Potential of South African Medicinal plants targeting the reduction of Aβ42 Protein as a treatment of Alzheimer's disease

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Abstract

Ethnopharmacological relevance: Twenty South African medicinal plant species were selected by conducting a literature review based on the relevant information of their reported traditional medicinal uses and scientific reports against Alzheimer's disease, dementia, anxiety, mental illness, depression, acetylcholinesterase inhibition, headache, epilepsy, convulsion, hysteria, central nervous system disorders and sedative effects.

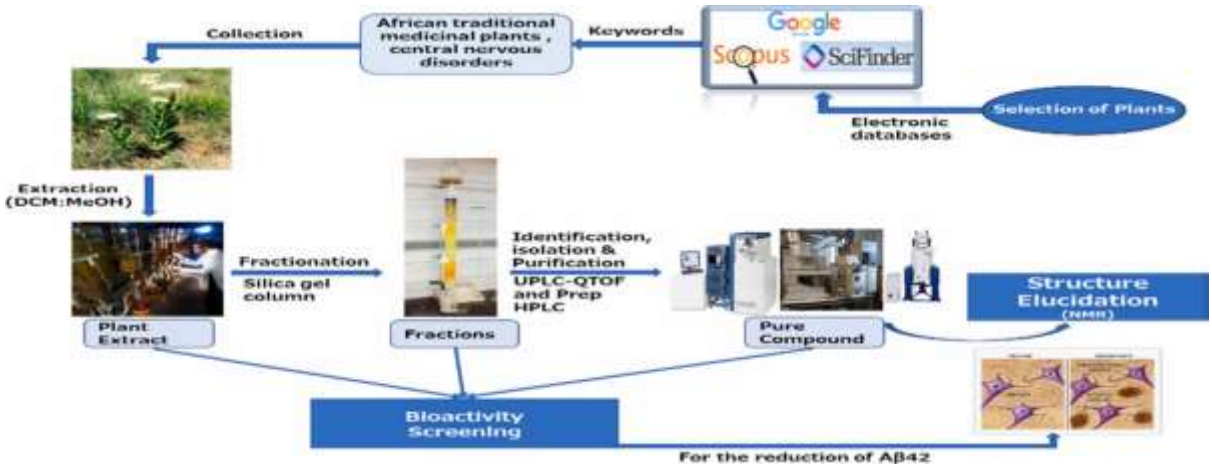
Aim of Study: The goal of this study was to investigate the biological activity of the traditionally used medicinal plant extracts against Alzheimer's disease by *in vitro* screening of the extracts to determine their potential to decrease levels of Aβ42 protein.

Material and Methods: Different plant parts (leaves, stem, bark, and stalks) of twenty selected plants were collected from the Manie van der Schijff Botanical Garden, University of Pretoria. Plant parts were dried, ground and then extracted using DCM:MeOH (1:1). We measured the levels of β-amyloid precursor protein proteolytic products in HeLa cells stably transfected with APP carrying the Swedish mutation using ELISA.

Results: Of 33 plant extract 10 (30.3%) were found active based on the potential to significantly reduce the production of Aβ42. Amongst them extracts of leaves of *Xysmalobium undulatum* (Apocynaceae), leaves of *Cussonia paniculata* (Araliaceae) and leaves of *Schotia brachypetala* (Fabaceae) potently decreased the production of Aβ42 by 77.3 ± 0.5%, 57.5 ± 1.3%, and 44.8 ± 0.1%, respectively. *X. undulatum* and *S. brachypetala* enhanced non-amyloidogenic processing of β-amyloid precursor protein, thereby decreasing Aβ42 level. We also showed that *C. paniculata* induced the decrease of Aβ42 level through inhibiting APP processing. In addition, we isolated two cardenolides, compound [A] and [B], from *X. undulatum* and found that they potently decreased the Aβ42 production.

Conclusion: These data suggest that the extract of *X. undulatum*, *C. paniculata*, and *S. brachypetala* have potential to be developed for Alzheimer's disease treatment. These active extracts and compounds are considered for further studies which examine their efficacy towards the reduction of Aβ42 through inhibiting APP process.

