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Phytochemistry and Pharmacology of the Family *Amaryllidaceae*: An Overview of Research at RCPGD

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Abstract

Professor Johannes van Staden, Director of the Research Centre for Plant Growth and Development-University of KwaZulu Natal, conducts research in the field of plant physiology, biotechnology, and ethnomedicine. The research span over a wide range of plant families growing in the southern African region. The plant family *Amaryllidaceae*, known for its ornamental and pharmacological values, received much attention by his research group. This review covers research conducted by his group on the chemistry of some members of *Amaryllidaceae* and biological activities of their constituents.

Keywords

Amaryllidaceae, chemistry, biological activity, alkaloids

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Members of the family *Amaryllidaceae* J. St.-Hil. are mostly bulbous perennial or biennial plants that are widely distributed in the tropics and the warm parts of the temperate regions of the world. They are richly represented in the southern African region and to a lesser extent in the Andean South America and the Mediterranean.[1,2] The family comprises about 1000 species belonging to 60 genera of which an estimated 300 species in 20 genera are of African origin.[3,4] The *Amaryllidaceae* plants centered in the southern African region belong mostly to 3 major tribes namely *Haemantheae* Pax (Hutchinson), *Cyrthantheae* Salisb., and *Amarylldeae* J. St.-Hil., although some members of the latter have a pantropical distribution, eg, *Crinum*.[4,5] The most African tribe *Amarylldeae* is divided into 2 subtribes: the subtribe *Crininae* comprising *Boophone* Herb., *Crinum* L., *Ammocharis* Herb., and *Cybistetes* Milne-Redh. & Schweick, while the subtribe *Amaryllidinae* comprising *Amaryllis* L., *Nerine* Herb., *Brunsvigia* Heist., *Crossyne* Salisb., *Hessea* Herb., *Strumaria* Jacq., and *Carpolyza* Salisb.[2,6]

Members of the family *Amaryllidaceae* are known for their ornamental and horticultural value. Many varieties are cultivated for their ornamental appeal including those of *Narcissus* L. in Europe,[7] *Hippeastrum* Herb. in South America and the Indian subcontinent,[8] others are wild sourced ornamental bulbs such as the genus *Galanthus* L.[9] Species belonging to *Amaryllidaceae* have also been used in traditional medicine by indigenous people throughout the world to cure ailments and diseases.[2,10,11] Literature reports suggest widespread use of *Amaryllidaceae* by different ethnic groups in South Africa such as Khoi-San, Sotho, Tswana, Xhosa, and Zulu.[10] This is due to the fact that traditional medicine forms an integral part of southern African culture.[2] Included among various ailments and diseases are stomach ailments, skin diseases, headaches, dizziness, wounds, chest and bladder pain, renal and liver complaints, infertility, aching joints, rheumatism, snake bites, facilitation of child birth during labor, hysteria, and as narcotics.[12-15]

The rationale behind the use of *Amaryllidaceae* in traditional medicine and their medicinal efficacy is attributed largely to the presence of a unique type of alkaloids. The alkaloids are present exclusively in this family and isolated from each member of *Amaryllidaceae* investigated so far. These alkaloids are structurally related due to their biogenesis from the common precursor norbelladine **1**. These alkaloids have been classified into distinct ring types namely crinine **2**, lycorine **3**, galanthamine **4**, tazettine **5**, homolycorine **6**, and montanine **7** ring types.[2,16,17]

The use of the amaryllidacious plants in both the ornamental industry and the traditional medicine formed the basis for

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the research activities conducted by Professor van Staden's group. Their ornamental value is the driving force for research in plant physiology, mainly tissue culture and micropropagation as well as plant growth regulators. Research on pharmacological activities and phytochemical screening has been conducted to establish the rational usage of these plants in traditional medicine and the molecules behind their activity. This review will highlight research conducted by the group on the chemistry and biological activities of species belonging to the family *Amaryllidaceae* from 1990 to 2018.

The study of *Amaryllidaceae* alkaloids began in the late 1990s with the investigation of the alkaloidal content of *Crinum bulbispermum*.[18] Since then, 36 alkaloids have been isolated from 9 species belonging to 4 genera endemic to South Africa. The structures of these alkaloids span over 3 main ring types namely crinine **2**, lycorine **3**, and tazettine **5**. Other ring type such as cherylline **8** was also found in some of these species. A list of trivial names of these alkaloids, their distribution, biological activities, and source is given in Table 1. Although most of the listed alkaloids are known compounds, of particular interest is the isolation of 8α-ethoxy precriwelline **9**, *N*-desmethyl 8α-ethoxy pretazettine **10**, and *N*-desmethyl-8β-ethoxy pretazettine **11** for the first time from *C. bulbispermum*.[18] The compounds are characterized by the presence of an ethoxy group at position 8 of the tazettine type ring.

It is worth mentioning that the compounds were extracted using hot ethanol and it is possible that these compounds are artifacts of the extraction process. Extraction of hemiacetals with methanol leads to methylation and it is likely the same that has happened here with ethanol.[38]

Two new alkaloids were isolated from *Crinum moorei* namely mooreine **12** and 3-[4'-(8'-aminoethyl)phenoxy] bulbispermine **13**.[28] Of particular interest is the isolation of distichaminol **14**, a derivative of distichamine **15** an alkaloid only isolated from the genus *Boophone*, from *Boophone haemanthoides*[23] and the isocarbostyril narciprimine **16** isolated from the endemic southern African *Cyrtanthus contractus*. Narciprimine **16** has been only isolated from *Zephyranthes*, *Narcissus*, and *Lycoris* genera, endemic to America, Europe, and Asia, respectively.[30] Some of the findings included isolation and detection of various nonalkaloidal compounds. Tyramine **17** which is one of the components for the biosynthesis of norbelladine **1**, a precursor for *Amaryllidaceae* alkaloids, was isolated from *Crinum macowanii*[27]; isogeunitol **18**, isoeugenitol glycoside **19**, and 9*Z*-octadec-9-enamide **20** from *Gethyllis ciliaris*.[36] Moreover, hydroxybenzoates, hydroxy-cinnamates, and flavonoids were identified in *Cryptostephanus vansonii*[4] and *Scadoxus puniceus*[37] using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS).

