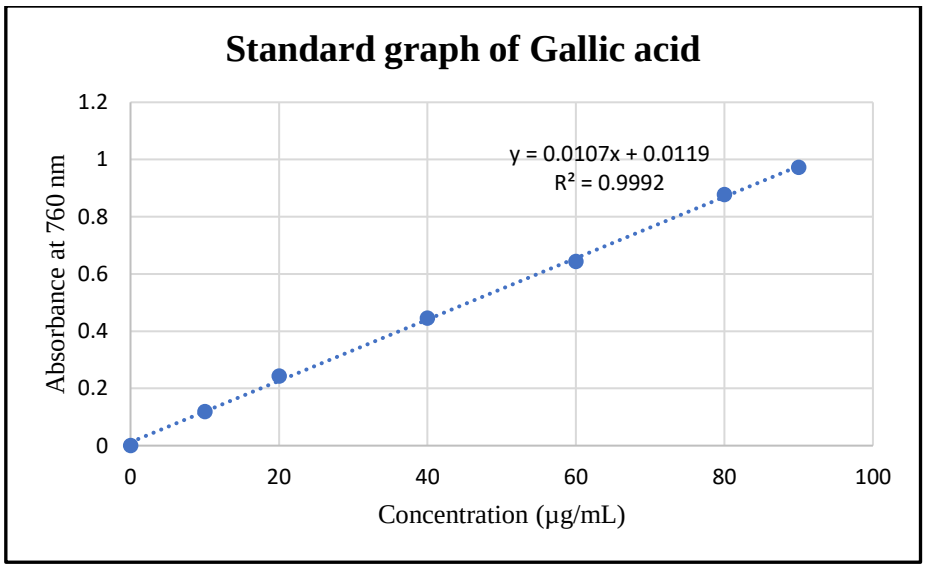

Figure 6: Standard graph of Gallic acid for TPC estimation

The Total Phenolic Content in bulb and leaf samples of selected *Crinum* species were quantified. A significant reduction was observed in the TPC of bulb samples in comparison with that of the leaf samples (Figure 7). The mean value of TPC from the leaf samples of both *C. viviparum* (119.4 ± 0.17 mg GAE/g E) and *C. solapurense* (118.82 ± 0.15 mg GAE/g E) was relatively similar, while *C. asiaticum* exhibited slightly lower value (110.97 ± 0.10 mg GAE/g E). *C. solapurense* is aquatic species, *C. viviparum* is semi-aquatic and *C. asiaticum* is terrestrial. These results suggest that the habitat may influence the TPC values in these species, with semi-aquatic and aquatic species exhibiting higher TPC values than the terrestrial species. In contrast, the bulb of terrestrial species, *C. asiaticum* (85.93 ± 0.06 mg GAE/g E) showed higher TPC than bulbs of both the aquatic and semi-aquatic species (Figure 5).

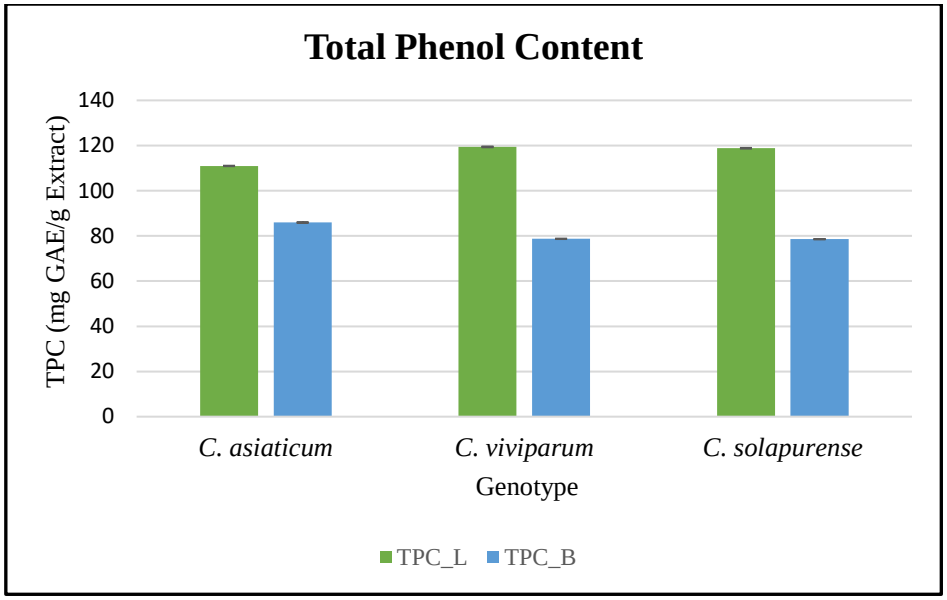

Figure 7: Total Phenolic Content quantified from leaves and bulbs *Crinum* genotypes. Concentrations were calculated from standard graph and expressed in mg GAE/g extract. Error bars represent standard error.

4.1.2. Total Flavonoid Content (TFC)

The Total Flavonoid Content is measured as equivalents of Quercetin using the aluminium chloride method. It was expressed as mg QE/g extract using the equation obtained from the calibration curve (Figure 8): y=0.0207x+0.0533, R^2=0.9981, where X is the Quercetin equivalent (QE) and Y is the absorbance at 415 nm. The absorbance values for different concentrations of standard Quercetin were plotted in the standard graph (Figure 8).

Calibration Curve of Quercetin

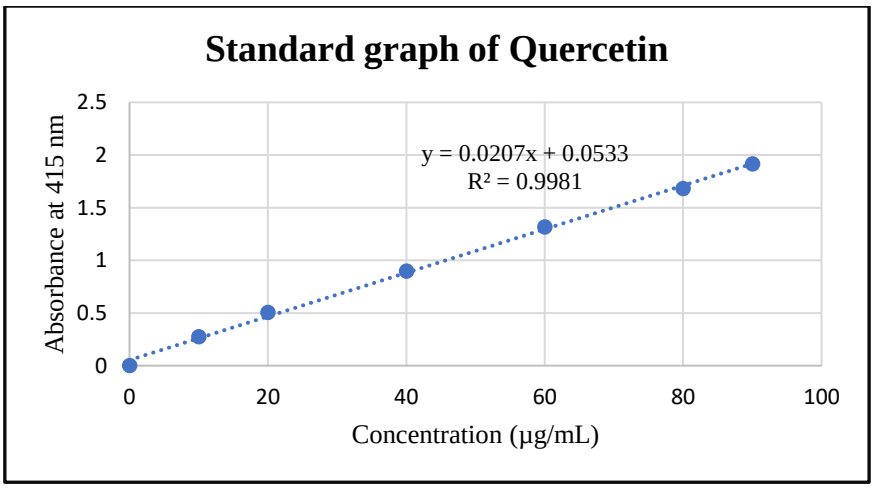

Figure 8: Standard graph of Quercetin for TFC estimation

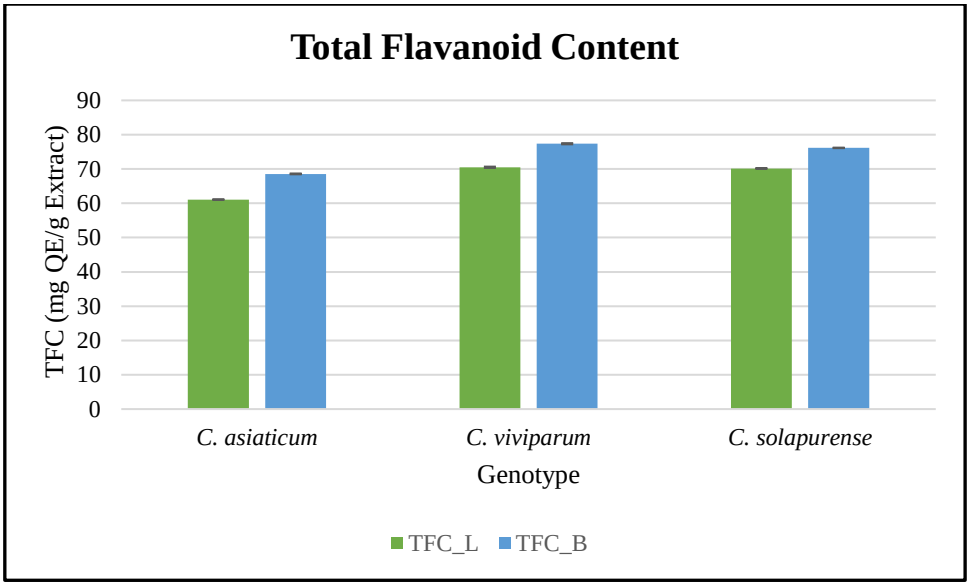

Figure 9: Total Flavonoid Content quantified from leaves and bulbs of selected *Crinum* species. Concentrations were calculated from standard graph and expressed in mg QE/g extract. Error bars represent standard error.

