Bangladesh J. Bot. **52**(2): 403-410, 2023 (June) DOI: https://doi.org/10.3329/bjb.v52i20.68287

VARIATION IN CHEMICALS AND ANTIMICROBIAL ACTIVITIES OF *NICOTIANA GLAUCA* GRAHAM

MANAR ALSENIDI AND MAHMOUD MOUSTAFA*

Department of Biology, College of Science, King Khalid University, Abha 9004, Saudi Arabia

Keywords: Nicotiana glauca, Antimicrobial activities, GC-MS, HPLC

Abstract

Nicotiana glauca was recorded as one of the invasive plant species which is dramatically spreading in many countries. Extracts of methanol, hexane, acetone, ethanol and petroleum ether of leaves of *Nicotiana glauca* obtained from seven locations were screened for their antimicrobial activities against *Klebsiella oxytoca, Shigella* sp., *Staphylococcus aureus, Micrococcus luteus* and *Candida albicans*. Forty seven chemical compounds were characterized and vitamins B_1, B_{12}, B_2 and folic acid were also found. All studied locations had toxic chemicals to human namely anabasine. The similarity index among locations was about 69.01% and the most dominant compound were 2-Pentanone, 4-hydroxy-4-methyl, 1,3-Dioxolane- 2-methanol, 2,4-dimethyl and 2-Acetoxyisobutyryl chloride. Interestingly, the plant had anabasine and showed low antimicrobial effect against specific human pathogens. Data of permanent quadrats showed the plant spread in a progressive manner.

Introduction

Saudi Arabia is considered as one of the richest in bio diversities in the Arabian Peninsula, because it is characterized by different ecosystems and has different types of plant of Asia, Africa and Mediterranean regions. A total of 2284 species from naturalized and alien plants species had been documented from different locations of Saudi Arabia (El-Ghanim *et al.* 2010). *Nicotiana glauca* Graham belonging to Solanaceae is one of the invasive plants in the western Mediterranean region (Bogdanvoic *et al.* 2006). The allelochemicals collected from invasive plants can inhibite the normal growth of adjacent plants through direct or indirect means (Callaway and Aschehoug 2000, Ridenour and Callaway 2001).

All species of the genus *Nicotiana* are characterized by the production of pyridine alkaloids, but the number and abundance of the different alkaloids are highly variable within the genus (Saitoh *et al.* 1985, Sisson and Severson 1990). *Nicotiana glauca* locations form dense monodominant stands due to high seeds viability, high rates of fruiting and frequent recruitment of seedlings growth (Ollerton *et al.* 2012). It produces large amount of tiny seeds (0.6 mm long) and one plant produces between 10,000 to 1,000,000 seeds per year (Florentine *et al.* 2006). It was reported that the major alkaloid chemicals in *Nicotiana glauca* plant is anabasine (Lisko *et al.* 2013). Anabasine was reported to be highly toxic and can cause death to humans because it has a destructive effect to the respiratory tissues (Sims *et al.* 1999). Therefore, the aim of this work was to determine the antimicrobial agents present in the leaf and bark of *Nicotiana glauca* against some human pathogenic microbes.

Materials and Methods

Leaves of *Nicotiana glauca* plants were collected from seven different locations namely, Alsamer (N 18° 13' 29.5284", E 42° 30' 41.9544" and altitude 7.272 feet), Aseer Mall (N 18° 14' 35.4048", E 42° 36' 43.7076" and altitude 6.845 feet), King Abdullah Road (N 18° 11' 7.3284", E

*Author for correspondence: <mfmostfa@kku.edu.sa>.

42° 38' 27.7506" and altitude 6.955 feet), Almahalah (N 18° 19' 15.4194", E 42° 35' 23.1678" and altitude 6.762 feet), Lasan (N 18° 14' 13.2432", E 42° 35' 24.6516" and altitude 7.000 feet), Al-sarhan (N 18° 11' 13.3038", E 42° 39' 27.8022" and altitude 6.888 feet) and Almoadafeen (N 18° 14' 12.3144", E 42° 35' 46.7484" and altitude 7.015 feet).