It is well established that alkaloid production and distribution vary with time of the year and the developmental stage of the plant. In many instances, the site of biosynthesis may differ from the site of accumulation.[17] Therefore, a number of studies on ontogenic variations of *Amaryllidaceae* alkaloids have also been conducted on some of the South African members of the family.[38–40] In a study investigating the seasonal and plant parts variation of *C. macowanii* over 2 consecutive years, bulbs were found to have high alkaloid content compared to leaves, roots, and flowering stalks. Crinine **2** and lycorine **3** were the major alkaloids detected in *C. macowanii*. Bulbs contained the highest concentrations of crinine **2** and lycorine **3** and only traces of cherylline **8**, crinamidine **22**, and 1-epideacetylbowdenisine **23**. Crinine **2** was the only alkaloid detected in the leaf, while the root is the major source of crinamine **24**. No trend was observed in the seasonal variation of these alkaloids. For instance, bulbs had the highest crinine **2** levels during winter followed by spring. In the roots, the crinine **2** content was high in spring in the first year of study; however, in the second year, the lowest level was detected in spring. The same applies to the seasonal levels of other alkaloids including crinamidine **22** and 1-epideacetylbowdensine **23**. The inconsistency in alkaloid variation on annual basis is a reflection of the heterogeneity of the population sampled. Investigations on the cytology of *C. macowanii* revealed variation in the chromosome number. Chromosome numbers of $2n = 72$, $2n = 44$, and $2n = 30$ were reported for plants of *C. macowanii*.[41]

The same pattern of variation was observed in alkaloid content of *C. moorei* between different plant parts, seasons of the year, and even consecutive years. For instance, the highest concentration of alkaloid was found in the leaves followed by the flowering stalks. Alkaloids possessing the ethano bridge in a β-position and lacking a double bond between positions 1 and 2 such as crinamidine **22**, undulatine **26**, and 1-epideacetylbowdenisine **23** were found in the leaves and flowering stalks. Lycorine **3** and 1-*O*-acetyllycorine **28** were found in higher concentrations in the roots. Crinine **2** and its derivatives buphanisine **45** and powelline **27** were found in higher concentrations in the bulb.[40] Seasonal and year-to-year variations in alkaloid content of *C. moorei* were also observed. Bulbs had the highest levels of lycorine **3**, crinine **2**, and undulatine **26** in winter during the first year of the study, while bulbs had the highest concentrations of lycorine **3** and crinine **2** in summer and had the lowest concentration of undulatine **26** in winter.[40] The high variability of alkaloid content in these species was due to high variation among plants of the same species collected in the same season. This variability is attributed to the fact that most members of the family *Amaryllidaceae* are self-sterile and seeds are seldom produced after self-fertilization.[42]

Variations in other nonalkaloidal constituents were also investigated. Different plant parts of *C. vansonii* were found to contain different levels of hydroxybenzoic acids, hydroxy-cinammates, and flavonoids. For instance, the flavonoids naringenin and genistein were identified in at least 1 plant part (leaf, basal leaf, rhizome, and root). Similar results

Table 1. List of Plant Species Investigated Including Their Constituents and Biological Activities.

Species	Alkaloid constituents	Biological activity	Reference
Boophone disticha (L.f.) Herb.	Buphanamine **37**	SSRI	19-22
	Buphanadrine **36**	SSRI, antibacterial	
	Buphanisine **45**	SSRI, cytotoxic	
	Crinine **2**		
	Distichamine **15**	SSRI, antibacterial, cytotoxic	
Boophone haemanthoides F.M. Leight	Crinine **2**		23
	Buphanisine **45**		
	Buphanidrine **36**		
	Ambelline **40**		
	Undulatine **26**		
	Lycorine 3	Cytotoxic	
	Distichamine **15**	Cytotoxic	
	Distichaminol **14**		
Crinum bubispermum ((Brum. f.) Milne-Redhead and Schweickerdt)	Crinamine **24**	AChE, SSRI	18,24,25
	Bulbispermine **49**		
	6-Hydroxycrinamine **29**	AChE	
	3-*O*-Acetylhamayne **30**	AChE	
	8α-Ethoxy precriwelline **9**	AChE	
	N-Desmethyl 8α-ethoxy pretazettine **10**	AChE	
	N-Desmethyl-8β-ethoxy pretazettine **11**	AChE	
Crinum lugardiae N.E.Br.	Crinine **2**		26
	Hamayne **46**		
	Lycorine **3**		
Crinum macowanii Bak.	Crinine **2**		24,27
	Bulbispermine **49**		
	Cherylline **8**		
	Hamayne **46**	AChE	
	Lycorine **3**	AChE	
	Tyramine **17**		
Crinum moorei (Hook. f.)	Crude extract	COX-1, -2, AChE, antioxidant	24,25,28,29
	Cherylline **8**	AChE, SSRI	
	Crinine **2**	AChE, SSRI	
	3-*O*-Acetylcrinine **44**		
	Crinamidine **22**	AChE	
	Epibuphanisine **25**	AChE, SSRI	
	Epivittatine **35**	AChE, SSRI	
	1-Epideacetyl-bowdensine **23**		
	Lycorine **3**		
	1-*O*-Acetyllycorine **28**	AChE, SSRI	
	Mooreine **12**		
	Powelline **27**	SSRI	
	Undulatine **26**		
	3-[4'-(8'-Aminoethyl)phenoxy] bulbispermine **13**		
Cryptostephanus vansonii I. Verd.	Crude extract	AChE, antibacterial, cytotoxic	4
Cyrtanthus contractus N.E.Br.	Crude extract	COX-1, -2, AChE, cytotoxic, antibacterial	21,30,31
	Narciprimine **16**	Cytotoxic, AChE	
Cyrtanthus falcatus R.A. Dyer	Crude extract	COX-1, -2, antibacterial	25,32,33
	Epipapyramine **48**		
	Maritidine **38**	SSRI	
	O-Methylmaritidine **39**	SSRI	
	Papyramine **47**		
	Tazettine **5**	SSRI	
Cyrtanthus mackenii Hook. f.	Crude extract	COX-1, -2, antibacterial	33