Graphical abstract



The picture of normal brain and Alzheimer's brain was adapted from an online resource: INTECH open accesses. URL: https://www.intechopen.com/books/neurodegenerative-diseases/therapeutic-interventions-in-alzheimer-disease

1. Introduction

Alzheimer's disease, a multifactorial disorder is a subtle, progressive and neurodegenerative disease that destroys human memory, other important mental functions and interrupt the daily activities in the ageing population. The main causes of the disease are accumulation of amyloid plaques and neurofibrillary tangles (Hardy & Higgins, 1992). Due to the raising life standards, busy and stressful lifestyle and more earnings, the Alzheimer's patients will grow from 47 million to 130 million by 2050 (Nguyen et al., 2017). The Food and Drug Administration (FDA) has approved five drugs for Alzheimer's disease. These are donepezil, galanthamine, rivastigime, tacrine from a class of drugs called cholinesterase inhibitors and memantine, an *N*-methyl-D-asparate antagonist. However, these drugs temporarily slow down the progression of Alzheimer's disease and improve symptoms but also have some side effects. Thus, it becomes a significant area of research, as none of these drugs directly target the amyloid plaques which are considered as the hallmark of Alzheimer's disease.

Amyloid plaques contain mainly amyloid β (Aβ) peptide generated by the proteolysis of a large β-amyloid precursor protein (APP). APP can be processed by the proteolytic enzymes called secretases (α, β and γ) in two different pathways. The processing of APP by α-secretase in non-amyloidogenic pathway produces a soluble fragment sAPPα which has neuroprotective activities. The other pathway is amyloidogenic pathway in which APP is processed by β-secretase and γ-secretase, resulting in the production of neurotoxic Aβ peptide (39-43 amino acids) (Vardy et al., 2005). Aβ40 (amyloid peptide containing 40 amino acids) and Aβ42 (amyloid peptide containing 42 amino acids) are the major isoforms, but Aβ42 is the major contribution to the Alzheimer's disease (X. Sun et al., 2015). Some gene mutations increase the production of Aβ42, and thus its accumulation in the brain induces aggregation to form amyloid plaques (Annaert & De Strooper, 2002).

In today's world, Alzheimer's disease has become one of the prominent diseases. Natural products derived from plants are the source for many potent pharmaceuticals and they have also shown over the years to be a powerful promise in the field of Alzheimer's disease. One of the drugs approved by FDA for the treatment of Alzheimer's disease, Galanthamine, is a natural product belonging to *Amaryllidaceae* family of alkaloids (Marco & Carreiras, 2006).

South Africa exhibits rich plant diversity with more than 20,000 different species and 10.8% of African flora is known to be used in traditional medicine(B-E Van Wyk, 2011).We selected 20 traditionally used African plants from the University of Pretoria Gardens to evaluate their potential for treatment of Alzheimer's disease through the reduction of Aβ42. We tested the effect of plant extracts on Aβ42 production in HeLa cells stably transfected with the Swedish mutant form of APP. The most promising samples were the extracts of leaves of *Xysmalobium undulatum*, leaves of *Cussonia paniculata*, and leaves of *Schotia brachypetala* which all showed very good of Aβ42 reduction as compared to the negative control.

2. Materials and methods

2.1. Selection and collection of plant material

A literature study was done with the help of Google scholar, SciFinder and Scopus for the selection of the plants by using the keywords African medicinal plants and traditional medicinal plants in combination with the memory loss, mental illness, depression, Alzheimer's disease, dementia, anxiety, epilepsy, forgetfulness, convulsion, hysteria, central nervous system disorders, sedative effects, acetylcholinesterase inhibition. The identified plants were

prioritized by a scoring system following the key criteria- plant part used, strength of the traditional use in relative to memory loss, plant toxicity, plant availability, published information against acetylcholinesterase. Twenty plants were selected based on the highest priority scores and different parts (leaves, stem, bark, roots, stalks and flower) and were collected. Two Plants, *Commelina africana* and *Ziziphus mucronata* were collected from the Dinokeng Nature Reserve, Pretoria and remaining of the 18 plants were collected from Maine *vander*Schijff Botanical Garden, University of Pretoria. Voucher specimen were deposited and identified at the Plant Herbarium at University of Pretoria (Table 1).