The Total Flavonoid Content was assessed in bulb and leaf samples of selected *Crinum* species and, revealing a significant increase in TFC values of bulb samples compared to the leaf samples (Figure 9). The mean TFC of the bulb samples of both *C. viviparum* (77.36 ± 0.17 mg QE/g E) and *C. solapurense* (76.15 ± 0.08 mg QE/g E) were closely comparable, while *C. asiaticum* exhibited notably lower value (68.56 ± 0.12 mg QE/g E). *C. solapurense* is aquatic species, *C. viviparum* is semi-aquatic and *C. asiaticum* is terrestrial. These findings indicate that the semi-aquatic and aquatic species display higher TFC levels than the terrestrial species. A similar trend was observed in the leaf samples, where *C. viviparum* (70.47 ± 0.22mg QE/g E) and *C. solapurense* (70.12 ± 0.13 mg QE/g E) showed significantly higher TFC values than *C. asiaticum* (61.08 ± 0.11 mg QE/g E) (Table 5).

4.1.3.. Total Alkaloid Content (TAC)

The Total Alkaloid Content is measured as equivalents of Lycorine using the Spectrophotometric method with Bromocresol green (BCG). It was expressed as mg LE/g extract using the equation obtained from the calibration curve (Figure 10): $y=0.0159x+0.0117$, $R^2=0.998$, where X is the Lycorine equivalent (LE) and Y is the absorbance at 470 nm. The absorbance values for different concentrations of standard Lycorine were plotted in the standard graph (Figure 10).

Calibration Curve of Lycorine

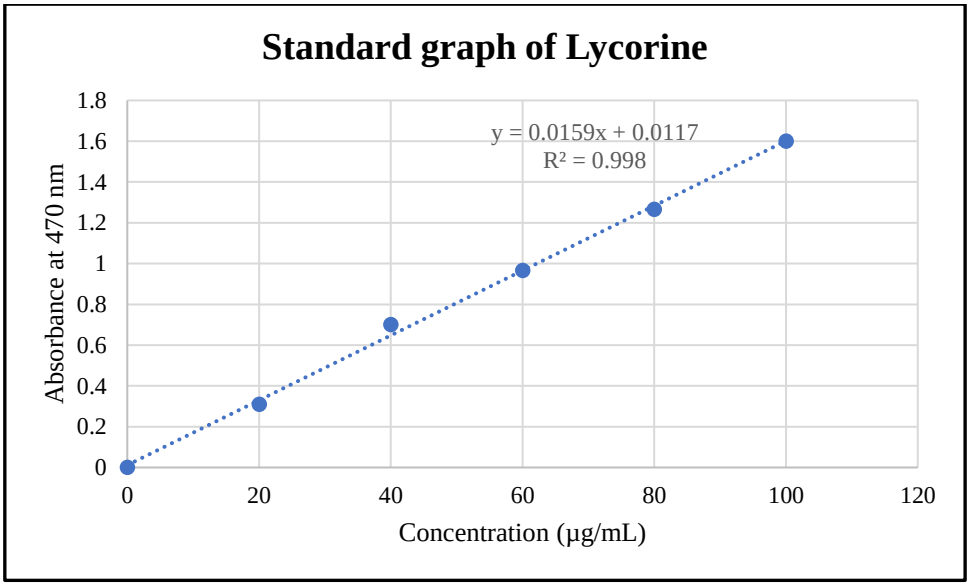

Figure 10: Standard graph of Lycorine for TAC estimation

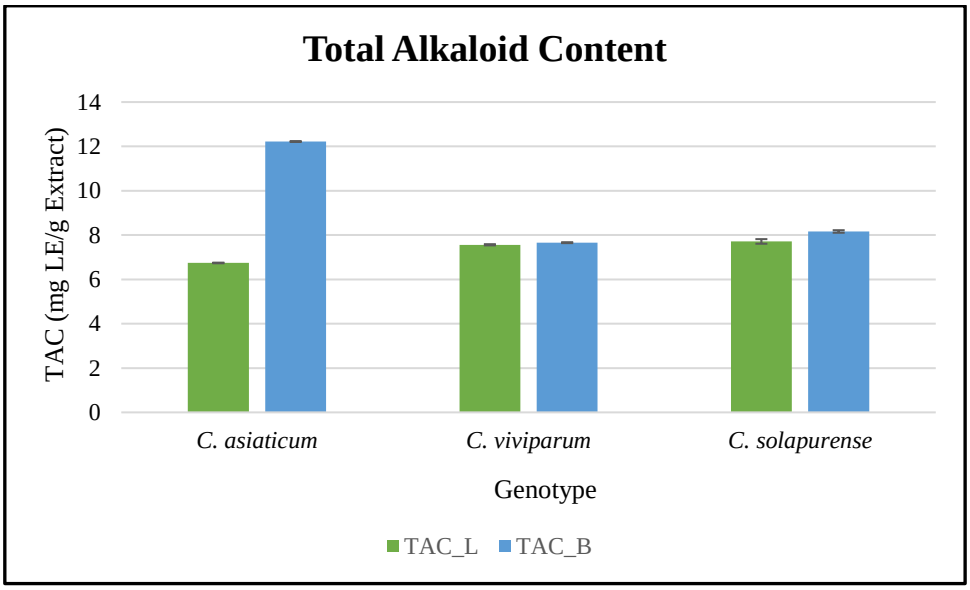

Figure 11: Total Alkaloid Content quantified from leaves and bulbs of selected *Crinum* species. Concentrations were calculated from standard graph and expressed in mg LE/g extract. Error bars represent standard error.

The Total Alkaloid Content (TAC) was measured in the bulb and leaf samples of selected *Crinum* species. The bulb samples exhibited higher TAC values than leaves (Figure 11). The terrestrial species *C. asiaticum* had the highest TAC value in the bulb (12.22 ± 0.02 mg LE/g) but had the lowest TAC value in the leaf (6.74 ± 0.01 mg LE/g

E). In contrast, *C. solapurense* (aquatic) and *C. viviparum* (semi-aquatic) exhibited moderate TAC values in both bulb (8.16 ± 0.06 mg LE/g E; 7.71 ± 0.10 mg LE/g E) and leaf (7.66 ± 0.02 mg LE/g E; 7.56 ± 0.02 mg LE/g E) (Table 5).

4.2. Antioxidant activities

For the present study, the *in vitro* antioxidant activities of methanolic extracts of selected *Crinum* species were investigated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay, 2,2'-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and Ferric reducing antioxidant property activity (FRAP).

Table 6: Antioxidant activity analysis (DPPH, ABTS and FRAP) of selected *Crinum* species.

Sl. No.	Genotype	Plant part	DPPH assay mg AAE/g E (mean ± SEM)	ABTS assay mg BHTE/g E (mean ± SEM)	FRAP assay mg Fe (II) E/g E (mean ± SEM)
1	*C. asiaticum*	Leaf	**60.88 ± 0.22**	20.74 ± 0.38	240.01 ± 0.19
		Bulb	**60.32 ± 0.30**	**33.14 ± 0.16**	**186.18 ± 0.23**
2	*C. viviparum*	Leaf	37.93 ± 0.28	25.02 ± 0.18	**278.29 ± 0.16**
		Bulb	38.78 ± 0.28	23.68 ± 0.22	161.02 ± 0.28
3	*C. solapurense*	Leaf	38.17 ± 0.28	**25.27 ± 0.23**	265.43 ± 0.23
		Bulb	38.07 ± 0.25	20.32 ± 0.15	165.49 ± 0.17