(Continued)

Table 1. Continued

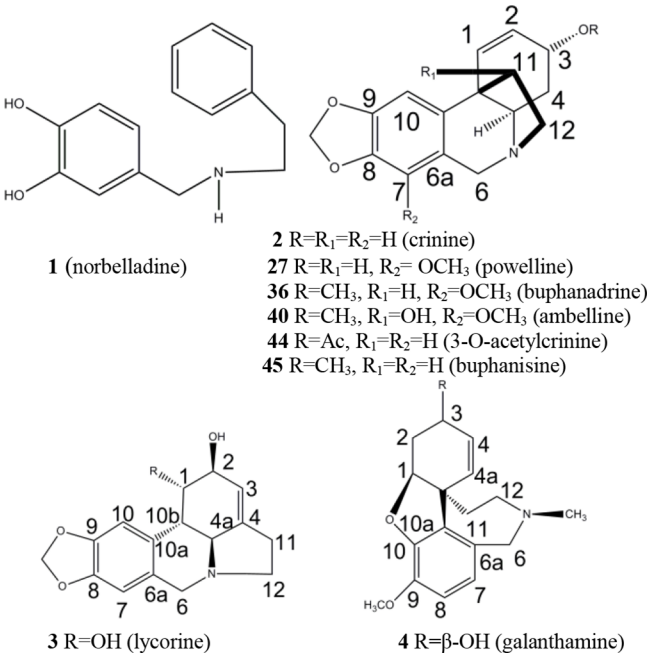
Species	Alkaloid constituents	Biological activity	Reference
Cyrtanthus obliquus Ait.	Crude extract (bulb)	Antibacterial	34
Cyrtanthus suaveolens Schonland	Crude extract	COX-1, -2, antibacterial	33,35
	Captan **43**		
Gethyllus ciliaris linn. f.	Crude extract	COX-1, -2, antibacterial (bulb) *Escherichia coli*	33,36
	Isoeugenitol **18**	COX-1	
	Isoeugenitol glycoside **19**		
	9*Z*-Octadec-9-enamide **20**		
Gethyllis multifolia L. Bolus	Crude extract	COX-1, -2	33
Gethyllis villosa linn. f.	Crude extract	COX-1, -2	33
Scadoxus puniceus (L.) Friis & I.Nordal	Haemanthamine **33**	AChE	37
	Haemanthidine **34**	AChE	
	Metolachlor **21**	AChE	

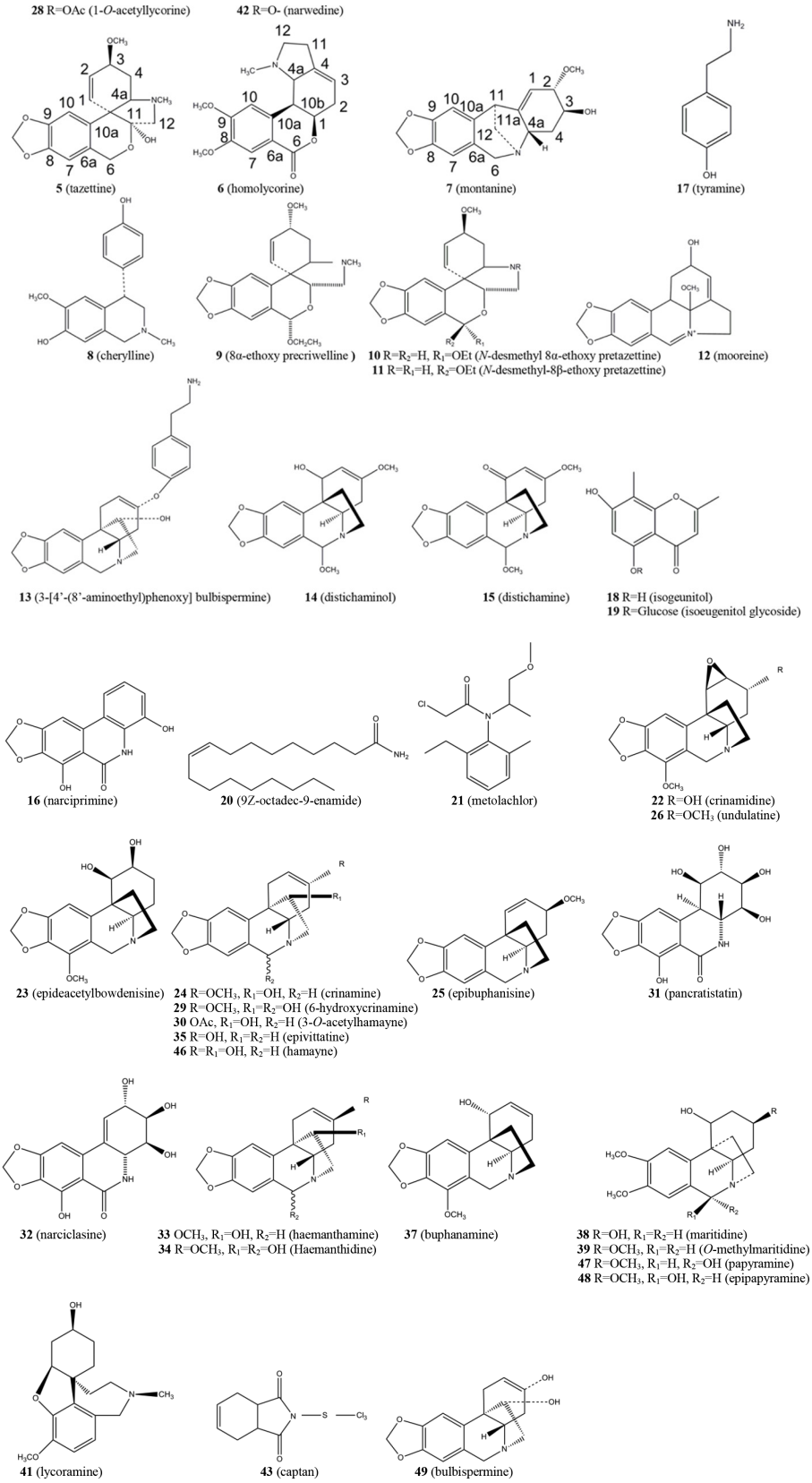
AChE, acetylcholinesterase; COX, cyclooxygenase; SSRI, selective serotonin re-uptake inhibitors.