Table 1[1]. Plants species selected and collected with voucher specimen numbers

Sample No.	Family	Plant species	Plant part used	Voucher specimen	Extraction yield (calculated from dry plant material) as *w/w%*
1	Apiaceae	*Centella asiatica* (L.) Urb.	Leaves + Stalks	124298	13.9
2	Apocynaceae	*Mondia whitei* (Hook.f.) Skeels	Leaves	124299	10.3
3			Stem		7.6
4	Apocynaceae	*Stapelia gigantea* N.E.Br.	Stem	124308	12.5
5			Seed		23.3
6			Seed Pods		3.1
7	Apocynaceae	*Tabernaemontana elegans* Stapf	Leaves	124300	13
8	Apocynaceae	*Xysmalobium undulatum* (L.) W.T.Aiton	Leaves	124301	7.6
9	Araliaceae	*Cussonia spicata* Thunb.	Leaves	124302	20.9
10			Stem		6
11	Araliaceae	*Cussonia paniculata* Eckl. & Zeyh.	Leaves	124309	5.7
12			Stem		7.7
13	Asphodelaceae	*Bulbine natalensis* Baker	Leaves	124310	24.5
14	Celastraceae	*Catha edulis* (Vahl) Endl.	Leaves	124303	13
15			Stem		6.9
16	Commelinaceae	*Commelina africana* L.	Leaves	124311	11.2
17	Crassulaceae	*Cotyledon orbiculata* L.	Leaves	124312	2.2
18			Stem		6.1
19	Fabaceae	*Schotia brachypetala* Sond.	Leaves	124313	9.2
20	Lamiaceae	*Tetradenia riparia* (Hochst.) Codd	Leaves	124304	12.5
21			Stem		5.4

[1] All plant species name have been confirmed as acceptable on http://www.theplantlist.org/

22	Maliaceae	*Trichilia dregeana* Sond.	Leaves	124305	9.9
23			Stem		6.8
24	Plumbaginaceae	*Plumbago auriculata* Lam.	Leaves	124306	19.3
25			Stem		7.1
26	Rhamnaceae	*Ziziphus mucronata* Willd.	Leaves + Stalks	124314	10.5
27	Rutaceae	*Ruta graveolens* L.	Leaves	124315	22.8
28			Stem		10.5
29	Rutaceae	*Zanthoxylum capense* (Thunb.) Harv.	Leaves	124316	7.2
30			Stem + Thorn		9.1
31	Scrophulariaceae	*Buddleja salvifolia* (L.) Lam.	Leaves	124307	10.5
32			Stem		6
33	Vitaceae	*Cissus quadrangularis* L.	Stem	124317	9.6

2.2. Extraction methods

Plant parts were cut into small pieces and then dried in oven at 60°C. The drying time varied with the nature of plant and the plant part. Dried plant material was ground to a coarse fine powder. The powdered plant material (2g) was extracted with dichloromethane/methanol (DCM/MeOH) (1:1). Plant extracts were concentrated using a rotary vacuum evaporator and then dried under high vacuum. All the extracts were stored in a cold room prior to screening.

2.3. Cell culture

HeLa cells stably transfected with APP carrying Swedish mutation (APPsw) were cultured at 37°C, 5% CO_2, in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum containing 100 units/ml penicillin, 100 µg/ml streptomycin, 260 µg/ml Zeocin, 400 µg/ml G418.

2.4. sAPPα, sAPPβ, Aβ peptide assay

APPsw-transfected HeLa cells at 80% confluence in a 35 mm dish were cultured with sample solubilized in dimethyl sulphoxide (DMSO) for 8 h in serum free medium. The conditioned medium was analyzed by a sandwich ELISA (Invitrogen) for detection of Aβ42 and Aβ40 according to the supplier's instruction. The levels of sAPPα and sAPPβ-sw were measured from the conditioned medium using the specific ELISA (IBL).

