

Phytochemical and Elemental Exploration of *Nothoscordum gracile* (Aiton) Stearn for Its Medicinal Potential

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ABSTRACT

The genus *Nothoscordum* contains 30 species. *Nothoscordum gracile* (Aiton) Stearn is a perennial herb of family Amaryllidaceae. Objective of the present study is to analyze phytochemical and elemental details of this species. Phytochemical screening of aqueous and ethanol extracts of leaves and bulbs have shown the presence of different phytoconstituents. Fourier Transform Infrared spectroscopy (FTIR) and Wavelength Dispersive X-Ray Fluorescence (WD-XRF) analysis have described the functional groups and elemental profile of this plant. Preliminary phytochemical study has revealed the presence of various phytoconstituents. Extraction of these compounds depends upon the solvent and plant part used. The Wavelength Dispersive X-Ray Fluorescence study has suggested the presence of macro, micro and trace elements in leaves and bulbs of this species. As the plant contains phytochemicals such as alkaloids, flavonoids, glycosides, gums and mucilages, phenolics *etc.* and elements like Potassium, Calcium, Phosphorus, Sulphur, Magnesium *etc.*, it is likely be of medicinal importance.

KEY WORDS: Medicines, *Nothoscordum gracile*, phytochemicals, FTIR, WD-XRF.

1. INTRODUCTION

Nothoscordum gracile (Aiton) Stearn is a member of family Amaryllidaceae. The plant is commonly known as false garlic or wild onion due to its resemblance with onion plant. *Nothoscordum gracile* (Aiton) Stearn is the accepted name where as *Nothoscordum inodorum* (Aiton) G. Nicholson, *Nothoscordum fragrans* (Vent.) Kunth, *Allium gracile* Ait and *Allium fragrans* Vent are its synonyms (Quattorochi, 1999). The genus '*Nothoscordum*' contains 13 species out of which seven were new and five had previously been placed in *Ornithogalum* or *Allium* (Kunth, 1843). The generic name '*Nothoscordum*' has been derived from '*nothos*' = 'false' and '*scordon*' = 'onion' as the plant was similar to *Allium* (Stearn, 1986). Due to this similarity, the morphological characterization of this species is of utmost importance for distinction between species. The species *Nothoscordum gracile* has been originated in Central and South America. It is widely distributed throughout the America and naturalized in many warm-temperate regions of Europe, Africa, Asia and Australia. Recent studies have claimed that this genus contains more than 30 species. The wide distribution is likely be due to dispersal of its seeds and bulbils production (Howard, 2001; Greuter and Raus, 2008; Souza, 2012). It was designated as a devilish weed in 1993. Now it has been categorised as a cosmopolitan weed (Howard, 1993; Gargiullo, 2008). During the present investigation, efforts have been made to study the different phytochemicals along with the detection of various elements of medicinal importance.

2. MATERIALS AND METHODS

Collection of plant material: The plant material was collected from P. N. Mehra Botanical Garden, Panjab University, Chandigarh in the year 2014. The leaves and bulbs were washed thoroughly first in tap water and then with distilled water. The washing was done carefully to avoid any damage to the material. The plant was identified by consulting the herbarium Department of Botany, Panjab University, Chandigarh and Department of Botany, Punjabi University, Patiala. The collected plant materials were sterilised with absolute alcohol to make them free from contaminations. The leaves and bulbs were separated and dried completely in shade at room temperature. Both were crushed and powdered using an electric blender separately. The powdered plant materials were kept in polythene bags till further use. The voucher specimen has been deposited in herbarium of Department of Botany, Panjab University, Chandigarh (PAN 20621).

Preparation of aqueous extract: The aqueous extracts of powdered material were prepared by using a standard method with slight modifications (Gupta, 1996). The leaf and bulb powders were weighed 20gm each using an electronic weighing balance. Both powders were mixed in 100ml of distilled water separately in 250ml flask. The mixtures were kept at room temperature on an orbital shaker for 24hrs. The mixtures were then filtered through five folds of muslin cloth followed by filtration through Whatman's Filter No. 1. The filtrates were collected in vials and kept in refrigerator at 4°C.

Preparation of ethanol extract: The ethanol extract of both powders were prepared by following methods with minor modifications (Yadav and Agarwala, 2011). 10gm of each powder was kept in Soxhlet apparatus and extracted by using 130ml of ethanol. The extraction was carried out till the powder turns colourless. The extracts were evaporated at room temperature so that it reaches to 1/3rd of the original volume. The extracts were collected in vials and stored in refrigerator at 4°C till further use.