were observed for the levels of these constituents detected in extracts of *S. puniceus*.[4,37]

The presence of *Amaryllidaceae* alkaloids in different members of the family has been the focus of many pharmacological investigations. However, in-depth studies on interspecific variations in *Amaryllidaceae* alkaloids are rare.[43] A study was conducted to investigate the interspecific variation in alkaloid content of *Crinum bulpespermum*, *C. macowanii,* and *C. moorei* using the above-mentioned data on ontogenic variations.[43] Of the 13 alkaloids used, the presence of epibuphanisine **25** and undulatine **26** separated *C. moorei* from *C. bulbispermum* and *C. macowanii*. While the presence of cherylline **8**, crinamidine **22**, 1-epideacetylbowdenisine **23**, powelline **27**, and 1-*O*-acetyllycorine **28** separated *C. macowanii* and *C. moorei* from *C. bulbispermum*. 6-Hydroxycrinamine was detected only in *C. bulbispermum*. Quantitatively, the levels of crinine **2**, powelline **27**, undulatine **26**, 1-epideacetylbowdenisine **23**, crinamidine **22**, and 3-*O*-acetylhamayne **30** were significantly higher in the bulbs, leaves, and flowering stalks of *C. moorei* than those of *C. bulbispermum* and *C. macowanii*. The roots of *C. moorei* had a significantly high level of cherylline **8**, powelline **27**, crinamidine **22**, and 1-epideacetylbowdenisine **23** than in *C. bulbispermum* and *C. macowanii*. The study also found that leaves of *C. moorei* had the highest alkaloid content throughout the year but no alkaloids were detected in the leaves of both *C. bulbispermum* and *C. macowanii*. Principal component analysis of these alkaloids revealed that *C. macowanii* is much closely related to *C. bulbispermum* than *C. moorei*.[43] These findings are in agreement with findings from morphological characters. *Crinum moorei* differs morphologically from all South African *Crinum* species. The plant forms a false stem from the thickened and hardened leaf-bases. The bulb has leaves at the apex which do not die back and grow out again the following year.[44] Other alkaloids that are unique to certain genera or species could be used as taxonomic markers. For instance, Nair et al suggested that distichamine **15** which is detected only in members of the genus *Boophone* namely *Boophone disticha* and *B. haemanthoides* is of significance as a distinctive chemotaxonomic marker for the genus *Boophone*.[6]

Extracts from members of the plant family *Amaryllidaceae* and their alkaloidal constituents have exhibited various biological activities such as antimicrobial, antitumor, antiparasitic, anti-inflammatory, and central nervous system diseases.[2,17] However, the pharmacological importance of the family received much attention after approval of the selective, reversible acetylcholinesterase (AChE) inhibitor galanthamine **4** for the management of Alzheimer's disease by the FDA and many European countries. Other promising molecules are the isocarbostyrils pancratistatin **31** and narciclasine **32** for their demonstrated anticancer and cell-line specific activities.[16,45]




28 R=OAc (1-O-acetyllycorine)
42 R=O- (narwedine)
5 (tazettine)
6 (homolycorine)
7 (montanine)
17 (tyramine)
8 (cherylline)
9 (8α-ethoxy precriwelline)
10 R=R2=H, R1=OEt (N-desmethyl 8α-ethoxy pretazettine)
11 R=R1=H, R2=OEt (N-desmethyl-8β-ethoxy pretazettine)
12 (mooreine)
13 (3-[4'-(8'-aminoethyl)phenoxy] bulbispermine)
14 (distichaminol)
15 (distichamine)
18 R=H (isogeunitol)
19 R=Glucose (isoeugenitol glycoside)
16 (narciprimine)
20 (9Z-octadec-9-enamide)
21 (metolachlor)
22 R=OH (crinamidine)
26 R=OCH3 (undulatine)
23 (epideacetylbowdenisine)
24 R=OCH3, R1=OH, R2=H (crinamine)
29 R=OCH3, R1=R2=OH (6-hydroxycrinamine)
30 OAc, R1=OH, R2=H (3-O-acetylhamayne)
35 R=OH, R1=R2=H (epivittatine)
46 R=R1=OH, R2=H (hamayne)
25 (epibuphanisine)
31 (pancratistatin)
32 (narciclasine)
33 OCH3, R1=OH, R2=H (haemanthamine)
34 R=OCH3, R1=R2=OH (Haemanthidine)
37 (buphanamine)
38 R=OH, R1=R2=H (maritidine)
39 R=OCH3, R1=R2=H (O-methylmaritidine)
47 R=OCH3, R1=H, R2=OH (papyramine)
48 R=OCH3, R1=OH, R2=H (epipapyramine)
41 (lycoramine)
43 (captan)
49 (bulbispermine)