2.5. Cell viability measurement

Cell viability was measured by using an EZ-Cytox kit (Daeil Lab Co, Ltd, Republic of Korea) according to the manufacturer's instructions. APPsw-transfected HeLa cells in a 96 well plate were cultured with sample solubilized in DMSO for 8 h, and then incubated with EZ-Cytox solution for 1 h at 37°C. The absorbance was detected at 450 nm using a microplate reader.

2.6. Statistical analysis

Data was expressed as mean ± SEM. Statistical comparisons between controls and treated experimental groups were performed using the Student's *t*-test. $P < 0.05$ was considered statistically significant.

2.7. Bioguided fractionation using column chromatography and preparative HPLC

The crude extract of *X.undulatum* leaf was subjected to gravity silica gel (0.063-0.2nm) column chromatography using glass column of 107cm length and 7cm internal diameter with a stepwise gradient of DCM:Hexane (50:50-80:20), DCM (100%), DCM:Methanol (95:05-70:30) for elution. The solvent gradient was at a flow rate of 2ml/min. The fractions were monitored by using TLC silica gel plate and tested for Aβ42 reduction. The active fractions were further purified by using preparative HPLC-MS on a Waters chromatographic system with waters PDA (2998) and MS detector (Waters, Milford, MA, USA). The active fraction (70mg) was diluted to 1ml with methanol. X Bridge preparative C18 column (19×250 mm, i.d., 5µm particle size, Waters) was used for separation. The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) at a flow rate of 20ml/min. Gradient elution was applied as follows: 95% A : 5% B keeping for 1 min, 50% A: 50% B (1.00-8.00 min), 15% A : 85% B (8:00-13:00 min), 95% A : 5% B (13:00-13:20 min). Injection volume was 150µl. Data were collected using MassLynx4.1^{TM} (Waters, USA) software. The preparative HPLC system was interfaced with a QDa mass spectrometer. Negative ion mode was selected. The probe temperature and source temperature were set at 600ºc and 120ºc .The capillary and cone voltage were set to 800 and 10 V respectively. Data was collected between 100 and 650 *m/z*. The eluents were fractionated into 2.5ml/tube using a fraction collector. The target compounds were collected in various fractions, subsequently combined and concentrated to give residues, which were later analyzed by UPLC-QTOF-MS.

2.8. Ultra-performance liquid chromatography QTOF mass spectrometry

The crude extracts, fractions and compounds were analyzed using a Waters Acquity UPLC system (Waters corp.,MAUSA), equipped with a binary solvent delivery system and an autosampler. The instrument was centrally operated by MassLynx 4.1 software (Waters Inc., Milford, Massachusetts, USA) for data acquisition. The Separation was achieved on Waters BEH C18 1.7 µm particle size (2.1 mm × 100 mm). The mobile phase consisted of solvent A: water with 0.1% formic acid and solvent B: methanol with 0.1% formic acid). The gradient elution was optimized as follows: 3% B (0-0.1 min), 100% B (0.3-14.00 min), 100% B (14.00-16.00 min), 3% B (16.00-16.50 min), 3% B (16.50-20.00 min). The flow rate was 0.3 ml/min for the entire run, giving a total run time of 20 min. and the injection volume was 5µl. The instrument was calibrated by direct infusion of 5 nM sodium formate solution at a flow rate of 10µl/min over a mass range of 50-1200 Da. The following MS source parameter were set for both positive and negative mode: Source temperature 100 ºC, sampling cone 15 V, extraction cone 4.0 V, desolvation temperature 400 ºC, cone gas flow 10.0 L/h, desolvation gas flow 700 L/h, capillary 2.0 kV. Both positive and negative modes were obtained, but the results were analyzed from the negative mode as the higher intensity peaks were obtained from negative mode. Compounds were tentatively identified based on their accurate mass generating from MassLynx V 4.1, iFit value, MS/MS fragmentations (product ions), and by the use of Metlin , Metfusion, Pubchem , Chemspider, Chemical Entities of Biological Interest (ChEBI) and Massbank libraries.