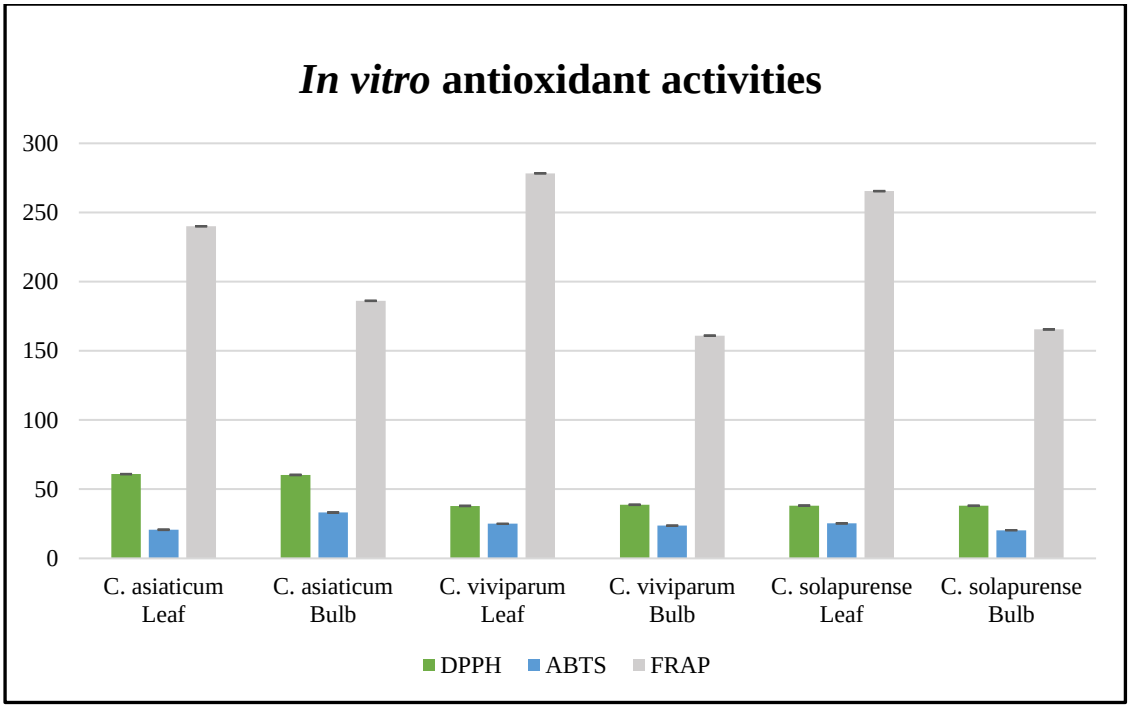

Figure 12: Antioxidant activity analysis (DPPH, ABTS and FRAP) of methanolic extracts of leaves and bulbs of *Crinum* genotypes. Error bars represent standard error.

4.2.1. DPPH free radical scavenging activity

The *in vitro* antioxidant assay of the bulb and leaf extracts of selected *Crinum* species from three different ecological habitats were evaluated by measuring their ability to scavenge the 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. It was expressed as mg AAE/g extract using the equation obtained from the calibration curve (Figure 13): $y=0.4084x+45.589$, $R^2=0.9991$, where X is the Ascorbic acid equivalent (AAE) and Y is the absorbance at 517 nm. The absorbance values for different concentrations of standard Ascorbic acid were plotted in the standard graph (Figure 13).

The DPPH free radical scavenging activity in bulb and leaf samples of selected *Crinum* species were assayed. The leaf and bulb samples show no significant difference in the DPPH assay in each species (Figure 14). The terrestrial species *C. asiaticum* exhibited higher DPPH assay in both leaf (60.88 ± 0.22 mg AAE/g E) and bulb (60.32 ± 0.30 mg AAE/g E). In contrast, no considerable difference was observed in DPPH assay of *C. solapurense* (aquatic) and *C. viviparum* (semi-aquatic) exhibiting moderate values in both bulb (38.78 ± 0.28 mg AAE/g E; 38.07 ± 0.25 mg AAE/g E) and leaf (37.93 ± 0.28 mg AAE/g E; 38.17 ± 0.28 mg AAE/g E) (Table 6 & Figure 12).

Calibration Curve of Ascorbic acid

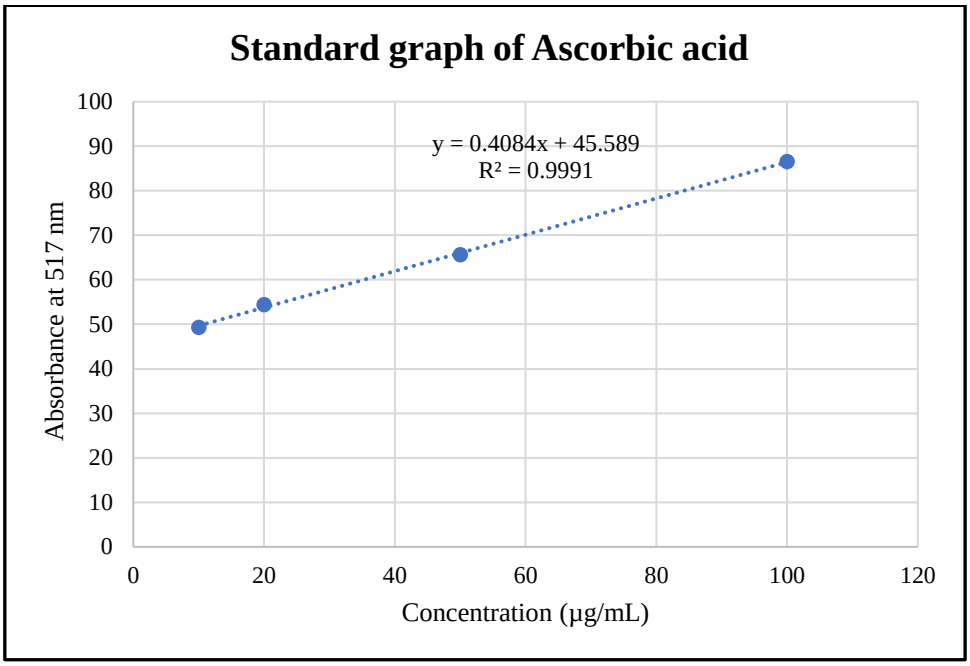

Figure 13: Standard graph of Ascorbic acid

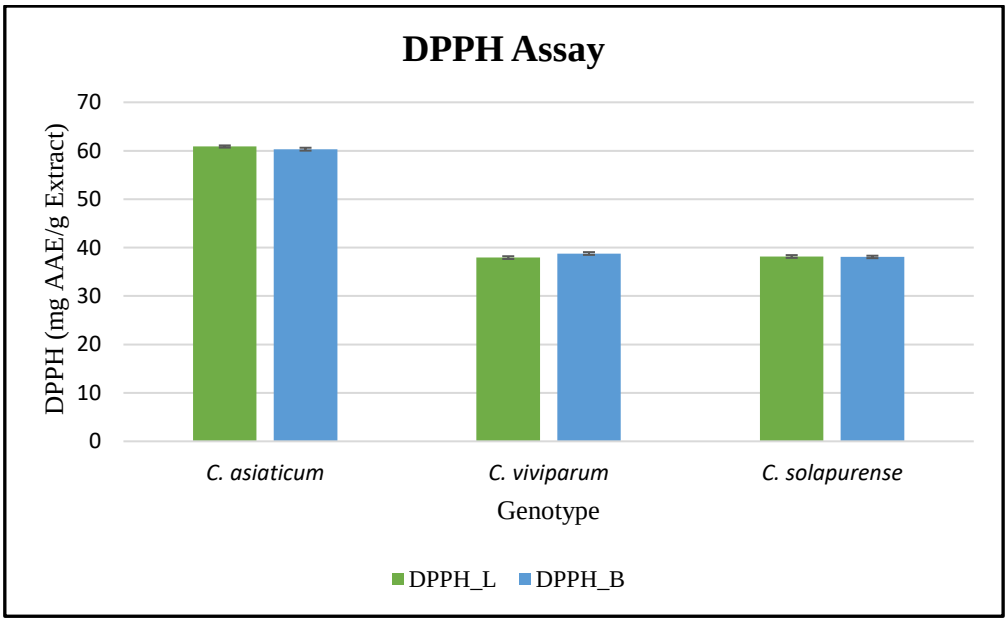

Figure 14: DPPH free radical scavenging activity assessed from leaves and bulbs of selected *Crinum* species. Concentrations were calculated from standard graph and expressed in mg AAE/g extract. Error bars represent standard error.

4.2.2. ABTS radical scavenging activity

The *in vitro* antioxidant assay of the bulb and leaf extracts of selected *Crinum* species from three different ecological habitats were evaluated by measuring their ability to scavenge the 2,2'-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cations. It was expressed as mg BHTE/g extract using the equation obtained from the calibration curve (Figure 15): y=0.5982x+29.978, R^2=0.9978, where X is the Butylated hydroxy toluene (BHT) and Y is the absorbance at 734 nm. The absorbance values for different concentrations of standard BHT were plotted in the standard graph (Figure 15).

The ABTS free radical scavenging activity was assayed in bulb and leaf samples of selected *Crinum* species (Figure 16). *C. asiaticum* (terrestrial) possessed the highest antioxidant activity, particularly in the bulbs (33.14 ± 0.16 mg BHTE/g E) followed by *C. viviparum*(semi-aquatic) (23.68 ± 0.22 mg BHTE/g E) and *C. solapurense* (aquatic) (20.32 ± 0.15 mg QE/g E). Contrarily, the leaves of *C. asiaticum* had the lowest antioxidant activity with a value of (20.74 ± 0.38 mg BHTE/g E) and the leaves of *C. viviparum* (25.02 ± 0.18 mg BHTE/g E) and *C. solapurense* (25.27 ± 0.23 mg BHTE/g E) recorded remarkable similarity in antioxidant activity (Table 6 & Figure 12). The terrestrial species (*C. asiaticum*) show the highest ABTS assay among the other bulbs and lowest ABTS assay among leaves.