The approval of galanthamine **4** for the management of Alzheimer's disease fuelled research on the *Amaryllidaceae* plants throughout the world with the Research Centre for Plant Growth and Development (RCPGD), University of KwaZulu Natal as no exception. Research on central nervous system disorders started in 2003 with the introduction of 2 biological assays: the AChE and serotonin re-uptake inhibitory assays. The AChE enzyme is responsible for the degradation of acetylcholine at the central cholinergic synaptic junction leading to impairment of cognitive functions including memory loss and inability to perform basic daily life activities.[46,47] Several members of *Amaryllidaceae* have been screened for their AChE inhibitory activity. Extracts from *B. disticha*, *Crinum campanulatum*, *Crinum graminicola*, *C. macowanii,* and *C. moorei* inhibited AChE activity. The activity is attributed to galanthamine **4** as all extracts tested contained a band corresponding to galanthamine **4** in thin layer chromatography plates.[48,49] Extracts from *C. contractus*,[30] *S. puniceus* (IC_{50} = 70 μg/mL,[37] and different parts of *C. vansonii* (IC_{50} = 7.7-25.6 μg/mL) inhibited AChE as well.[4] Bulbs of *C. moorei* had an IC_{50} as low as 2.9 μg/mL.[29] Narciprimine **16** (IC_{50} = 78.9 μM) has been identified as the compound responsible for activity from *C. contractus,*[30] while haemanthamine **33** (IC_{50} = 23.7 μM), haemanthidine **34** (IC_{50} = 23.1 μM), and the chlorinated amide metolachlor **21** (IC_{50} = 11.5 μM) are behind the activity of *S. puniceus*.[37] Metolachlor is a known synthetic herbicide, its presence in crude extracts of *S. puniceus* is likely due to contamination of the plant material in the field and raises concern about the quality of commercially available medicinal plants.

Screening of several alkaloids isolated from a number of South African *Amaryllidaceae* species for activities against AChE showed differences related to different ring types. Lycorine- and galanthmine-type alkaloids were the most active ones against AChE compared to crinine- , tazettine-, and cherylline-type alkaloids which showed only weak activity against AChE. The alkaloid 1-*O*-acetyllycorine **28** (IC_{50} = 0.96 μM) exhibited inhibitory effects 2-fold more potent than that of the currently used drug galanthamine **4** (IC_{50} = 1.9 μM). However, the alkaloids crinine **2** (IC_{50} = 461 μM), crinamine **24** (IC_{50} = 300 μM), epivittatine **35** (IC_{50} = 239), 6-hydroxycrinamine **29** (IC_{50} = 490 μM), *N*-desmethyl 8α-ethoxy pretazettine **10** (IC_{50} = 234 μM), *N*-desmethyl-8β-ethoxy pretazettine **11** (IC_{50} = 419 μM), and lycorine **3** (IC_{50} = 213 μM) had weak activity.[24]

The surface electrostatic potential of these alkaloids was calculated in a structure activity relationship study to investigate the correlation between the surface charge distribution of the alkaloids and their IC_{50}. The surface charge distribution of 1-*O*-acetyllycorine **28** was found to be much closer to the most active group, the galanthamine-type alkaloids than to those of the lycorine-type alkaloids. Moreover, superpositioning of galanthamine **4** on 1-*O*-acetyllycorine **28** indicated that the 1-*O*-acetyl group and the nitrogen atom of the later superimpose on the hydroxyl group and the nitrogen atom of galanthamine **4**, respectively.[45] Greenblatt et al[50] investigated the mechanism of galanthamine **4** binding to the active site of AChE. The group reported that the double bond of the cyclohexene ring of galanthamine **4** stacks against the indole-ring binding site, while the *O*-methyl group of galanthamine **4** occupies the acetyl-binding pocket of acetylcholine. However, from the analysis and superpositioning of 1-*O*-acetyllycorine **28** and other related lycorine-type alkaloids on galanthamine **4**, it appears that the methoxy group of galanthamine **4** partially aligns with the methylenedioxy group of the lycorine-type alkaloids, while the double bond of the cyclohexene ring of galanthamine **4** does not align with any part of the lycorine-type alkaloids. This indicates that the mechanism of binding of 1-*O*-acetyllycorine **28** to AChE enzyme might not be the same as that of galanthamine **4**.[45]

Depression is a disabling illness that causes significant impairment and incurs large costs in the form of lost productivity and health care expenses. The neurotransmitters serotonin, noradrenalin, and dopamine are believed to be strongly implicated in the neuropathology of the disease.[51] Several antidepressants are known as selective serotonin re-uptake inhibitors (SSRI) as they exert their effect by selective inhibition of serotonin re-uptake by binding to a specific site on the neuronal serotonin transporter thereby inhibiting the transportation of serotonin from the synaptic gap back to the neuron.[52] Few South African *Amaryllidaceae* species have been investigated for their affinity for the serotonin re-uptake transport protein.[52] Extracts of the leaves and bulbs of *B. disticha* have exhibited strong affinity to the SSRI site, while leaf extracts of *Brunsvigia grandiflora* and root extracts of *G. ciliaris* have moderate to low affinity to SSRI site, respectively.[52] In addition, ethanolic extracts of *B. disticha* showed functional inhibition of serotonin transporter, noradrenalin transporter, and dopamine transporter upon screening in a functional inhibition assay using COS-7 cells.[51] The activity was confirmed *in vivo* using the tail suspension test and the forced swim test in both mice and rats.[51] A bioassay guided fractionation of extracts from *B. disticha* led to the identification of buphandrine **36** (IC_{50} = 274 μM) and buphanamine **37** (IC_{50} = 1799 μM) as the active constituents.[19]

