Isolation and identification of *Sceletium tortuosum* (L.) N.E.Br. constituents – first results

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Introduction

Sceletium tortuosum (L.) N.E.Br. (Aizoaceae) is a succulent plant from South Africa. Indigenous people use it as a medicinal herb for hunger and thirst relief, treatment of insomnia and other ailments, and for mood elevation. *S. tortuosum* contains alkaloids of four different structural types: mesembrine type, *Sceletium* A_4 type, joubertiamine type and tortuosamine type (see figure 1 below). Most abundant are mesembrine type alkaloids, which have also promising activities: serotonin reuptake inhibition (mesembrine being the most active) and phosphodiesterase 4B inhibition (mesembrenone being the most active) [1, 2]. These two mechanisms of action suggest potential use of *S. tortuosum* constituents in treatment of depression.
In this contribution, we present the first results of isolation and identification of nine compounds from a methanolic extract of whole dried plant.

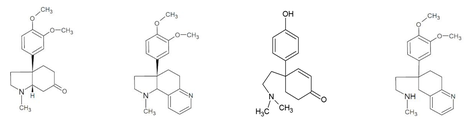

Figure 1: Four structural types of alkaloids found in *S.tortuosum* and other *Sceletium* species.

Methodology

We extracted 500 g of dried plant material with methanol (3×for 24 h, 1×in ultrasound bath for 30 min). Methanolic extract (231 g after evaporation) was subjected to liquid-liquid extraction, which yielded n-hexane part ST1A (13.5 g), chloroform part ST1B (9.3 g), ethyl acetate part (2.1 g) and water part (205.3 g). *n*-hexane part ST1A was applied to a silica gel column and eluted with benzene–chloroform = 40:60 (v/v), fractions of 100 mL were collected and combined based on TLC analysis, giving rise to 36 fractions. All subsequent separation steps were conducted in this manner. Fraction ST1A24 (1.4 g) was again applied to a silica gel column and eluted with chloroform–acetone–n-hexane = 95:3:2 (v/v) + 0,1 % formic acid, resulting in final 24 fractions. Fraction ST1A24/9 (90.3 mg) yielded compounds A-E after preparative RP-HPLC (with a gradient of acetonitril and 0.2% formic acid, ELSD detection). Chloroform part ST1B was applied to a silica gel column and eluted with benzene–chloroform = 25:75 (v/v), resulting in final 22 fractions. Fraction ST1B21 (5.6 g) was again applied to a silica gel column and eluted with chloroform–methanol–n-hexane = 90:8:2 (v/v/v), giving rise to 31 fractions. Fraction ST1B21/3 (1.7 g) was once more applied to a silica gel column and eluted with chloroform–acetone = 85:15 (v/v), yielding 23 fractions. Fraction ST1B21/3/6 (17 mg) was subjected to RP-HPLC (with a gradient of acetonitril and 0.2% formic acid, UV detection) and compounds F and G were isolated. Fraction ST1B21/3/10-11 (397 mg) was purified with RP-HPLC yielding compound H. Compound I crystallized spontaneously from fraction ST1B21/3/10-11.
Analytical and preparative HPLC was performed on a Dionex UltiMate 3000 with VWD and ELSD detectors. UV spectra were measured on an Agilent 1100 with DAD detector. Mass spectra were measured on a Shimadzu Nexera X2 coupled with a Shimadzu LCMS 8030 Triple Quadrupole Mass Spectrometer.

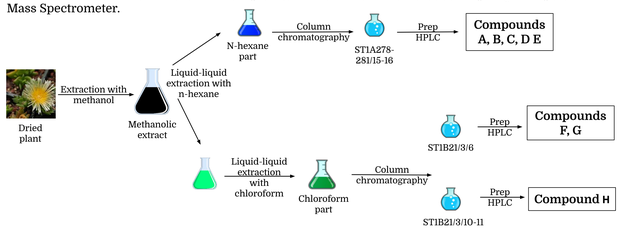

Figure 2: Scheme of separation and isolation of compounds from the dried *S. tortuosum* sample.

Results

Isolation of compounds A-E

Five compounds (A, 3.5 mg; B, 20 mg; C, 2.5 mg; D, 2.8 mg and E, 3.2 mg) were isolated from the fraction ST1A24/9 by preparative HPLC with ELSD detection. Compounds were collected manually based on their retention times; detection was performed only every 10th run to check the retention times remain the same. All isolated compounds were checked for purity on HPLC.