Calibration Curve of BHT

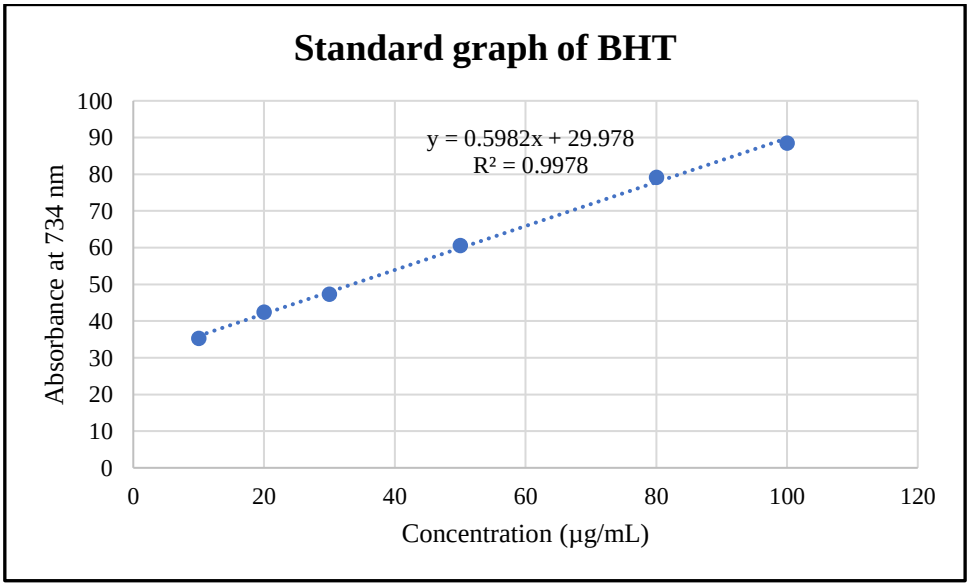

Figure 15: Standard graph of BHT

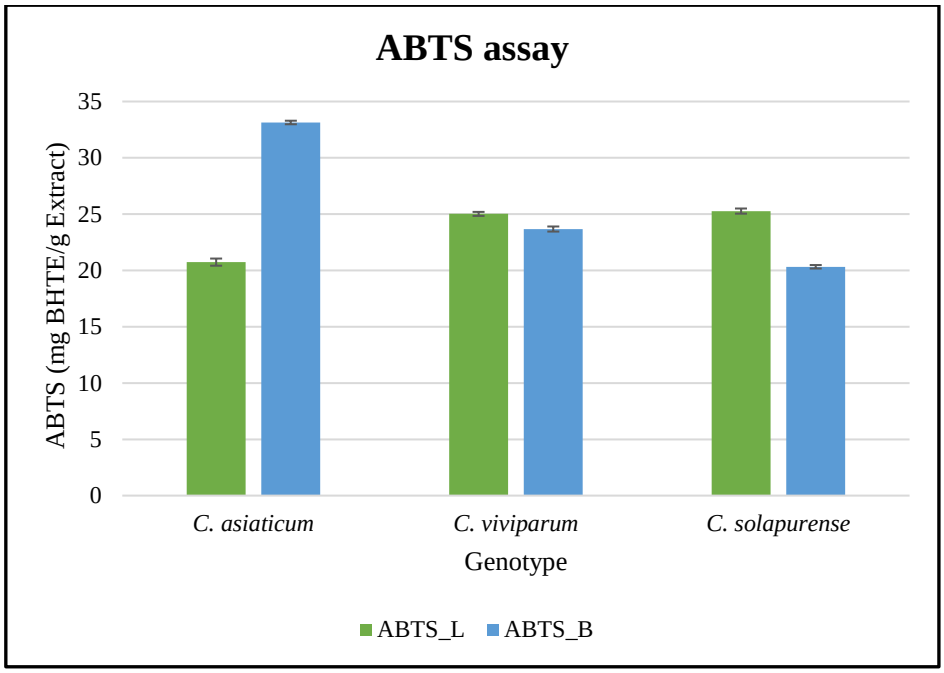

Figure 16: ABTS radical scavenging activity assessed from leaves and bulbs of selected *Crinum* species. Concentrations were calculated from standard graph and expressed in mg BHTE/g extract. Error bars represent standard error.

4.2.3. Ferric Reducing Antioxidant Property Activity (FRAP)

The ferric reducing antioxidant power (FRAP) assay is a common ET-based technique that quantifies how much antioxidants reduce the ferric ion (Fe3+)-ligand complex to the strikingly blue ferrous (Fe2+) complex (Zhong & Shahidi, 2015). It was expressed as mg Fe (II) E/g extract using the equation obtained from the calibration curve (Figure 17): y=0.0036x+0.0043, R^2=0.9932, where X is the Fe (II) and Y is the absorbance at 700 nm. The absorbance values for different concentrations of standard Fe (II) were plotted in the standard graph (Figure 17).

Ferric Reducing Antioxidant Property Activity (FRAP) was assessed in bulb and leaf samples of selected *Crinum* species. The results revealed a significant increase in FRAP activity of leaf samples compared to the bulb samples (Figure 18). Notably, the terrestrial species (*C. asiaticum*) show highest antioxidant activity in bulb (186.18 ±0 .23 mg Fe (II) E/g E) and lowest in leaf (240.01 ± 0.19 mg Fe (II) E/g E), while the semi-aquatic species (*C. viviparum*) possess highest antioxidant activity in leaf (278.29

± 0.16 mg Fe (II) E/g E) and lowest in bulb (161.02 ± 0.28 mg Fe (II) E/g E) (Table 6 & Figure 12).

Calibration Curve of Fe (II)

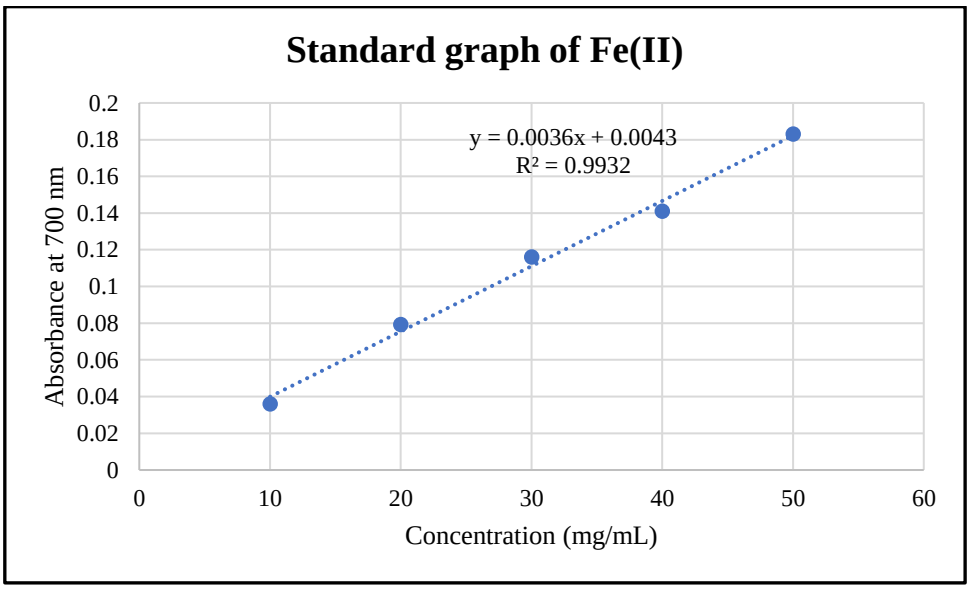

Figure 17: Standard graph of Fe (II)

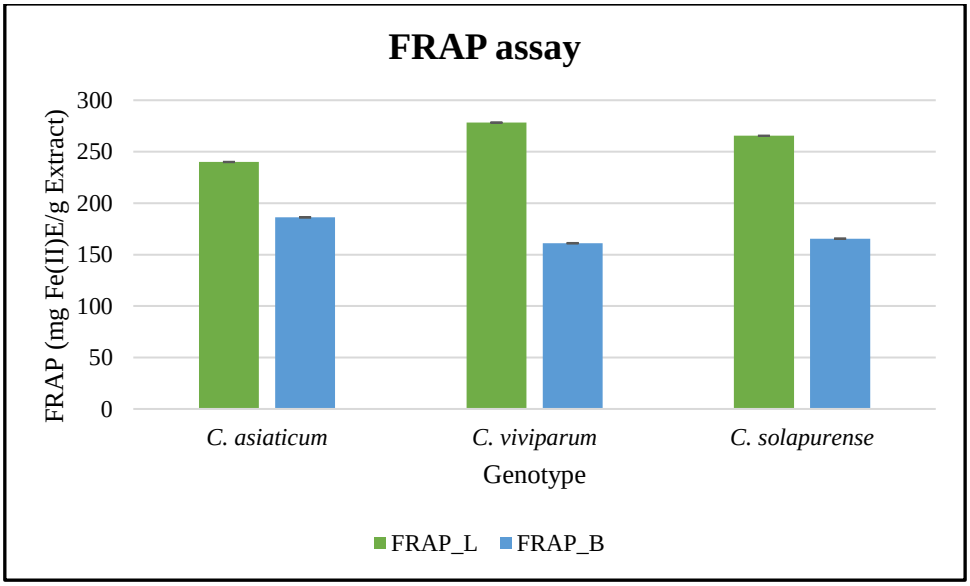

Figure 18: Ferric Reducing Antioxidant Property Activity (FRAP) assessed from leaves and bulbs of selected *Crinum* species. Concentrations were calculated from standard graph and expressed in mg Fe (II)E/g extract. Error bars represent standard error.