Phytocomponents of *Nicotiana glauca* leaves were evaluated using Perkin-Elmer Gas chromatography-Mass spectrometry. As described previously by Malarvizhi and Ramakrishnan (2011), the extract was subjected to GC-MS analysis and then all phytocomponents compounds were identified. The mass spectrums of unknown compounds were compared with the known spectrum stored in the data base of NIST library.

HPLC analyses of the leaves extracts of *Nicotiana glauca* were performed by using a Shimadzu model HPLC system (Shimadzu Corporation, Kyoto, Japan). Shimadzu HPLC system composed of a solvent delivery module (LC-10AD) integrated with a double plunger reciprocating pump, ultraviolet-visible spectrophotometry (UV-Vis**)** detector (SPA-10A), column oven (CTO-10A) and 20-µl injection loop was used.

Seven grams of powdered leaves were mixed with 25 ml of methanol, acetone, ethanol, hexane and petroleum ether. Samples with solvent were placed in rotary shaker at 100 rpm at 25°C for 48 hrs, then the solvents were evaporated from each sample at 60°C. Each extract was weighed and then dissolved in the dimethyl sulfoxide (DMSO) a concentration 1g/ml and kept at 4°C for antimicrobial activity assay against *Klebsiella oxytoca, Shigella* sp*., Staphylococcus aureus, Micrococcus luteus* and *Candida albicans*.

The 20 ml from sterilized Mueller-Hinton sterile agar was poured in sterile Petri dishes and were kept for 60 min for well solidifying the culture media. The medium in each plate was inoculated equally using a sterile loop with 0.1ml from each examined pathogenic microorganisms. A hole of six mm in diameter was made using a sterile cork-borer and then 0.1 ml of the plant extract was added to each hole. All the plates were incubated at 29°C for 24 hrs. The sensitivities of the pathogenic microbe to *Nicotiana glauca* plant extracts were achieved by calculating the diameter of inhibition zone around the well. To test the significance of data one-way analysis of variance was carried out by using Minitab for Windows version 15.

Permanent quadrates were applied for three years to examine the rate of invasiveness of *Nicotiana glauca* plants. In each sample site 30 permanent quadrates of 10 meter square were marked from April 2017 to April 2020 to know the rate of invasion of the plants. Absolute density had been calculated for a species by using the formula (Riaz *et al*. 2007, Alwadi and Moustafa 2016).

Results and Discussion

GC-MS chromatogram analysis of the acetone, ethanol and methanol extracts of leaves of *Nicotiana glauca* grown at various locations in Asir region, KSA, showed the presence of various phytochemicals (Table 1). According to the peak area the most dominant compound in the acetone extract of King Abdullah Road leaves was 2**-**Pentanone, 4-hydroxy-4-methyl**-**(89.20%) while 16-Heptadecenal (0.53 %) was found to be in less amount. In Alsamer location, 2**-**Pentanone, 4-hydroxy-4-methyl**-** (83.51%) was major chemicals while 1,6-Heptadiene, 2-methyl-6-phenyl-(1.77%) represents the lowest value. 2-Pentanone, 4-hydroxy-4-methyl-(98.99%) represent the highest percentage and 1,6-Heptadiene, 2-methyl-6-phenyl-(1.01%) represent the lowest percentage in lasan locations. In Aseer Mall location, 2-Pentanone, 4-hydroxy-4-methyl-(98.98%) was the major chemicals in acetone extract while 16-Heptadecenal (0.86%) was very less. The most common compound is 2**-**Pentanone, 4-hydroxy-4-methyl**-** (95.89%) and Phytol (0.74%) was very less in the acetone extract of plant growing in Almahalah location. In Al-sarhan location

Table 1. Comparison among phytochemicals resulting from GC-MS analysis of solvents extract of *Nicotiana glauca* leaves from various locations.