The activity of *Amaryllidaceae* alkaloids from *B. disticha* prompted the screening of several *Amaryllidaceae* alkaloids isolated from a number of *Crinum* and *Cyrtanthus* species for their affinity to the SSRI site. Of the 21 alkaloids tested, cherylline **8** (IC_{50} = 3.4 μM) had the highest affinity to SSRI followed by epivittatine **35** (IC_{50} = 12.1 μM). Cherylline **8** shares some structural features with the antidepressant drug sertraline, while the activity of epivittatine **35** was attributed to the presence of a 1,3-dioxole moiety in common with the used SSRI drug paroxetin which could explain their high affinity to SSRI. Other alkaloids with affinity to the serotonin transporter are mostly crinine-type alkaloids namely powelline (IC_{50} = 20 μM), maritidine **38** (IC_{50} = 20 μM), epibuphanisine **25** (IC_{50} = 78.2 μM), and *O*-methylmaritidine **39** (IC_{50} = 40.1 μM) which had moderate activity. While crinine **2** (IC_{50} = 267 μM), crinamine **24** (IC_{50} = 608 μM), and 1-*O*-acetyllycorine **28** (IC_{50} = 452 μM) had weak affinity to the protein.[25]

The search for antimicrobial active crude extracts and molecules from the *Amaryllidaceae* plants is inspired by the widespread use of these plants in traditional medicine for the treatment of wounds and infections by different South African cultural groups. For example, Tswana people in South Africa use *C. bulbispermum* for the treatment of kidney and bladder infections. The Zulu use *C. macowanii* for the treatment of urinary tract problem. Sotho and Khosa, on the other hand, use leaves of *B. disticha* for the treatment of skin diseases.[13-15] Of the *Amaryllidaceae* species evaluated for antibacterial activity, *Cyrtanthus falactus*, *Cyrtanthus mackenii*, *Cyrtanthus suaveolens*, and *G. ciliaris* inhibited the growth of 5 bacterial strains and including both Gram-negative and Gram-positive strains.[33] The screening was done using the disc diffusion assay and the antibacterial activity of these extracts was expressed as a ratio to that produced by the positive control neomycin. All 3 *Cyrtanthus* species inhibited the bacterial growth of at least 1 strain with the ratio to neomycin within the range of 0.01 to 0.91 with the dichloromethane extracts of the underground parts of *C. suaveolens* scoring the highest ratio to neomycin. *Gethyllis ciliaris*, on the other hand, inhibited only the growth of the Gram-negative *Escherichia coli*. Low antibacterial activity was observed for other *Amaryllidaceae* plants. For example, bulb extracts of *Haemanthus albiflos* inhibited only the growth of *E. coli* and *Klebsiella pneumonia,*[53] and *Cyrtanthus obliquuos* had weak activity against *Bacillus subtilis* and *Staphylococcus aureus*.[54] *Cryptostephanus vansonii*, however, inhibited the activity of all 4 strains with minimum inhibitory concentration (MIC) values ranging from >0.5 to 0.25 mg/mL.[4] The work has been extended to *B. disticha*, another member of the *Amaryllidaceae* family where the ethanolic bulb extracts inhibited the growth of both the Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli* and *K. pneumonia*) strains. The MIC values ranged from 0.13 to 0.25 mg/mL. Bioassay-guided fractionation led to the isolation of the active alkaloids buphanidrine **36** and distichamine **15** (MIC = 0.063-0.13 mg/mL).[20] The low activity of 2 alkaloids is in agreement with the previous studies in the laboratory where none of 21 *Amaryllidaceae* alkaloids investigated inhibited bacterial growth at a concentration of 250 μg/mL.[55]

Seasonal variation is one of the major contributing factors to variations in secondary metabolites production by plants which may in turn affect their pharmacological properties for the benefit of the people. Ethanol bulb extracts of *C. contractus* collected monthly over a year were investigated for antibacterial activity against *B. subtilis*, *S. aureus*, *E. coli,* and *K. pneumonia*. The extracts collected in May and July were the only extracts that had good antibacterial activity (<1.0 mg/mL) against at least 2 bacterial strains.[31]

Few studies carried out at RCPGD focused on antifungal activity mostly against the opportunistic fungus *Candida albicans*[31,34,53,54] and to a lesser extent on skin fungi such as *Trichophyton mentagrophytes*, *Trichophyton tonsurans,* and *Micrococcus canis*.[53] Extracts of *Cyrtanthus obliquus* had significant antifungal activity against *C. albicans* with an MIC value as low as 0.195 mg/mL.[54] While extracts of *C. contractus* and *H. albiflos* were not active.[31,53]