4.3. Acetylcholinesterase (AchE) inhibitory activity

The acetylcholinesterase activity was determined by a Spectrophotometric method. The enzyme activity is measured by following the increase of yellow colour produced from thiocholine when it reacts with dithiobisnitrobenzoate ion. The AChE inhibitory activity was assessed by measuring absorbance at 412 nm using Galantamine as standard.

Table 7: AChE inhibition % of methanolic extract of Bulbs and leaves of selected *Crinum* species.

S. No	Genotype	Plant part	AChE Inhibition % of plant extract (100 µg/ml)	IC50 Value (µg/ml)
1	*C. asiaticum*	Leaf	**55.21 ± 1.21**	**82.98 ±0.04**
		Bulb	**83.74 ± 2.04**	**12.63 ± 0.061**
2	*C. viviparum*	Leaf	14.34 ± 1.14	108.5 ± 0.07
		Bulb	72.91 ± 3.04	41.69 ± 0.003
3	*C. solapurense*	Leaf	48.99 ± 2.13	102.54 ± 0.03
		Bulb	70.69 ± 2.06	34.54 ± 0.012
4	GAL (Std)		98.33 ± 1.01	0.569 ± 0.013

Acetylcholinesterase (AChE) inhibitory activity was assessed in bulb and leaf samples of selected *Crinum* species. The results demonstrated a significantly higher AChE inhibitory activity in the bulb samples compared to the leaf samples (Figure 19). The methanolic extracts of the *C. asiaticum* exhibited the highest inhibitory activity in both bulb (83.74 ± 2.04%) and leaf (55.21 ± 1.21%). The bulbs of *C. viviparum* (72.91 ± 3.04%) and *C. solapurense* (70.69 ± 2.06%) showed relatively similar inhibitory activities. In contrast, the leaf samples of *C. viviparum* (14.34 ± 1.14%) showed a significant decrease in AChE inhibitory activity when compared to *C. solapurense* (48.99 ± 2.13%) and *C. asiaticum*. The terrestrial species exhibit more AChE inhibition % than semi-aquatic and aquatic species (Table 7).

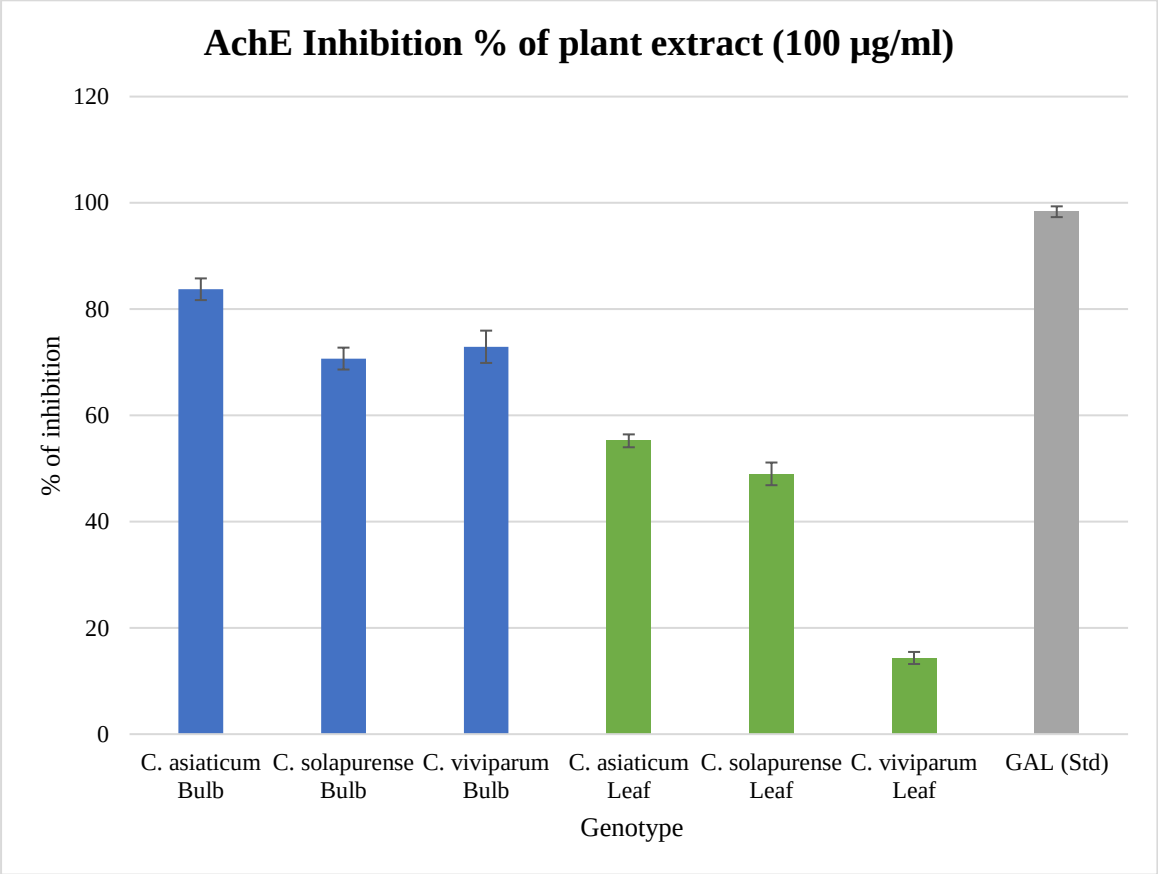

Figure 19: AChE inhibition % determined from leaves and bulbs of selected *Crinum* species using Galantamine as standard. Error bars represent standard error.

5. DISCUSSION

Crinum, a biochemical-rich genus within the Amaryllidaceae family, is well known for its therapeutic properties and aesthetic appeal and are found in various regions of India, particularly in tropical and subtropical areas. The therapeutic potential of *Crinum* species is largely attributed to their rich secondary metabolite content, particularly alkaloids, flavonoids, saponins, terpenoids, and phenolic compounds. The plant's rich phytochemical profile accounts for their various medicinal properties such as antioxidant, anti-Alzheimer, anti-tumour, anti-diabetic, immune- stimulating, analgesic, cytotoxic, antiviral, anti-inflammatory, anti-malarial and antimicrobial properties (Refaat *et al*., 2013; Emran, 2012; Meerow *et al*. 2003; Araújo *et al*., 2023). Thus, members of the genus *Crinum* are commonly used in treatment of various painful and inflammatory disorders such as rheumatism, earache, lumbago, edema, headache, worm infestation, vomiting, swelling, backache, wounds and haemorrhoids (Refaat *et al*., 2013)

The present study aims to assess comparative analysis of methanolic extracts from the leaves and bulbs of three *Crinum* species from distinct ecological conditions: *Crinum asiaticum* (terrestrial), *Crinum viviparum* (semi-aquatic) *Crinum solapurense* (aquatic). The study will evaluate their phytochemical diversity (alkaloids, flavonoids, and phenols), antioxidant activity (DPPH, ABTS, and FRAP), and Acetylcholinesterase inhibition property. This research will contribute valuable insights into the pharmacological potential of *Crinum* species and support their conservation and sustainable utilization.

Despite numerous studies on *Crinum* species, significant research gaps persist, particularly regarding their phytochemical profiles. Most studies have focused on the leaves, overlooking the potential of bulbs as reservoirs of bioactive compounds. Additionally, comprehensive comparative analyses of *Crinum* species from different habitats—aquatic, semi-aquatic, and terrestrial—remain largely unexplored. Understanding how environmental factors influence phytochemical diversity and antioxidant activity is crucial for optimizing the therapeutic applications of *Crinum* species.

5.1. Phytochemical quantification

In the present study, total phenolic content (TPC) was found to be higher in the leaf extracts compared to the bulb extracts across all *Crinum* species examined. Among the leaf samples, *C. viviparum* and *C. solapurense* exhibited relatively similar TPC levels, whereas *C. asiaticum* showed slightly lower values. Interestingly, in bulb extracts, *C. asiaticum* displayed a higher TPC than the bulbs of *C. viviparum* and *C. solapurense*, suggesting organ-specific variation in phenolic accumulation within species (Figure 20).