Acetone solvent	Locations	Ethanol solvent	Locations	Methanol solvent	Locations
2-Pentanone, 4-hydroxy-4-methyl-	KA-AS-LA-AR-AM	Dimethoxy(dimethyl) silane	KA-AS-LA-AR-AM-AH-AD	Undecane	KA-AS-LA-AR-AM-AH-AD
1,6-Heptadiene, 2-methyl-6-phenyl-	KA-AS-LA-AR	Triacetin	KA-AS-LA-AM	Estragole	KA
1-Methyl-5-phenylbicyclo[3.2.0] heptane	KA-AS	Dodecanoic acid, propyl ester	KA	Anabasine	KA-AS-LA-AR-AM-AH-AD
Tetratetracontane	KA-AS-AD	16-Heptadecenal	KA-AR-AH-AD	Hexadecanoic acid, methyl ester	KA-LA-AR-AM-AH-AD
Anabasine	AM	Benzene,(1-hexyl-1-heptenyl)	KA-AM	Pentadecanoic acid	KA
Phytol	AM-AD	Pentadecanal	KA-AH	9,12-Octadecadienoic acid, methyl ester	KA
Octadecanamide	AM	Tetradecanamide	KA	Phytol	KA-AR-AM-AH-AD
Nonadecanamide	AM	13-Docosenamide,(Z)	KA-AS	Oleic acid	KA
4-Hydroxy-2-pentanone	AH-AD	9-Octadecenamide,(Z)	KA	Eicosanoic acid	KA
1,3-Dioxolane-2-methanol, 2,4-dimethyl-	AH	Nonadecanamide	KA-AS	Tetrachloroethylene	AS
16-Heptadecenal	AH-AD	Dodecanoic acid, 1-methylethyl ester	AS-LA-AR-AM	n-Hexadecanoic acid	
Pentadecanal	AH	Octadecanamide	AS	9,12-Octadecadienoic acid (Z,Z)-	
Heneicosane	AH	Nonane, 1-iodo-	AR	Triacetin	
2-Acetoxyisobutyryl chloride	AD	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	AR	Acetic acid, [o-(trimethylsiloxy) phenyl]- trimethylsilyl ester	AS
2,2-Dimethylbutane	AD	Eicosanoic acid, ethyl ester	AR-AM-AH-AD	Pentadecanal	AS
		1,6-Heptadiene, 2-methyl-6-phenyl	AR-AM	1,6-Heptadiene, 2-methyl-6-phenyl-	LA
		Eicosane	AR	Octadecenoic acid, methyl ester	AR
		Anabasine	AM-AH	Dodecanoic acid, 1-methylethyl ester	AR-AH
		Diethyl phthalate	AM	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	AR-AM-AH
		1H-Indene,2,3-dihydro-1,1,3-trimethyl-3-phenyl-	AM	11,14,17-Eicosatrienoic acid, methyl ester	AR-AM-AH
		Phytol	AM-AH-AD	Hexadecanoic acid, 15-methyl-, methyl ester	AD
		Propanoic acid, 2-hydroxy-, ethyl ester	AH		
		1,1,1,3,5,5,7,7,7-Nonamethyl-3-(trimethylsiloxy)tetrasiloxane	AH		
		Tetratetracontane	AH		

KA= King Abdullah Road, AS =Alsamer, LA =Lasan, AR =Aseer Mall, AM =Almahalah, AH= Al-sarhan, AD= Almoadafeen.

1,3-Dioxolane-2-methanol, 2,4-dimethyl with 87.91% and 16-Heptadecenal with 0.13% was found. In Almoadafeen location 2-Acetoxyisobutyryl chloride with 86.85% while 16-Heptadecenal with 0.11 % was found (Table 1).

The similarity index among seven locations illustrating the highest similarity value of phytocomponents was 69.01% between Al-samer and Lasan (Table 2). Al-sarhan location has lowest similarity value (31.87%) with King Abdullah Road and Al-samer locations. A dendrogram was grouped the seven accessions into three main clusters (Fig. 1). The first cluster contained the chemotype of *Nicotiana glauca* from King Abdullah Road, Al-samer and Lasan that showed the highest similarity index value (69.01%) between Al-samer and Lasan. The second cluster included Aseer Mall and Almahalah locations with similarity index value (53.85%), also the third cluster included Al-sarhan and Almoadafeen location with same similarity index value.