Figure 3: Column chromatography of ST1A

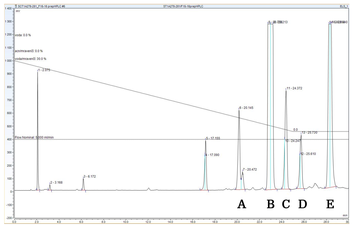

Figure 4: ELSD chromatogram of fraction ST1A24/9

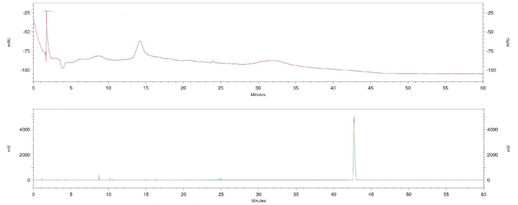

Figure 5: UV (254 nm) and ELSD chromatograms of compound B

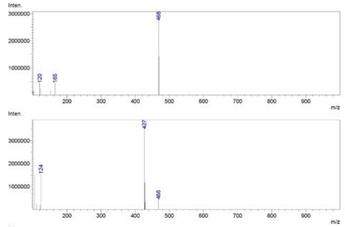

Figure 6: ESI-MS spectrum of compound A in the positive mode

Isolation of compounds F-I

Two compounds (F, 2.6 mg; G, 3.7 mg) were isolated from the fraction ST1B21/3/6 and one compound (H, 55.3 mg) from ST1B21/3/10-11 by preparative HPLC with UV detection. One compound (I, 32.7 mg) spontaneously crystallized from ST1B21/3/10-11 dissolved in tetrahydrofuran. All isolated compounds were checked for purity on HPLC. Compounds H and I seem to have the same structure (based on their retention times, UV spectra and assumed molecular masses). However, NMR analysis is needed to further substantiate this claim.

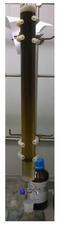

Figure 7: Column chromatography of ST1B

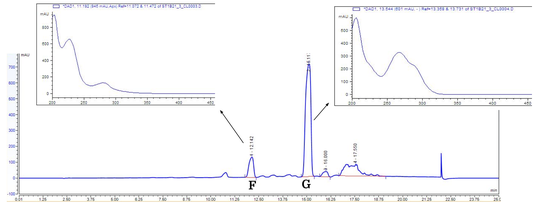

Figure 8: Chromatogram of fraction ST1B21/3/6, with UV spectra of compounds F and G

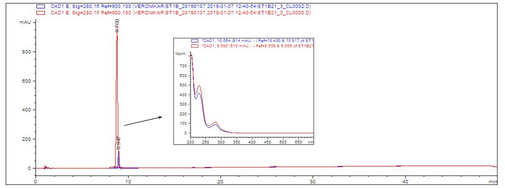

Figure 9: Overlaid chromatograms and UV spectra of compounds H and I

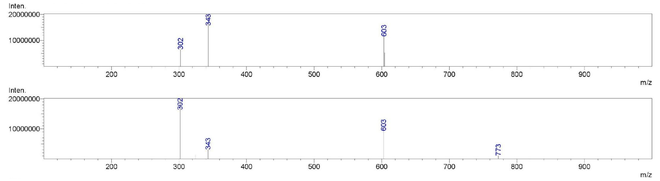

Figure 10: ESI-MS spectrum of compound H in the positive mode

Table 1: Overview of isolated compounds

Compound	Mass [mg]	Appearance	UV light absorption	Solubility	Assumed molecular mass
A	3.5	white to yellow amorphous substance	no	chloroform	467, N present
B	20	white amorphous substance	no	chloroform	data not sufficient
C	2.5	white amorphous substance	no	chloroform	410
D	2.8	white amorphous substance	no	chloroform	270
E	3.2	yellowish amorphous substance	no	chloroform	284
F	2.6	yellowish liquid	yes λ_{max} = ~204, ~227, ~280 nm	tetrafydro-furan	274
G	3.7	yellowish liquid	yes λ_{max} = ~206, ~267 nm	tetrafydro-furan	data not sufficient
H	55.3	yellow oily substance	yes λ_{max} = ~208, ~228, ~280 nm	tetrafydro-furan	602
I	32.7	white-yellowish crystals	yes λ_{max} = ~205, ~228, ~280 nm	tetrafydro-furan	602

Conclusion

Eight compounds have been isolated from methanolic extract of *Sceletium tortuosum* by preparative HPLC (A-H), one compound (I) crystallized spontaneously from its mother fraction dissolved in tetrahydrofuran. Interestingly, compound A contains nitrogen; compounds H and I have very similar retention times (8.7 and 8.9 min, respectively) and their spectral data shows they could be identical. Structures of all compounds will be elucidated in our future work.

Acknowledgements

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References

[1] Gericke, N.; Viljoen, A. M. *J Ethnopharmacol* **2008**, *119*, 653–663
[2] Harvey, A.; Young, CL.; Viljoen, AM.; Gericke, NP. *J Ethnopharmacol* **2011**, *137*, 1124–1129