These findings are in partial agreement with earlier studies. For example, Lekhak *et al*. (2022) and Ghane *et al*. (2018) also reported high TPC in methanolic extracts of root and leaf of *C. asiaticum* respectively, though their observed values varied across accessions. Similarly, Goswami and Ray (2017) documented substantially higher TPC in aqueous leaf extracts of *C. asiaticum*, indicating that extraction solvent can significantly influence phenolic yield. Comparative studies on other species, such as *C. latifolium* and *C. ornatum*, also support this variability, with some showing higher phenolic content in bulb extracts, as seen in *C. latifolium* (Aziz *et al*., 2013) and *C. ornatum* (Mohammed *et al*., 2014).

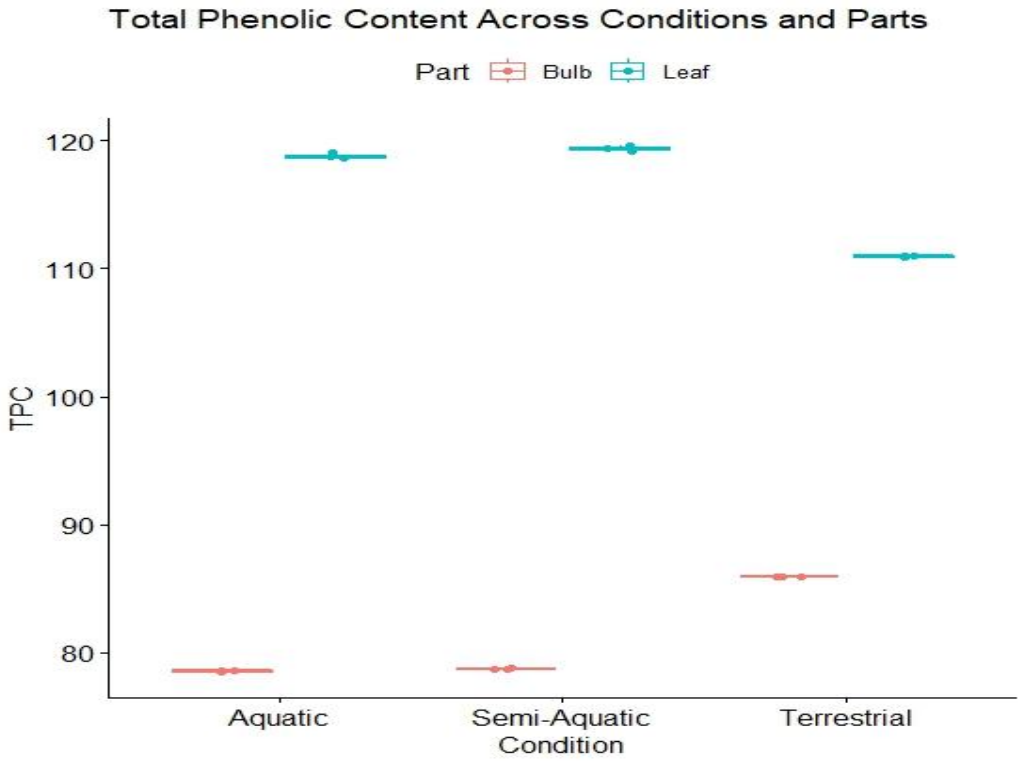

Figure 20: Total Phenolic Content of *Crinum* species Across Conditions and Parts.

In the present investigation, the Total Flavonoid Content was assessed to be higher in bulb samples compared to the leaf samples across selected *Crinum* genotypes. Among the bulb samples, TFC levels of both *C. viviparum* and *C. solapurense* were closely comparable, while *C. asiaticum* exhibited notably lower value. A similar trend was observed in the leaf samples, where *C. viviparum* and *C. solapurense* showed significantly higher TFC values than *C. asiaticum* (Figure 21).

These findings concurred with that of Lekhak *et al*. (2022) reporting lower TFC in water extracts of roots of *C. asiaticum* than other species examined. In contrast to our finding, Ghane *et al*., (2018) and Goswami and Ray (2017) recorded high TFC in aqueous extracts and Ethyl acetate extracts leaves of *C. asiaticum* respectively, indicating that extraction solvent can significantly influence flavonoid yield. Comparative studies on other species, such as *C. latifolium* and *C. amoenum*, also support this variability, by exhibiting higher TFC in the leaves as seen in *C. amoenum* (Lekhak *et al*., 2022) and in *C. latifolium* (Kumar *et al*., 2022). Whereas the roots of *C. bulbispermum* was reported having the highest TFC value by Adewusi and Steenkamp (2011).

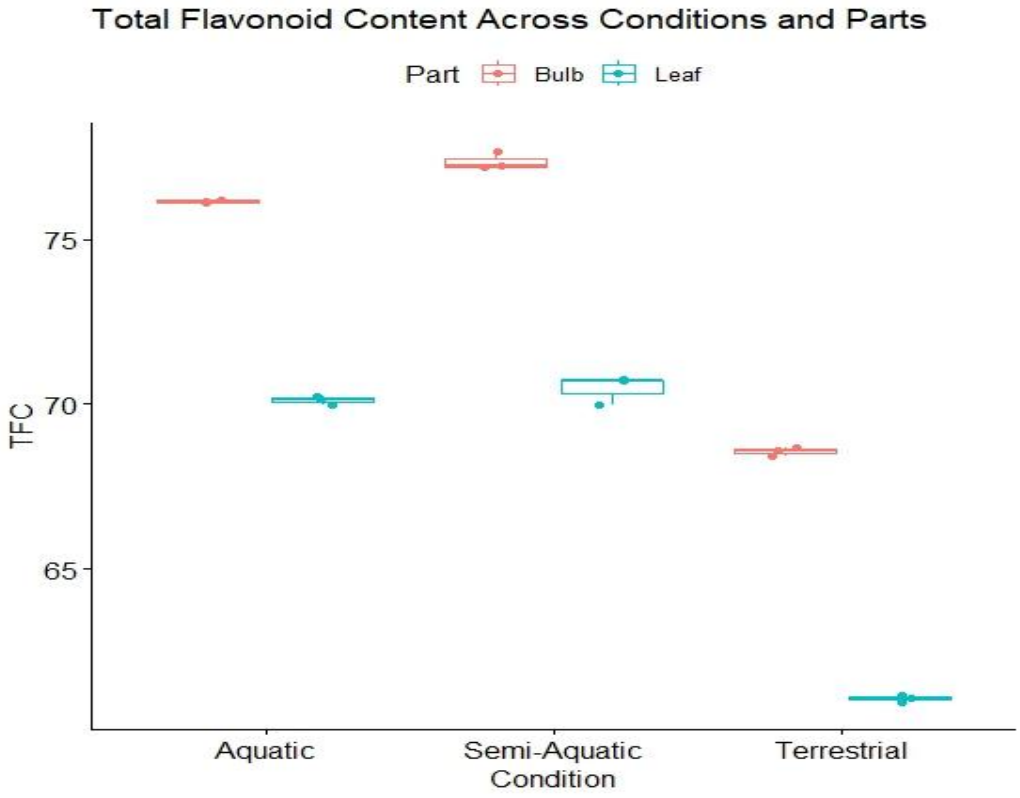

Figure 21: Total Flavonoid Content of *Crinum* species Across Conditions and Parts.

The bulb samples exhibited higher Total Alkaloid Content than the leaves across all examined *Crinum* species. *C. asiaticum* had the highest TAC value in the bulb but had the lowest TAC value in the leaf. In contrast, *C. solapurense* and *C. viviparum* exhibited moderate and relatively similar TAC values in both bulbs and leaves, suggesting organ-specific variation in alkaloid accumulation within the species (Figure 22).

These findings are in partial agreement with earlier studies, for instance, higher TAC values were reported in methanolic extracts of roots of *C. viviparum* (Lekhak *et al*., 2022) and leaves of *C. solapurense* (Ghane *et al*., 2018), whereas lower TAC values were recorded in methanolic and water extracts of *C. asiaticum* roots (Lekhak *et al*., 2022). Similar to our findings, Ghane *et al*., 2018 recorded higher TAC in the aqueous leaf extracts *C. asiaticum*. Comparative studies on *C. macowanii* also recorded higher TAC value in bulb than other plant parts (Elgorashi *et al*., 2002). Bulbs of *C. asiaticum* is reported to have high TAC value in several earlier studies which contributed to their antibacterial activity, making it a candidate for development of potential drugs for several diseases (Rahman *et al*., 2011; Refaat *et al*., 2013). Oloyede *et al*. (2010) also reported that the presence of alkaloids are responsible for the effective free radical scavenging activities in *C. ornatum*.

The variation in TPC, TFC and TAC observed across species, organs, and solvent systems may be attributed to genetic differences, tissue-specific metabolic activity, and the polarity of solvents used for extraction. This reinforces the importance of standardized extraction protocols when comparing phytochemical profiles across species. Overall, the current findings contribute to the growing body of knowledge on phytochemical diversity in *Crinum* species and suggest that bulb tissues, particularly in *C. viviparum* and *C. solapurense*, could serve as promising sources of flavonoid and phenolic compounds, and that of *C. asiaticum* as a prospective candidate for alkaloid extraction for future pharmacological studies.