Table 2. Similarity index among seven locations of *Nicotiana glauca.*

	King Abdullah Road	Al-samer	Lasan	Aseer Mall	Al-mahalah	Al-sarhan	Al-moadafeen
King Abdullah Road	100						
Alsamer	53.85	100					
Lasan	51.90	69.01	100				
Aseer Mall	42.86	50.00	64.38	100			
Almahalah	33.33	39.53	51.90	53.85	100		
Al-sarhan	31.87	31.87	46.34	48.15	44.58	100	
Almoadafeen	41.18	44.58	57.89	48.15	44.58	53.85	100

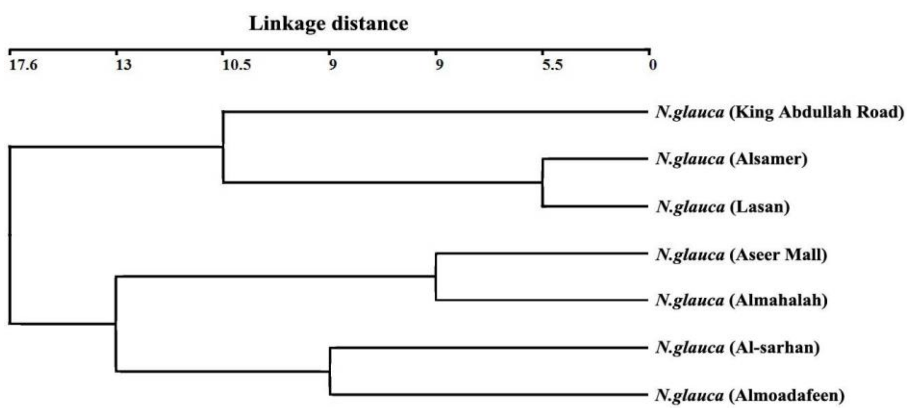

Fig. 1. The dendrogram constructed from phytocomponents found in *Nicotiana glauca* leaves grown in seven locations.

Results showed variations in the vitamins content among the different locations of *Nicotiana glauca* samples (Fig. 2). Alsamer location showed the highest content of vitamin B_1 (0.33mg/g), while Aseer mall location (0.22 mg/g) had the lowest. Almoadafeen location had the highest content of folic acid concentration (6.24 mg/g), while the lowest concentration was found in Lasan location (3.57mg/g). Aseer mall location showed the highest vitamin B_{12} content (27.54mg/g) and

Almahalah location showed the lowest vitamin B_{12} content (0.48 mg/g). The highest content of vitamin B_2 (0.14 mg/g) was found in Al-sarhan location while the lowest content of vitamin B_2 (0.07mg/g) in Almahalah location.