Phytochemical analysis: The aqueous and ethanol extracts of leaves and bulbs were studied for the presence of various phytochemicals like alkaloids, carbohydrates, flavonoids, glycosides, gums and mucilages, phenolics, phlobatannins, reducing sugars, saponins, steroids, tannins and terpenoids (Thakur and Sidhu, 2014).

Fourier Transform Infrared (FTIR) Spectroscopy: Fourier Transform Infrared spectroscopy was done for the detection of different functional groups present in the powder samples of plant. The FTIR analysis was carried out using Perkin Elmer Spectrum 400 FT-IR/FT-FIR spectrometer in range 4000-400 cm^{-1} at Sophisticated Analytical Instrumentation Facility (SAIF)/Central Instrumentation Laboratory (CIL)/University Centre for Instrumentation and Microelectronics (UCIM), Panjab University, Chandigarh.

Wavelength Dispersive X-Ray Fluorescence (WD-XRF) analysis: Wavelength Dispersive X-Ray Fluorescence analysis was conducted to identify the various macro and micro elements present in leaves and bulb powder by using WD-XRF Tiger S8 at SAIF, CIL and UCIM, Panjab University, Chandigarh.

3. RESULTS AND DISCUSSION

The plant is a perennial herb, looks like onion and grows on sandy and uncultivated fields (Fig. 1a). It also occurs in gardens, lawns and wastelands. Irrespective of its morphological resemblance with onion, the odour is either absent or weak when the plant material is crushed. The root system consists of an ovoid shaped underground white coloured bulb. The bulb contains bulbils that easily get separated when dried. Fibrous roots arise from base of the bulb (Fig. 1b). The leaves are somewhat white near the base which are sheathing type and green towards the pointed tip, linear, strap like, flat, basal and have parallel venation (Fig. 1c). The flowering stem is smooth, one or two in number, long, erect, cylindrical, hollow, unbranched and green in colour. It bears white coloured flowers at the terminal position. The flowers are arranged in a cluster of 10-15 on pedicels in an umbel inflorescence that grows from two overlapping leaf like bracts called spathe (Fig. 1d & e). Each flower is infundibulate, bisexual, hypogynous, actinomorphic and incomplete with perianth arranged in two whorls of three tepals each (Fig. 1f & g). Thus there are total six tepals in each flower. These are pale green at the base and pointed at the tips, polytepalous, persistent and with a midvein marking. Stamens are six in number and fused with tepal at the base (Fig. 1h), inferior, persistent. Filament is broader in middle whereas tapering towards the both ends. (Fig. 1i). Ovary is superior, trilocular having axile placentation with terminal and persistent style (Fig. 1j). Fruit is green coloured capsule that turns pale brown towards maturity with 10-12 black coloured, obovoid and angled seeds (Fig. 1k & l).

The results pertaining to phytochemical screening of aqueous and ethanol extracts of leaves and bulbs are given in Table 1. Phytochemicals like alkaloids, flavonoids, glycosides, gums and mucilages, phenolics, phlobatannins, saponins, tannins, terpenoids have been observed in leaves. Some of them were recorded in aqueous and others in ethanol extract. The ethanol extract of bulbs contain more phytochemicals. Hence, it is clear that extraction of phytoconstituents is solvent dependent. The phytochemicals such as carbohydrates, reducing sugars and steroids were absent in leaves but found in bulbs. Similarly, phlobatannins were absent in bulbs but present in traces in leaves. This shows that the number of phytoconstituents also depends upon the plant parts used. Earlier, Brunengo, 1987, reported the presence of steroidal sapogenins in *N. gracile* while performing GC and GC-MS studies.