The majority of studies dedicated to in vitro screening of plant extracts for anti-inflammatory activity followed an ethnobotanical lead.[33,54,56] Plants traditionally used to treat pain, fever, swelling of the body, asthma, and joint pain have been targeted in a course of continued investigation of South African plants for anti-inflammatory activity. Initially, crude extracts were screened for their inhibition of cyclooxygenase (COX) enzyme activity. Cyclooxygenase converts arachidonic acid to prostaglandins which are involved in the complex process of inflammation and are responsible for the sensation of pain.[57] A number of South African *Amaryllidaceae* species inhibited COX-1 activity including *B. disticha* (55%-65%).[56,58] Others were screened against both COX-1 and COX-2 activities including bulb extracts of *C. moorei* which exhibited an inhibition of 84% to 99% against COX-1 and 52% to 75% inhibitory activity against COX-2,[29] and bulb extracts of *C. obliquus* inhibited COX-1 activity by 50% to 75% and COX-2 by 15% to 85%.[54] Dichloromethane (DCM) and methanolic extracts from different parts of *Cyrtanthus falcatus*, *C. mackenii*, *C. suaveolens, G. ciliaris*, *Gethyllis multifolia,* and *Gethyllis villosa* inhibited both COX-1 and COX-2 activities. The DCM extracts had strong activity against both COX-1 (93%-100%) and COX-2 (73%-96%) when tested at a concentration of 250 μg/mL. The methanolic extracts however were less active against both enzyme activities.[33] Bioassay-guided fractionation of extracts of *G. ciliaris* led to the isolation of the COX-1 inhibitor isogeunitol **18** (IC_{50} = 262 μM).[36]

The seasonal variation in bulb extracts from *C. contractus* collected monthly over a year on COX activity was investigated. The extracts exhibited moderate to high activity throughout the year (≥40%) against both COX-1 and COX-2 enzymes. Extracts from bulbs collected during August, September, and December had high activity against both enzymes, with those obtained in September exhibiting the highest activity against COX-1. The high activity in September coincided with the high alkaloidal content within the duration of the study period which may attribute this high activity, in part, to high alkaloidal content.[31]

Bulbs of *H. albiflos* are used in South African traditional medicine to treat aching skin, while leaves are used for the treatment of wounds, sores, and ulcers.[13] In a search for agents that can prevent the development of skin scarring and dark spots as a result of the inflammatory process, a 3-step screening targeting pro-inflammatory enzymes was conducted on extracts of *H. albiflos*. First, extracts of *H. albiflos* were screened against phospholipase A2, an enzyme that catalyzes the release of arachidonic acid from the cell membrane phospholipid layer. Arachidonic acid serves as a precursor for eicosanoids which are responsible for acute skin inflammation. *Haemanthus albiflos* extracts had low inhibitory activity against phospholipase A2 (IC_{50} = 15.8-28.1 μg/mL). This low activity against phospholipase A2 necessitated exploration of other mechanisms by which the extracts may induce their effects on the skin scars and spots. The search then shifted to 15-lipoxygenase, an enzyme involved in the inflammatory process through

conversion of the released arachidonic acid to hydroxyeicosatetraenoic acid which results in skin psoriasis. Again, the extracts exhibited only low activity against the 15-LOX enzyme with IC_{50} values range of 35.4 to 64.6 μg/mL. The extracts, however, had noticeable activity against COX-1 (IC_{50} = 1.9-5.3 μg/mL) and COX-2 (IC_{50} = 4.3-14.9 μg/mL).[59]

Interestingly, in a screening program of 15 *Amaryllidaceae* alkaloids, isolated from *C. bulbispermum* and *C. moorei*, against COX-1 and COX-2 activities, most alkaloids exhibited only low activity against the former and no activity against the latter.[55] The results suggest that the assumption that the rationale behind uses of *Crinum* species in traditional medicine attributed largely to their alkaloid content needs to be carefully revised and revisited.[55] In addition, lectin-like protein isolated and partially purified from *C. moorei* was not active against COX-1 enzyme.[60]

The toxic and poisonous nature of different members of *Amaryllidaceae* has been utilized by different ethnic groups in traditional medicine and other cultural rites. For instance, the Khoi and San people of South Africa use bulbs of *B. disticha* as arrow poison.[13-15] Another example is the use of the outer scales of the bulb of *Crinum kirkii* in Kenya as a rat poison.[61] In fact, the medicinal utilization of the toxic properties of members of the family *Amaryllidaceae* for the treatment of tumors can be traced back to the times of Hippocrates and Pliny and continued by practitioners of the middle ages throughout the world.[62] It is the poisonous properties of certain members of the family *Amaryllidaceae* that led many investigators during the 19th century to seek for active compounds from this family. However, scientists had to wait until 1877 for the isolation of lycorine **3** as the first alkaloid from the family[63] although lycorine **3** is known for its antiproliferative properties against a wide range of cancerous cell lines. However, the discovery of the potent, cell line specific anticancer isocarbostyril pancratistatin **31**, which has shown most promise for clinical development as an anticancer drug, that attracted attention to *Amaryllidaceae* as a potential source of anticancer drugs. This inspired the group at the Research Centre to embark on the screening of *Amaryllidaceae* alkaloids for their cytotoxic activity against a panel of normal and cancerous cell lines namely human acute lymphoblastic leukemia, breast adenocarcinoma, cervical adenocarcinoma, and the normal human fibroblasts cell lines.[21,23]

Of the alkaloids lycorine **3**, crinine **2**, buphanisine **45**, buphanidrine **36**, ambelline **40**, undulatine **26**, distichamine **15**, and distichaminol **14** isolated from *B. haemanthoides* screened for cytotoxic effects against the above-mentioned cell lines, only lycorine **3** and distichamine **15** had noticeable cytotoxic effects on all cell lines in a dose-dependent manner with IC_{50} values ranging from 1.8 to 8.9 μM for lycorine **3** and 2.3 to 12.4 for ditichamine **15**.[23] The low activity of the β-crinane alkaloids is in line with the previous studies on various cancerous cell lines.[21] The results of this study are a confirmation of earlier findings where both lycorine **3** and distichamine **15** significantly decreased the viability of the above cancerous and normal cell lines with the cervical adenocarcinoma cell line more sensitive to distichamine **15** (IC_{50} = 2.2 μM). The study also found that the galanthamine-type alkaloids galanthamine **4**, lycoramine **41**, and narwedine **42** had very low influence on the viability of both human cancer and normal cell lines.[21] Haemanthamine **33** was found to be active across the cell lines screened (IC_{50} = 2.1-8.1 μM), while narciprimine **16** was only active against acute lymphoblastic leukemia and homolycorine **6** was active against acute lymphoblastic leukemia, chronic myelogenous leukemia, and malignant melanoma. Buphanidrine **36**, however, was only active against the normal human fibroblast cell line.[21]