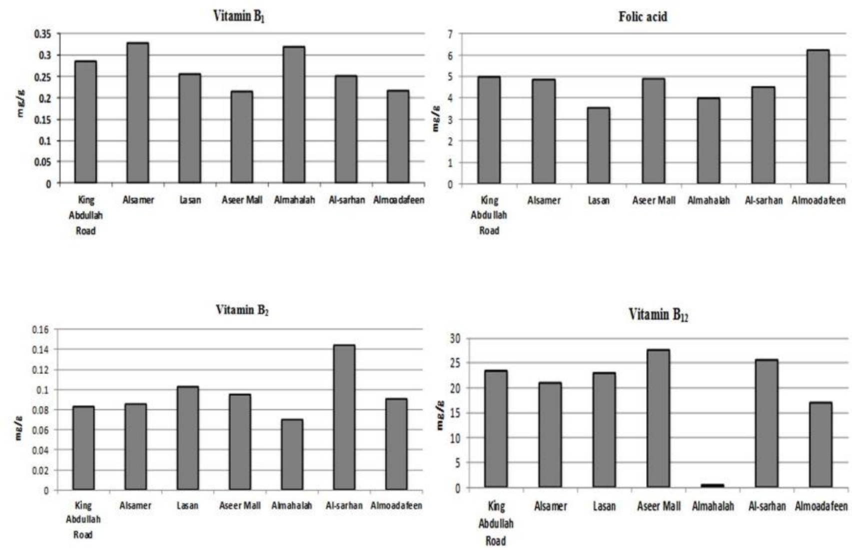

Fig. 2. Different vitamin contents found in *Nicotiana glauca* leaves grown in seven locations.

The present study confirmed that each location had a specific vitamin contents which considers also as chemotype for individual plant growing in a specific locations. These differences may be attributed to the differences in genotypes or to the growing conditions, such as specific location, temperature, rain and humidity. Balentine *et al.* (1997) described the variation in the amount of biosynthesis of phenolic compounds in tea shoots due to differences in sunlight and length of daytime. It was reported that *Copaifera guianensis* Desf., *Copaifera multijuga* Hayne and *Copaifera duckei* Dwyer plants had significant chemical variations and this variations not only among various plant genus but also within a given species and among individual plant trees (Cascon and Gilbert 2000). A study carried out on total phenolic content in *Camellia sinensis* during three harvest times proofed that the amount of total phenolics varied from each other (Anesini *et al.* 2008). Moustafa *et al.* (2016) showed that variation in altitudinal gradient could cause both genetics and chemical variations among individuals at the species level.

All studied locations were found to contain variable amounts of anabasine (a pyridine and piperidine alkaloid) especially in the methanol extracts. Alkaloids are the dominant class of constitutive secondary chemicals in *Nicotiana glauca*, but in contrast to the most *Nicotiana* species, the alkaloid in *Nicotiana glauca* tissues (seeds, roots, leaves, fruits and corollas) is anabasine instead of nicotine (Bush and Crowe 1989). The toxic impact of anabasine and nicotine can cause shivering, vomiting, nausea and diarrhea in very low concentrations as well as paralysis, respiratory compromise and the death in high dose (Wink 2000).

Results showed the plant spread progressively in the investigated area from 2017 to 2020 AD (Table 3). Plant absolute density ranged between 0.207 and 0.379 in Al-mahalah area, in King Abdullah Road from 0.379 and 0.448, in Al-samer from 0.310 to 0.379, in Lasan from 0.276 to 0.345, in Aseer Mall from 0.241 to 0.310, in Al-sarahn from 0.241 to 0.310 and in Al-moadafeen from 0.275 to 0.301. Hence, the plant distribution might affect the ecosystem in negative way. Stein *et al.* (2000) had summarized the consequences of invasive species on native plant species and in community structure. It was noted the rate of invasion of *Nicotiana glauca* plants in some investigated area is not so high, and this might due to that the seed germination of this species requires specific environmental condition to grow and spread (need further study). Therefore, developing strategy should be planned to prevent plant to spread more to a new area, which is frequently easier than controlling vast established populations (Goodell *et al.* 2000).

Table 3. Absolute density of seven locations of *Nicotiana glauca.*

Absolute density (AD)	King Abdullah Road	Al-samer	Lasan	Aseer Mall	Al-mahalah	Al-sarhan	Al-moadafeen
AD: 2017	0.379	0.310	0.276	0.241	0.207	0.241	0.275
AD: 2020	0.448	0.379	0.345	0.310	0.310	0.310	0.301

Results indicated that the plant extracts showed from leaves very little or no antimicrobial activities against tested pathogenic microorganisms (Fig. 3). Positive control showed inhibition activities against all the tested microorganisms. No antimicrobial activity was observed against any pathogenic microorganisms when dimethyl sulfoxide (DMSO) was used.