The previous phytochemical analysis of bulbs of *Allium sativum* had shown the presence of carbohydrates, glycosides and proteins in high concentrations. Alkaloids, saponins, reducing sugars, oils and steroids were in moderate amounts. Flavonoids and acidic compounds were low in concentrations whereas tannin, resin and terpenoids were absent (Ameah and Nwammoh, 2010). *Allium sativum* is useful in traditional medicines for heart diseases, blood pressure, diabetes etc. It also acts against various infectious agents (Ayaz and Alpsy, 2007). According to Howard and Kritchevsky, (1997) phytoconstituents such as sterols, flavonoids and sulphur found in fruits and vegetables can be important in reducing the risk of atherosclerosis. During present investigation, some of these chemical compounds have been recorded in variable amount. Therefore, detailed study of phytoconstituents in leaves, bulbs or in whole plant powder may throw light on some bioactive compounds. FTIR spectra of leaves and bulbs powder have been studied separately. Different absorption peaks has revealed the presence of various functional groups associated with the reported chemical compounds (Pavia, 2001). The FTIR spectra of leaves at 3274.50 cm^{-1} can be assigned to N-H stretch in amines and amides. The peaks at 2917.35 cm^{-1} , 2847.72 cm^{-1} are pertaining to O-H stretch in carboxylic acids. The peak at 1654.17 cm^{-1} indicates the presence of C=C in alkene. The peaks occurring at 1586.23 cm^{-1} and 1559.75 cm^{-1} can be assigned to N-H bend in amines and amides. The peak at 1459.54 cm^{-1} and 1364.77 cm^{-1} is due to C-H stretch in alkane and C-N stretch, respectively. Three peaks *i. e.* 1237.80 cm^{-1} , 1102.96 cm^{-1} and 1011.55 cm^{-1} have been reported for C-O stretching in alcohols, ethers, esters, carboxylic acids, anhydrides. The peak centered at 529.97 cm^{-1} revealed the presence of pentaborate anion (Yongzhong, 2001). The peak at 430.32 cm^{-1} represented the presence of S-S stretch in aryl disulfide (Pednekar and Raman, 2013).

FTIR spectra of bulbs have revealed the presence of three peaks which were almost similar to those obtained in leaves including 3274.13 cm^{-1} corresponded to N-H stretch in amines and amides, 2918.23 cm^{-1} and 2847.72 cm^{-1}

to O-H carboxylic acids. C=O stretch in aldehyde displayed a peak centered at 1735.19cm^{-1} . Similarly, peaks at 1629.33cm^{-1} and 1538.96cm^{-1} indicated C=C stretch in alkene and C=C stretch in aromatic compounds, respectively. The weak peak at 1258.24cm^{-1} revealed the C-O stretching in alcohols, ethers, esters, carboxylic acids and anhydrides. The peak at 1012.19cm^{-1} and 927.26cm^{-1} were the result of C-N stretching and C-O stretching in anhydrides, respectively. The comparative account of FTIR spectroscopy of leaves and bulbs suggested that pentaborate anion and disulfide linkage were present only in leaves. Similarly, only the bulbs has shown the presence of C=O stretch in aldehyde. Presence of C=O stretch can explain the presence of carbohydrates in bulbs. Fourier Transform Infrared (FTIR) spectroscopy of leaf and bulb powder of *Nothoscordum gracile* are given in Figs. 2a-b.

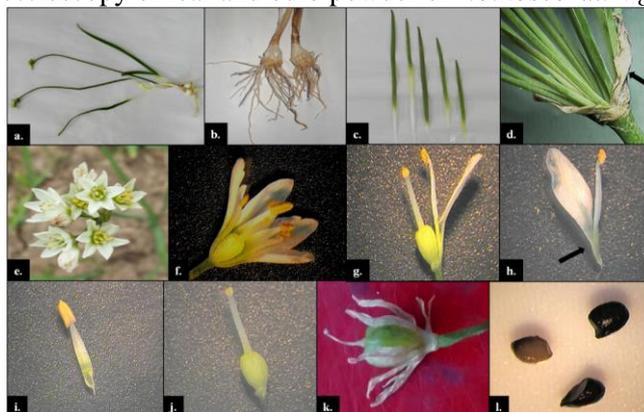


Fig. 1. *Nothoscordum gracile* (Aiton) Stearn: a. Whole plant; b. Bulb showing bulbils and fibrous roots; c. Leaves; d. Spathe subtending inflorescence; e. Blooming flowers; f. Vertical section of flower;

g. Superior ovary; h. Stamen adnate to tepal base; i. A stamen; j. A pistil; k. A fruit capsule with persistent tepals, stamens and pistil; l. Angled seeds

The micro and macro elements have been detected using WD-XRF spectroscopy in leaves and bulbs of *N. gracile* (Table 2). The leaves contain higher amount of K, Ca, Cl followed by P and S in moderate quantity and Cu, Rb and Br in traces. Whereas in bulbs, K is in higher concentration, P, S, Ca in moderate amount and Sr, Mo and Rb in traces. The elements like Potassium, Magnesium, Calcium, Sodium and Phosphorus were found effective in the management of cardiovascular diseases (McCarron, 1982; Houston and Harper, 2008). The concentration of K, Ca, Mg is more in leaves as compared to bulbs whereas, P is higher in bulbs. Therefore, individual plant parts or even the whole plant may provide some useful bioactive compounds of medicinal interest.