2.9. NMR analysis

Structure elucidation of isolated compounds was carried out using NMR spectroscopy. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker Fourier 400 spectrometer (^{1}H at 400 MHz and ^{13}C at 100 MHz) for compound A and 500 spectrometer (^{1}H at 500 MHz and ^{13}C at 125

MHz) for compound B. Chemical shifts are reported in ppm, referenced to residual solvent resonances (methanol-d4 δH 3.31, δC 49.0 ppm).

3. Results and Discussion

3.1. Collection and extraction of plant material

Twenty plants belonging to the different families (Table 1) were collected and their traditional uses are described in Table 2. All the traditional uses are related to some form of mental illness with none being directly used for the treatment of Alzheimer's disease. A total of 33 extracts were prepared for the different plants and their plant parts extracted with dichloromethane/methanol (DCM:MeOH) (1:1). The extraction yield of 33 plant extracts ranged from 3% to 25% labelled 1 to 33 shown in Table 1. The variation in the extraction yields depended on the plants species and the plant part collected.

Table 2. Reported traditional uses of selected 20 plant species

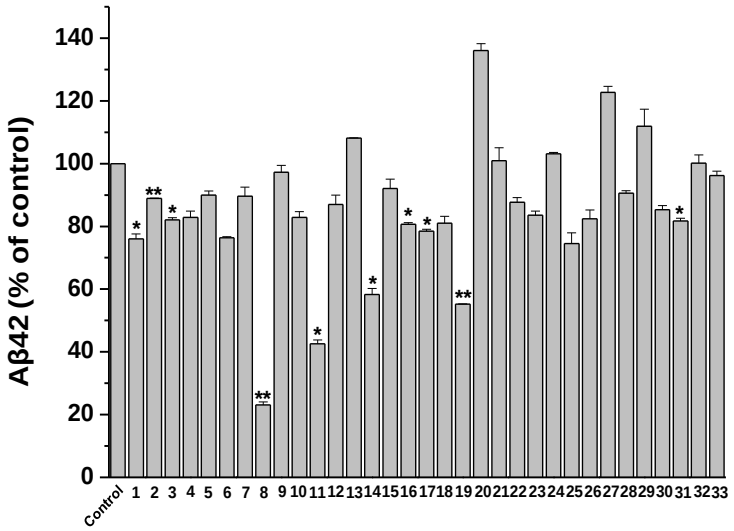
Plant species	**Traditional use**
Buddleja salvifolia	Traditionally used in the treatment of neurodegenerative disorders. Buddleja species together with *Heteromorpha trifoliate* & *Cussonia paniculata* are used for the treatment of early nervous and mental disorders in South Africa (Adewusi et al., 2011).
Bulbine natalensis	Tuber decoctions are used for the treatment of convulsions in South Africa (SOBIECKI, 2002).
Catha edulis	Fresh leaves reduce fatigue and relieve sleepiness. It increases mental power and communication skills but also causes hypertension and affects male fertility(Ben-Erik Van Wyk et al., 2009).
Centella asiatica	Dried powdered leaves reportedly produce a calming and sedative effect and in some parts of India it is given with milk to improve memory against dementia (Gary I Stafford et al., 2008).
Cissus quadrangularis	Boiled with water and used for the treatment of epilepsy in Dar es Salaam, Tanzania (Moshi et al., 2005).
Commelina africana	In Lesotho plant decoctions with Tephrosia capensis are used for heart complaints and nervous disorders (Gary I Stafford et al., 2008)
Cotyledon orbiculata	Leaf juice has been used for the treatment of epilepsy (Ben-Erik Van Wyk et al., 2009).
Cussonia paniculata	Used for the treatment of rheumatism, dysmenorrhea, colic and diseases associated with the nervous system (Tetyana et al., 2002)
Cussonia spicata	Boiled with water and used for the treatment of epilepsy in Dar es Salaam, Tanzania (Moshi et al., 2005).
Mondia whitei	Root infusions are taken by unspecified groups in South Africa to treat stress in adults (Gary I Stafford et al., 2008).
Plumbago auriculata	Used for headache in Zulu, Xhosa and Sotho medicine (Hutchings & van Staden, 1994).