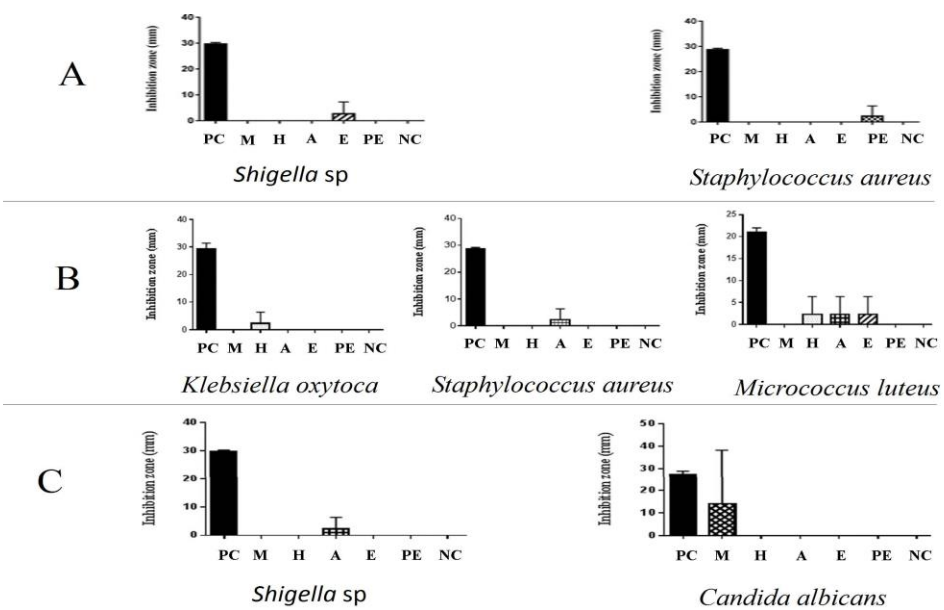

Fig. 3. Antimicrobial activities of leaf extracts of *Nicotiana glauca* collected from Alsamer location= A, Lasan location =B, Almoadafeen location= C. M= Methanol, H= Hexane, A= Acetone, E= Ethanol, PE= Petroleum ether, NC= Negative control, PC= Positive control.

The weak activity against tested microbes is probably due to loss some of the plant's active constituent through drying conditions or the inability of the solvents to dissolve some of the active principles. These differences might be due to the nature of solvents either polar or non-polar which were used for extraction the active principles from *Nicotiana glauca* plants. In addition, susceptibility variation among tested strains might be due to the nature of cell outer membrane that the active chemical could not affect. It was demonstrated that the activity cannot be attributed only to a single individual extract but due to the synergistic effects (Ncube *et al.* 2011). Also it was documented that the antimicrobial inhibiting activities of plant extracts could be due to the action of more than one chemical compounds present in plants and not by the action of individual compound (Da Silva *et al.* 2013).

In conclusion, *Nicotiana glauca* have many phytocomponents with variable amounts of toxic substances. Antimicrobial activity of applied solvents extracts showed very low antimicrobial activities. Further investigation on the properties of anabasine found in *Nicotiana glauca* plans is needed. Also, action should be taken to eliminate this plant mechanically or biologically as the plant established in many area.

Acknowledgements

Authors would like to thank the Deanship of Scientific Research at King Khalid University under grant no. (RGP1/244/42) and King Abdul Aziz City for Science and Technology, grant no. AT-1078-37.

References

Alwadi H and Moustafa M 2016. Altitudinal impact on the weeds species distribution in the semi-arid mountainous region of Abha, Saudi Arabia. J. crop weed. **12**: 87-95.

Anesini C, Ferraro GE and Filip R 2008. Total polyphenol content and antioxidant capacity of commercially available tea (*Camellia sinensis*) in Argentina. J. Agric. Food. Chem. **56**: 9225-9229.

Balentine DA, Wiseman SA and Bouwens LC 1997. The chemistry of tea flavonoids. Crit. Rev. Food Sci. Nutr. **37**: 693-704.

Bogdanović, S, Mitić B, Ruščić M and Dolina K 2006. *Nicotiana glauca* Graham (Solanaceae), a new invasive plant in Croatia. Acta Bot. Croat. **65**: 203-209.