Flow cytometry on acute lymphoblastic leukemia (CEM) cancer cells revealed that treatment with distichamine **15** increased the proportion of G2/M phase cells in a dose-dependent manner, with concomitant reductions in the proportion of G0/G1 and S cells. The proportion of cells with sub-G1 amounts of DNA (apoptotic cells) increased following a 24 hours treatment with distichamine **15**, relative to that observed in untreated cells. Narciprimine **16** showed similar effects but with insignificant increase in sub-G1 fraction. Thus, of 2 compounds, distichamine **15** was clearly capable of cell cycle disturbance and apoptosis induction in CEM cancer cells. Distichamine **15** also induced apoptosis of CEM through the activation of caspase 3/7. This finding was supported further by Western blot analysis which revealed a decreased level of the anti-apoptotic protein Mcl-1 which is necessary for cell viability.[21]

Screening of crude extracts for their cytotoxic activity against a panel of cancer and normal cell lines has also been envisaged. For instance, bulb extracts of *C. contractus* collected monthly over 12 months were investigated for their cytotoxic effects against human acute lymphoblastic leukemia, breast adenocarcinoma, cervical adenocarcinoma, and the normal human fibroblasts cell lines. Extracts of bulbs collected in May (IC_{50} = 11.7-37.9 μg/mL) and September (IC_{50} = 23.5-91.7 μg/mL) caused the highest level of cytotoxicity, while those collected in June and November (IC_{50} >100 μg/mL) had the least cytotoxic effect. The variation was attributed largely to variation in the quantity and composition of the total alkaloid content.[31] In addition, extracts of different plant parts of *C. vansonii* had modest cytotoxic activity against Vero Monkey Kidney cell line with IC_{50} values ranged from 12.6 to 62.6 μg/mL.[4]

Plants in general and those used in traditional medicine in particular are considered to be safe due to their long-term use by humans. However, research have shown that some plants used as food and as herbal medicine are mutagenic and carcinogenic.[64–66] This made it necessary to screen plants for their potential mutagenic effect and plants that show mutagenic potential should be considered safe only after rigorous toxicological testing. With this in mind, many South African plants used in the South African traditional medicine, including members of the family *Amaryllidaceae*, have been screened by van Staden and coworkers for their mutagenic potential.[21,54,64,67,68]

In a screening of 51 South African plant species for their potential mutagenic effect using Ames and Vitotox tests, DCM extracts of bulbs of *C. macowanii* were mutagenic to strain TA98, a frameshift mutation detecting strain, without metabolic activation in a dose-dependent manner. However, only the highest concentration tested (5 mg/mL) was mutagenic with metabolic activation, while bulb extracts of *B. disticha* were not mutagenic with and without metabolic activation.[65] Interestingly, DCM extracts of both species were highly toxic upon testing in the micronucleus test on human lymphocytes. However, methanolic extracts of 2 species induced a significant number of micronuclei when compared to the blank control.[67]

Investigation of dichloromethane and methanolic extracts of different parts of *C. falcatus*, *C. mackenii*, *C. suaveolens*, *G. ciliaris*, *G. multifolia*, and *G. villosa* for their mutagenic potentials in Ames test using *Salmonella typhimurium* TA98 strain indicated that only extracts from different parts of *C. falcatus* and *C. suaveolens* induced mutagenic effects.[33] In other studies, mutagenic effects were not detected for extracts from *C. obliquus* and *S. puniceus* toward *S. typhimurium* TA98 strain.[54] Bioassy-guided fractionation of the crude extracts of *C. suaveolens* led to the isolation of captan **43** as the active principles at a concentration of 100 mg/kg of dry plant material. The plant material was purchased from a nursery that uses different agricultural practises including pesticide application to increase production and manage pests. The use of captan **43**, which has been commercially available fungicide and bactericide in large-scale agriculture, formed part of these practices. Metolachlor **21** is another synthetic pesticide isolated from *S. puniceus*.[37] The detection of captan **43** in *C. suaveolens* and metolachlor **21** from *S. puniceus* raises concern about the safety of traditional medicinal plants grown commercially in South Africa and the need for the establishment of good agricultural practise guidelines and regulations.[35]

The RCPGD also published several reviews on the phytochemistry and biological activities on specific species and genera such as *B. disticha*[69] and *Crinum*.[10] Special reviews on biological activities of *Amaryllidaceae* alkaloids covered antibacterial,[70] antifungal,[71] and cytotoxic activities.[16,21,72–75]

In summary, the research group contributed considerably in expanding our knowledge on the chemistry and biological activities of the plant family *Amaryllidaceae*. Of particular interest is the unraveling of the potential of 1-*O*-acetyllycorine as a potent inhibitor of the AChE enzyme which is associated with the pathology of Alzheimer's disease. Another finding is the uncovering of the antiproliferative activity of distichamine and narciprimine and their potential as lead molecules for anticancer drug discovery. Other biological properties such as antimicrobial and anti-inflammatory activities have given important insights on the potential of the plant family in drug discovery.

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