Ruta graveolens	Leaf infusions are taken for epilepsy and hysteria, and convulsions and fits in children (Ben-Erik Van Wyk et al., 2009).
Schotia brachypetala	Roots and barks are used for nervous conditions(Wyk & Gericke, 2000). Bark decoction is used for the treatment of heartburn and to calm down the effects of too much drinking (Ben-Erik Van Wyk et al., 2009).
Stapelia gigantean	Zulu healers use hot stem infusions to treat hysteria (Gary I Stafford et al., 2008).
Tabernaemontana elegans	Used for the treatment of heart diseases, cancer, tuberculosis, stomach ache, infertility(*Medicinal Plants, Volume 1*, 2008).
Tetradenia riparia	Leaf infusions are taken for the treatment of respiratory ailments, mouth ulcers, fever, influenza, diarrhea, stomach ache, swollen legs and crushed leaves are used for headache (Ben-Erik Van Wyk et al., 2009).
Trichilia dregeana	Used as wound healing, for the treatment of dysentery, fever, stomach and intestinal complaints (Quattrocchi, 2012).
Xysmalobium undulatum	Plant is used for headache and abdominal pains in Botswana (Hutchings & van Staden, 1994). Traditionally, it is also used for the treatment of diarrhoea, dysentery, stomach cramps, indigestion (Ben-Erik Van Wyk et al., 2009).
Zanthoxylum capense	A species of *Zanthoxylum* is used for the treatment of mental illness in Gabon. It is also used as an epilepsy remedy among Europeans (Gary I Stafford et al., 2008).
Ziziphus mucronata	Decoctions are taken for pneumonia. It is also used for the treatment of the swellings of skin, wounds (Rabe & Van Staden, 1997).

3.2. The effect of the plant extracts on Aβ42 production

The 33 plant extracts were screened to determine their inhibitory properties for Aβ42 production in HeLa cells stably transfected with APPsw. Cells were incubated with 50 μg/ml extracts for 8 h, and the level of Aβ42 was measured from the conditioned media by using specific ELISA method. Galanthamine, which was reported to inhibit amyloid aggregation (Matharu et al., 2009) and release of Aβ42 was decreased by 37% with 10μM galanthamine in SH-SY5Y cells (Li et al., 2010) was used as a positive control (Supplementary Fig. S1). The Aβ42 production of galantamine was measured by treating cells with varying concentrations for 8 h. However, galanthamine at high concentration of 10 μM did not significantly affect the Aβ42 production in our experimental condition. We found that 10 extracts (30.3%) (Extract numbers: 1,2,3,8,11,14,16, 17, 19 and 31) significantly decreased the Aβ42 level as compared to the negative control as shown in Fig. 1. Among them, the extracts of leaves of *Xysmalobium undulatum* (No. 8), leaves of *Cussonia paniculata* (No. 11) and leaves of *Schotia brachypetala* (No. 19) potently reduced the secreted level of Aβ42 compared to the control. The level of Aβ42 was significantly decreased by 76.9 ± 1%, 57.5 ± 1.3% and 44.8 ± 0.1% with leaves of *X. undulatum*, leaves of *C. paniculata*, and leaves of *S. brachypetala*, respectively. Thus, we selected these three extracts for further studies.

Fig. 1. Effect of plant extracts on the level of Aβ42 in APPsw-transfected HeLa cells.
APPsw-transfected HeLa cells were incubated with 50 μg/ml extracts for 8 h, and the level of Aβ42 was measured from the conditioned media by using specific ELISA methods. The extracts of leaves of *X.undulatum*(No. 8), leaves of *C.paniculata*(No. 11), and leaves of *S.brachypetala*(No. 19) potently decreased the secreted level of Aβ42 (n = 2). *, $P<0.05$; **, $P<0.01$.