Bush L and Crowe M 1989. Nicotiana alkaloids. Toxicants of plant origin, ed. Cheeke PR, 1st ed., **1**: 87-107.

Callaway RM and Aschehoug ET 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. Sci. **290**: 521-523.

Cascon V and Gilbert B 2000. Characterization of the chemical composition of oleoresins of *Copaifera guianensis* Desf., *Copaifera duckei* Dwyer and *Copaifera multijuga* Hayne. Phytochem. **55**: 773-778.

Da Silva EBP, Soares MG, Mariane B, Vallim MA, Pascon RC, Sartorelli P and Lago JHG 2013. The seasonal variation of the chemical composition of essential oils from *Porcelia macrocarpa* RE Fries (Annonaceae) and their antimicrobial activity. Molecules **18**: 13574-13587.

El-Ghanim WM, Hassan LM, Galal TM and Badr A 2010. Floristic composition and vegetation analysis in Hail region north of central Saudi Arabia. Saudi J. Biol. Sci. **17**: 119-128.

Florentine S, Westbrooke M, Gosney K, Ambrose G and O'Keefe M 2006. The arid land invasive weed *Nicotiana glauca* R. Graham (Solanaceae): Population and soil seed bank dynamics, seed germination patterns and seedling response to flood and drought. J. Arid Environ. **66**: 218-230.

Goodell K, Parker IM and Gilbert GS 2000. Biological impacts of species invasions: implications for policy makers. National Research Council (US), Incorporating science, economics, and sociology in developing sanitary and phytosanitary standards in international trade. pp. 87-117.

Lisko JG, Stanfill SB, Duncan BW and Watson CH 2013. Application of GC-MS/MS for the analysis of tobacco alkaloids in cigarette filler and various tobacco species. Anal. Chem. **85**: 3380-3384.

Malarvizhi P and Ramakrishnan N 2011. GC-MS analysis of biologically active compounds in leaves of *Calophyllum inophyllum* L. Int. J. Chemtech Res. **3**: 806-809.

Moustafa MF, Hesham AE-L, Quraishi MS and Alrumman SA 2016. Variations in genetic and chemical constituents of *Ziziphus spina-christi* L. populations grown at various altitudinal zonation up to 2227 m height. J. Genet. Eng. Biotechnol. **14**: 349-362.

Ncube B, Finnie J and Van Staden J 2011. Seasonal variation in antimicrobial and phytochemical properties of frequently used medicinal bulbous plants from South Africa. S. Afr. J. Bot. **77**: 387-396.

Ollerton J, Watts S, Connerty S, Lock J, Parker L, Wilson I, Schueller S, Nattero J, Cocucci AA and Izhaki I 2012. Pollination ecology of the invasive tree tobacco *Nicotiana glauca*: comparisons across native and non-native ranges. J. Pollinat. Ecol. **9**: 58-95.

Riaz T, Khan SN, Javaid A and Farhan A 2007. Weed flora of gladiolus fields in Lahore, Pakistan. Pak. J. Weed Sci. Res. **13**: 113-120.

Ridenour WM and Callaway RM 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. Oecologia **126**: 444-450.

Saitoh F, Noma M and Kawashima N 1985. The alkaloid contents of sixty *Nicotiana* species. Phytochem. **24**: 477-480.

Sims DN, James R and Christensen T 1999. Another death due to ingestion of *Nicotiana glauca*. J. Forensic Sci. **44**: 447-449.

Sisson VA and Severson R 1990. Alkaloid composition of the Nicotiana species. Beitr. Tabakforsch. Int. **14**: 327-339.

Stein B, Kutner L and Adams J 2000. Precious heritage: the status of biodiversity in the United States: Oxford University Press, USA. pp. 159-199.

Wink M 2000. Interference of alkaloids with neuroreceptors and ion channels. Stud. Nat. Prod. Chem. **21**: 3-122.

(Manuscript received on 30 March, 2021; revised on 6 June, 2023)