Allium sativum contain K, Ca, Mg and P in the concentration of 16675.75mg/kg , 694.41mg/kg , 710.12mg/kg and 4777.88mg/kg , respectively (Akinwade and Olatunde, 2015). The concentration of K (60100mg/kg), Ca (13100mg/kg), Na (1900mg/kg) and Mg (2700mg/kg each) was more in leaves of *N. gracile* than bulbs where it was 19800mg/kg , 3000mg/kg , 800mg/kg and 1500mg/kg , respectively. Chlorine in an appropriate amount is useful in fluid balance, digestion and chloride shift that occurs in RBCs (Soetan, 2010). The amount of chlorine varies from 1900ppm in bulbs to 12200ppm in the leaves of the species under investigation. The elements such as Fe, Zn, Mn and Cu are acting as structural and catalytic cofactors for proteins (Andreini, 2008). During present investigation, only two heavy metals like Zinc and Copper have been found in *N. gracile*. Silicon is essential to maintain the bone health. Sulphur is used in the synthesis of sulphur containing amino acids, effective in allergy, pain syndrome and various urinary disorders (Jugdasingh, 2007; Parcell, 2002). These two are also present in *N. gracile*. It has been observed that, many phytoconstituents and elements, important in the management of human health are present in this species. These properties are likely being useful for the pharmaceutical researchers.

Table.1. Phytochemical screening in leaves and bulbs of *N. gracile*

Tests	Leaves		Bulbs	
	Aqueous	Ethanol	Aqueous	Ethanol
Alkaloids	-	++	-	++
Carbohydrates	-	-	±	++
Flavonoids	-	++	-	+++
Glycosides	-	++	-	++
Gums & mucilage	+	-	±	-
Phenolics	++	+++	+++	+++
Phlobatannins	±	-	-	-
Reducing sugar	-	-	-	++
Saponins	±	-	++	-
Steroids	-	-	-	+
Tannins	++	++	+++	+++
Terpenoids	++	±	-	+

Abbreviations: (-) absent; (±) present in traces; (+) only present; (++) moderately present; (+++) present in abundance

Table.2.WD-XRF analysis showing elemental detail of *N. gracile* (in ppm)*

Element (s)	Leaves	Bulb	Element (s)	Leaves	Bulb
¹¹ Na	1900	800	²⁶ Fe	300	400
¹² Mg	2700	1500	²⁹ Cu	19	18
¹³ Al	500	500	³⁰ Zn	83	50
¹⁴ Si	1500	1600	³⁵ Br	8	-
¹⁵ P	5700	6200	³⁷ Rb	21	9
¹⁶ S	9500	7500	³⁸ Sr	56	16
¹⁷ Cl	12200	1900	⁷⁰ Zr	1	-
¹⁹ K	60100	19800	⁴² Mo	-	14
²⁰ Ca	13100	3000	⁴⁴ Ru	16	19
²² Ti	39	29	C ₆ H ₁₀ O ₅	892300	956700
²⁵ Mn	31	18			

*The error in concentration values of the elements ranges from ~5% up to 10%.

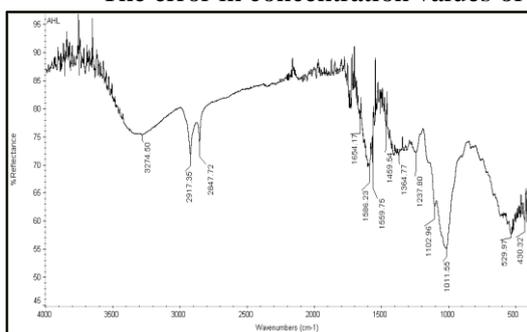


Fig.2a. Fourier Transform Infrared spectroscopy of leaf powder of *N. gracile*

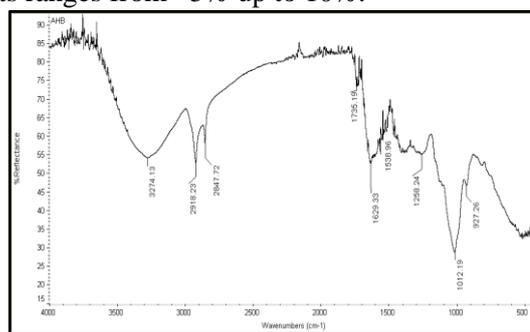


Fig.2b. Fourier Transform Infrared spectroscopy of bulb powder of *N. gracile*

4. CONCLUSION

Present study has revealed the phytochemical and elemental details of *Nothoscordum gracile*. The screening of various phytochemicals such as alkaloids, carbohydrates, flavonoids, glycosides, steroids, tannins *etc.* in different quantity has depicted the chemical composition of this plant. The FTIR analysis has strengthened the phytochemical observations by providing detailed information of various functional groups. The solvent and plant part used have their own effect on phytochemical characterisation. WD-XRF study has depicted many elements which are useful in the treatment of different human diseases. Therefore, this plant needs further critical evaluation so that its medicinal potential in pharmaceutical sector could be explored.