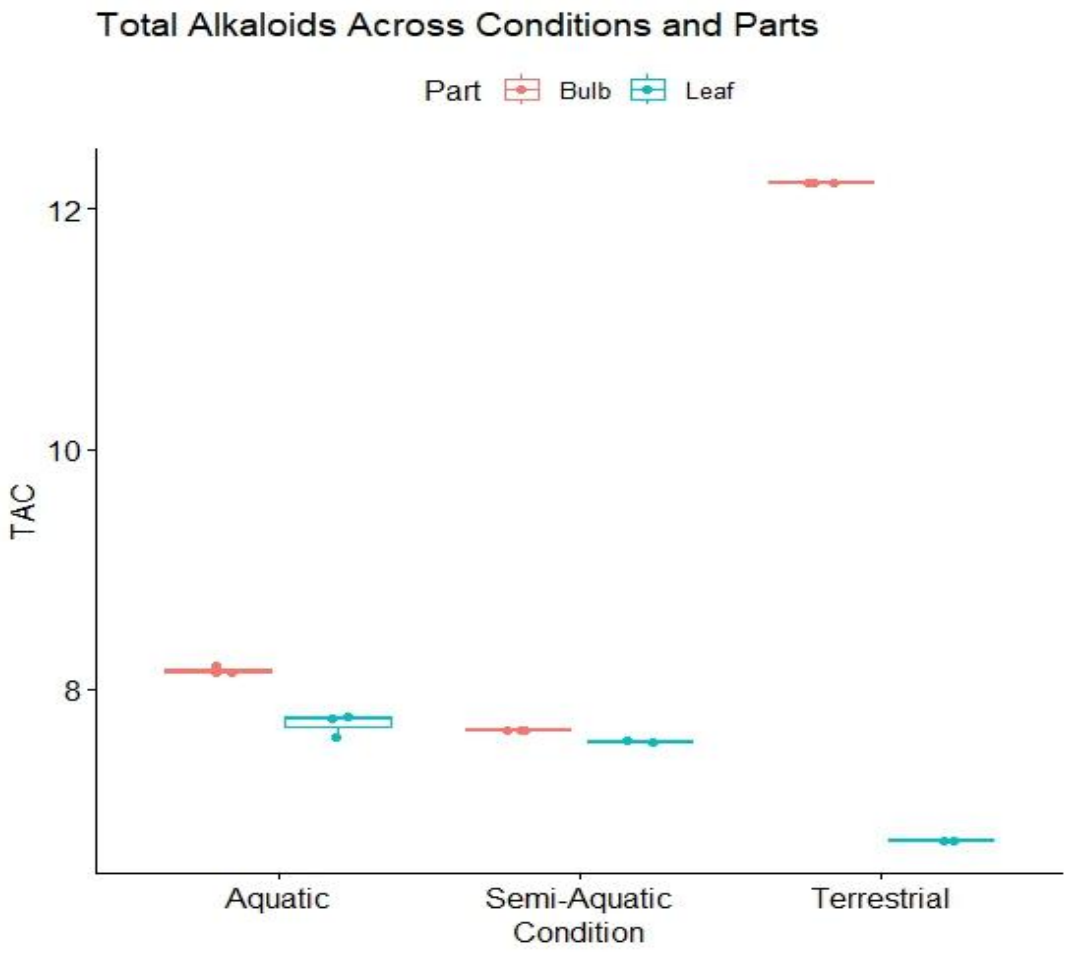

Figure 22: Total Alkaloid Content of *Crinum* species Across Conditions and Parts.

5.2. *IN VITRO* ANTIOXIDANT ACTIVITIES

The present study revealed that the leaf and bulb samples show no significant difference in the DPPH assay among the *Crinum* species examined. *C. asiaticum* exhibited higher DPPH assay in both leaf and bulb. Whereas, no considerable difference was observed in DPPH assay of *C. solapurense* and *C. viviparum* exhibiting moderate and highly comparable values in both bulbs and leaves.

The observed outcomes support earlier investigations. For instance, *C. asiaticum* have exhibited significant DPPH radical scavenging activity in both ethanolic and aqueous leaf extracts by earlier workers (Uddin *et al*., 2012; Goswami and Ray, 2017). Remarkable DPPH radical scavenging was reported from the methanolic extract of *C. asiaticum*, particularly in bulb (Rahman *et al*., 2011) and leaf (Ghane *et al*., 2018). Soni *et al*. (2015) also noted considerable DPPH scavenging activity from different solvent extracts of *C. asiaticum* bulb, indicating the high free radical scavenging activity of *C.*

asiaticum in various extraction solvents. Among the similar studies, highest radical scavenging activity measured from the methanolic and aqueous root extracts of *C. moorei* (Lekhak *et al*., 2022) and from the hydroalcoholic extracts of *C. latifolium* (Kumar *et al*., 2022). Lowest DPPH activity was observed in the aqueous extract of *C. lorifolium* by Ghane *et al*. (2018).

In the present investigation, significant variations are observed in the bulb and leaf samples of each *Crinum* species. Among the bulbs, *C. asiaticum* possessed the highest antioxidant activity and *C. solapurense* possessed the least. Interestingly, the leaves of *C. asiaticum* had the lowest antioxidant activity and the leaves of *C. viviparum* and *C. solapurense* recorded remarkable similarity, suggesting organ-specific variation in antioxidant activity within species.

Previous investigations align with these findings, evidenced by Ramesh, (2021) recording significant antioxidant activity in the methanolic flower extracts of *C. asiaticum*. However, in the study conducted by Lekhak *et al*. (2022), minimum ABTS scavenging activity in methanol extract were exhibited by *C. asiaticum* and the highest ABTS scavenging was noticed in water extracts of *C. lorifolium*. Similar studies conducted on *C. latifolium*, revealed significantly higher ABTS scavenging activity was observed in aqueous leaf extracts of *C. latifolium* (Ghane *et al*., 2018) and in the ethanolic bulb extracts of C. *latifolium* (Chen *et al*., 2018). Adewusi and Steenkamp (2011) recorded noteworthy ABTS scavenging activity in the methanolic extracts of bulb of *C. bulbispermum.*

The present study reveals that leaves possess significant increase in Ferric Reducing Antioxidant Property Activity (FRAP) compared to the bulb samples. Notably, *C. asiaticum* show highest antioxidant activity in bulb and lowest in leaf, while *C. viviparum* possess highest antioxidant activity in leaf and lowest in bulb, suggesting organ-specific variation in antioxidant activity within species.

Similar results obtained in earlier studies, noting significant FRAP activity in *C. asiaticum*, particularly in the methanolic extracts of leaf (Ghane *et al*., 2018) and methanolic extracts of root (Lekhak *et al*., 2022). Comparative studies on *C. lorifolium* recorded considerable FRAP activity in methanolic root extracts by Lekhak *et al*.

(2022). Alwode *et al*. (2019) reported that ethyl acetate extract of *C. jagus* bulbs contains compounds with the highest ferric reducing properties. Comparatively weak FRAP radical scavenging activity was reported in *C. mauritianum* (Neergheen *et al*., 2007).

Increased antioxidant activity in *Crinum* genus is attributed to its enhanced phytochemical profiles. The free radical scavenging and electron-donating abilities of the selected species is reflected by their elevated phenolic, flavonoid and alkaloid contents. The present study reveals that the total flavonoid and phenolic contents are directly proportional to the DPPH, ABTS and FRAP assays. For instance, the bulb of *C. asiaticum* and the leaves of *C. viviparum* and *C. solapurense* possess remarkable TPC and TFC values which align with their increased DPPH, ABTS and FRAP activities. Interestingly, the least DPPH, ABTS an FRAP assays of *C. asiaticum* leaves are linked to its reduced TPC and TFC values, suggesting organ-specific variation in antioxidant activity within species. Thus, the highest antioxidant activity was observed in the bulbs of terrestrial species and the leaves of semi-aquatic and aquatic species.

In general, the Ferric reducing antioxidant potential and ABTS radical scavenging activity shows high correlation with phenolic content and alkaloid content respectively. However, DPPH free radical scavenging activity relatively shows no correlation with low correlation with phenolic content and flavanoid content and possess mild correlation with alkaloid content suggesting that in addition to these phytochemical groups other secondary metabolites are also contributing the antioxidant potential of the methanolic extracts of *Crinum* species studied (Figure 23).

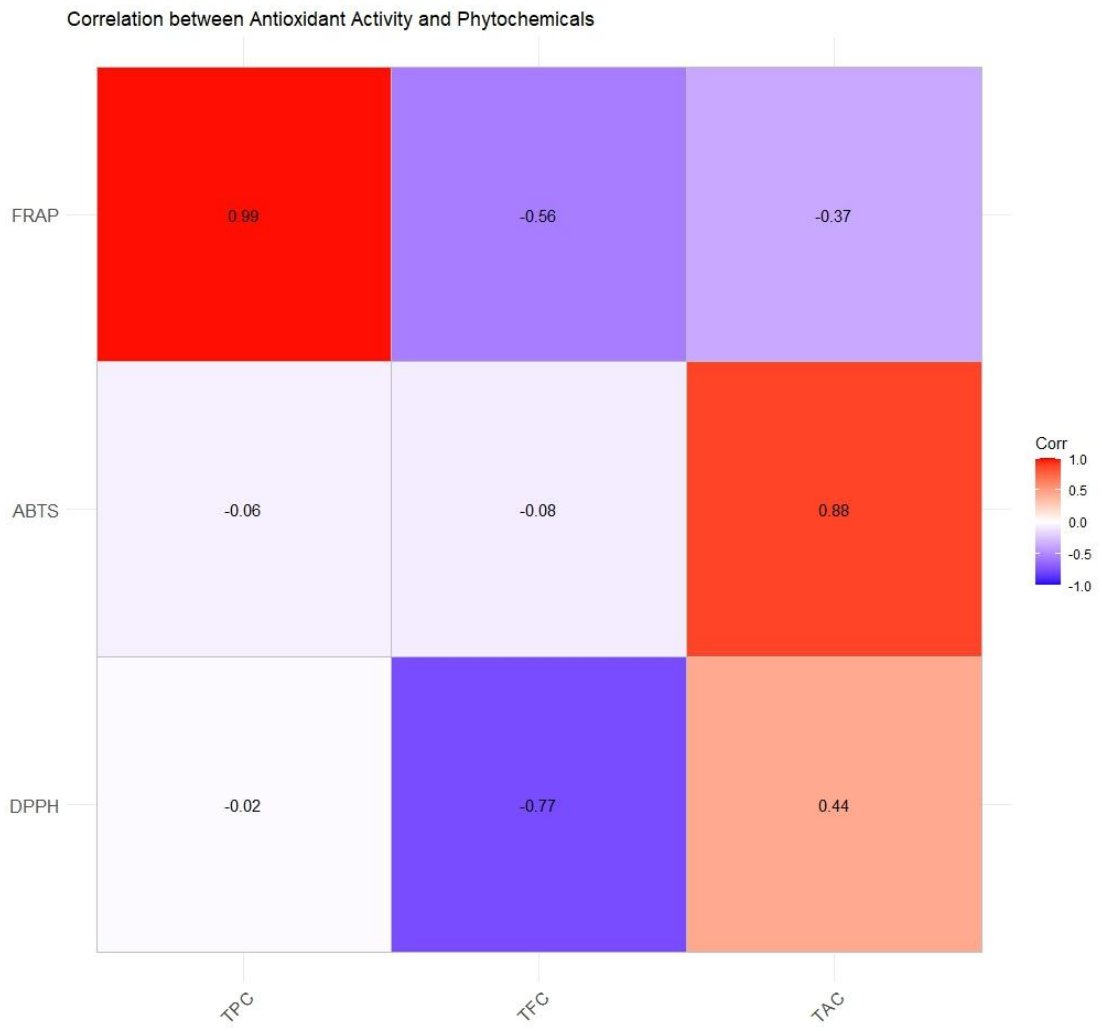

Figure 23: Correlation between Antioxidant Activity (DPPH, ABTS and FRAP) and Phytochemicals (TPC, TFC and TAC).

5.3. Acetylcholinesterase (AChE) inhibitory activity

Higher AChE inhibitory activity was observed in the bulb samples compared to the leaf samples. The methanolic extracts of the *C. asiaticum* exhibited the highest inhibitory activity in both bulb and leaf. The bulbs of *C. viviparum* and *C. solapurense* showed relatively similar inhibitory activities. In contrast, the leaf samples of *C. viviparum* showed a significant decrease in AChE inhibitory activity when compared to *C. solapurense* and *C. asiaticum*.

These findings align with Lekhak *et al*. (2022) reporting significant AChE inhibition in the methanolic root extract of *C. asiaticum* > *C. viviparum* > *C. solapurense*, while highest AChE inhibition activity was observed in *C. amoenum* and lowest in *C. lorifolium*. Ghane *et al*. (2018) and Duc *et al*. (2024) demonstrated high AChE inhibitory effects in methanolic leaf extract of *C. asiaticum*. Significant inhibition was also observed in *C. solapurense* (Ghane *et al*., 2018).

Comparative studies on other species, such as *C. latifolium* and *C. malabaricum*, also reported significantly higher AChE inhibition properties as seen in the methanolic leaf extracts of *C. latifolium* (Trang *et al*., 2024; Ghane *et al*., 2018) and in aqueous root extracts of *C. malabaricum* (Lekhak *et al*., 2022). An alkaloid, galantamine from different Amaryllidaceae species is an active and reversible inhibitor of AChE (Trang *et al*., 2024; Calderón *et al*., 2010; Tumiatti *et al*., 2008). AChE inhibition was also studied in methanolic bulb and root extracts of *C. bulbispermum* (Adewusi and Steenkamp, 2011) and in *C. jagus* (Ka *et al*., 2020). The AChE inhibitory activity of ethyl acetate: methanol alkaloid (L_EAMAF) fraction, derived from the ethanolic extract of *C. americanum* leaves showed high activity (Gomes *et al*., 2022).

AChE inhibition is considered as a significant mechanism for the therapy of Alzheimer's disease (Trang *et al*., 2024). The observed variation in AChE inhibition across species, organs, and solvent systems may be attributed to genetic differences, tissue-specific metabolic activity, and the polarity of solvents used for extraction. This reinforces the importance of standardized extraction protocols when comparing AChE inhibition property across species. Overall, the current findings contribute to the accumulation of evidences on the AChE inhibition of *Crinum* species and emphasize the potential of *C. asiaticum* bulbs as AChE inhibitors, contributing to the proven interventions for treating Alzheimer's disease symptoms.

Increased Acetylcholinesterase inhibitory activity (AChE) in *Crinum* genus is attributed to its enhanced alkaloid content. Remarkable inhibitory activity was observed in the bulbs and leaves of *C. asiaticum*, particularly higher in bulbs, making this terrestrial species a promising AChE inhibitor.

The alkaloid-rich genus *Crinum* is a reservoir of galantamine, haemanthamine, crinamine, and lycorine, making it a promising drug for the treatment of neurodegenerative disorders like Alzheimer's disease (Mimrot *et al*., 2024; Chaichompoo *et al*., 2024). These alkaloids, particularly galantamine, contributes to their Acetylcholinesterase inhibitory activity. Phenols and flavonoids isolated from *Crinum* species can be used as a leading molecule in drug discovery and pharmacological investigations due to its enhanced antioxidant property. For advanced research, Insilico methods can be used for the identification and validation of the structure of major components present in the phytochemicals that attributes to their therapeutic potential. Since *Crinum* species is enriched with medicinal properties, there arise a chance of over exploitation, which demands for *in situ* and *ex situ* conservation. Thus, *Crinum* species can be utilised for addressing global healthcare challenges by the sustainable utilization and commercialization of this plant.

6. CONCLUSION

The genus *Crinum* is well known for their therapeutically potential biochemicals. This bio constituents including alkaloids, flavonoids and phenols which possess remarkable antioxidant and enzyme inhibition activity. In India the species show diversity in habitat ranges from aquatic, semi aquatic to terrestrial. Most of these species are least studied and their bioactive components are lesser known. Prevailing researches have focused on the leaves, overlooking the potential of bulbs as reservoirs of bioactive compounds. Additionally, comprehensive comparative analyses of *Crinum* species from diverse ecological niches—aquatic, semi-aquatic, and terrestrial—remain largely unexplored. Moreover, some of the members, such as *C. solapurense* are recently discovered and are known from limited locations. Understanding their potential uses helps to the enrich the cause of their conservation.

The present study was contented to comparative estimation of the bioactive potential of three Indian *Crinum* species of varied ecological niches viz. *Crinum viviparum* (aquatic), *C. solapurense* (semi aquatic) and *C. asiaticum* (terrestrial). In this study the phytochemical contents such as TPC, TFC, TAC of the methanolic extract of bulbs and leaves of these species was quantified using colorimetric methods. And the antioxidant potential such as DPPH free radical scavenging activity, ABTS radical scavenging activity and Ferric reducing antioxidant property (FRAP) activity was studied. Additionally, the Acetylcholinesterase inhibition activity was studied to assess the neuroprotective potential of these species.

Our findings indicate that terrestrial species have highest bioactivity in terms of antioxidant activity and enzyme inhibition property, particularly in their bulbs. Phenolic and flavonoid contents contribute to antioxidant activity, whereas AChE inhibitory property attributes to the alkaloid content. Thus, the phytochemical diversity within the *Crinum* species makes it a potential candidate for future pharmacological investigations and drug discovery, enhancing the need for sustainable utilization and commercialization.

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