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REFERENCES

- Akinwade BA, Olatunde SJ, Comparative evaluation of the mineral profile and other selected components of onion and garlic, *International Food Research Journal*, 22, 2015, 332-336.
- Ameh GI, Nwammoh AO, Phytochemical and ethnobotanical evaluation of leaves of garlic bulb (*Allium sativum* L.), *Bio Resources*, 8, 2010, 618-621.
- Andreini C, Bertini I, Cavallaro G, Holliday GL, Thornton JM, Metal ions in biological catalyses: from enzyme databases to general principles, *Journal of Biological Inorganic Chemistry*, 13, 2008, 1205-1218.
- Ayaz E, Alpsy HC, Garlic (*Allium sativum*) and traditional medicine, *Turkiye Parazitoloji Dergisi*, 31, 2007, 145-149.
- Brunengo MC, Tombesi OL, Gros EG, Sterols from four species of Liliaceae, *Phytochemistry*, 26, 1987, 3088-3089.
- Gargiullo MB, Magnuson B, Kimball L, A field guide to plants of Costa Rica, Oxford University Press, New York, 2008.
- Greuter W, Raus Th. (ed.), *Med-Checklist Notulae*, 27, *Willdenowia*, 38, 2008, 465-474.

Gupta VP, Govindaiah, Datta RK, Plant extracts: A non-chemical approach to control *Fusarium* diseases of mulberry, *Current Science*, 71, 1996, 406-408.

Houston MC, Harper KJ, Potassium, Magnesium and Calcium: Their role in both the cause and treatment of Hypertension, *The Journal of Clinical Hypertension*, 10, 2008, 3-11.

Howard BV, Kritchevsky D, Phytochemicals and cardiovascular disease, *Circulation*, 95, 1997, 2591-2593.

Howard TM, A review of genus *Nothoscordum* in cultivation, *Winter*, 2, 1993, 2-13.

Howard TM, *Bulbs for warm climates*, Texa Press, Austin, 2001, 32-33.

Jugdaosingh R, Silicon and bone health, *Journal of Nutrition Health and Aging*, 11, 2007, 99-110.

Kunth CS, *Iphigenia*, *Enumeratio Plantarum* 4, J.G. Cotta, Stuttgart & Tubingen, 1843.

McCarron DA, Calcium, Magnesium and Phosphorus balance in Human and Experimental hypertension, *Hypertension*, 4, 1982, 27-33.

Parcell S, Sulphur in human nutrition and applications in medicine, *Alter Med Rev* 7, 2002, 22-44.

Pavia DL, Lampman GM, Kriz GS, *Introduction to spectroscopy*, 3rd edn., Fort Worth: Harcourt College Publishers, 2001, 579.

Pednekar PA, Raman B, The FT-IR spectrometric studies of vibrational bands of *Semecarpus anacardium* Linn. f. leaf, stem powder and extracts, *Asian Journal of Pharmaceutical Clinical Research*, 6, 2013, 159-198.

Quattrocchi U, *CRC World Dictionary of Plant names: Common names, Scientific names, Eponyms, Synonyms and Etymology*, CRC Press, USA, 1999.

Soetan KO, Olaiya CO, Oyewole OE, The importance of mineral elements for humans, domestic animals and plants: A review, *African Journal of Food Science*, 4, 2010, 200-222.

Souza LGR, Crosa O, Speranza P, Guerra M, Cytogenetic and molecular evidence suggest multiple origins and geographical parthenogenesis in *N. gracile* (Alliaceae), *Annals of Botany*, 109, 2012, 987-999.

Stearn WT, *Nothosordum gracile*, the correct name of *N. fragrans* and *N. inodorum* of Authors (Alliaceae), *Taxon*, 35, 1986, 335-338.

Thakur S, Sidhu MC, Phytochemical screening of some traditional medicinal plants, *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 5, 2014, 1088-1097.

Yadav RNS, Agarwala M, Phytochemical analysis of some medicinal plants, *Journal of Phytology*, 3, 2011, 10-14.

Yongzhong J, Shiyang G, Yan J, Yuan Z, Shuping X, FTIR spectroscopy of Magnesium tetraborate solution, *Chemical Papers*, 55, 2001, 162-166.