3.3. The effect of Xysmalobium undulatum on APP processing and cytotoxicity

In the present study, we confirmed the effect of the extracts of leaves of *X. undulatum* on Aβ42 production in a dose-dependent manner. Cells were incubated with 0.5, 1, 5, and 10 μg/ml extract for 8 h, and the level of Aβ42 was measured from the conditioned media by using specific ELISA kit. The level of Aβ42 was significantly decreased by extract of leaves of *X. undulatum* in a dose-dependent manner (Fig. 3A). Secreted level of Aβ42 was decreased by 71.5 ± 1.7% and 73 ± 1.1% at 5 and 10 μg/ml of extract, respectively. The extract of leaves of *X. undulatum* induced the decrease of cell viability (Fig. 2A). Although the extract of leaves of

X. undulatum has some cytotoxicity, it potently induced the decrease of Aβ42 production. We next measured the secreted levels of APP proteolytic products from the conditioned media using specific ELISA kits for Aβ40, sAPPβ-sw and sAPPα. Secreted level of Aβ40 was decreased by 66.4 ± 1.4% and 73.3 ± 1% at 5 and 10 μg/ml of extract, respectively (Fig. 3B). Secreted level of sAPPβ-sw was also reduced by 49.1 ± 4.8% and 52.7 ± 2% at 5 and 10 μg/ml of extract, respectively (Fig. 3C). In contrast, this extract significantly increased the secreted level of sAPPα in a dose-dependent manner (Fig. 3D). The sAPPα level was increased by two-fold at 10 μg/ml extract of leaves of *X. undulatum.* These data suggest that the extracts of leaves of *X. undulatum* increase the non-amyloidogenic processing of APP, while it decreases amyloidogenic processing of APP, leading to the reduction of Aβ level.

Fig. 2. Cytotoxicity of extracts of leaves of *X. undulatum*, leaves of *C.paniculata*, and leaves of *S.brachypetala*. Cells were incubated with indicated concentrations of extracts of leaves of *X.undulatum*, leaves of *C. paniculata*, and leaves of *S.brachypetala* for 8 h, and then incubated with EZ-Cytox solution for 1 h. (A) The extract of leaves of *X. undulatum* induced the decrease of cell viability (n = 6). (B, C) Both extracts of leaves of *C. paniculata* (B, n = 6) and *S. brachypetala*(C, n = 6) did not show cytotoxic effects except for high concentration of 100 μg/ml. **, P<0.01; ***, P<0.001.

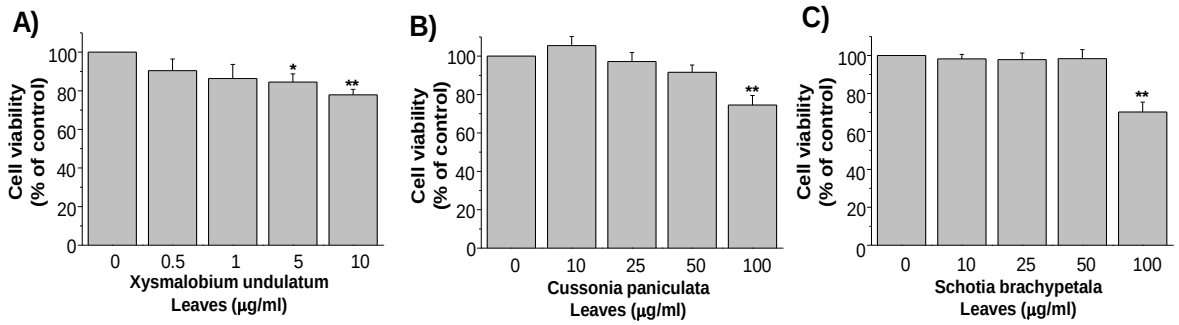
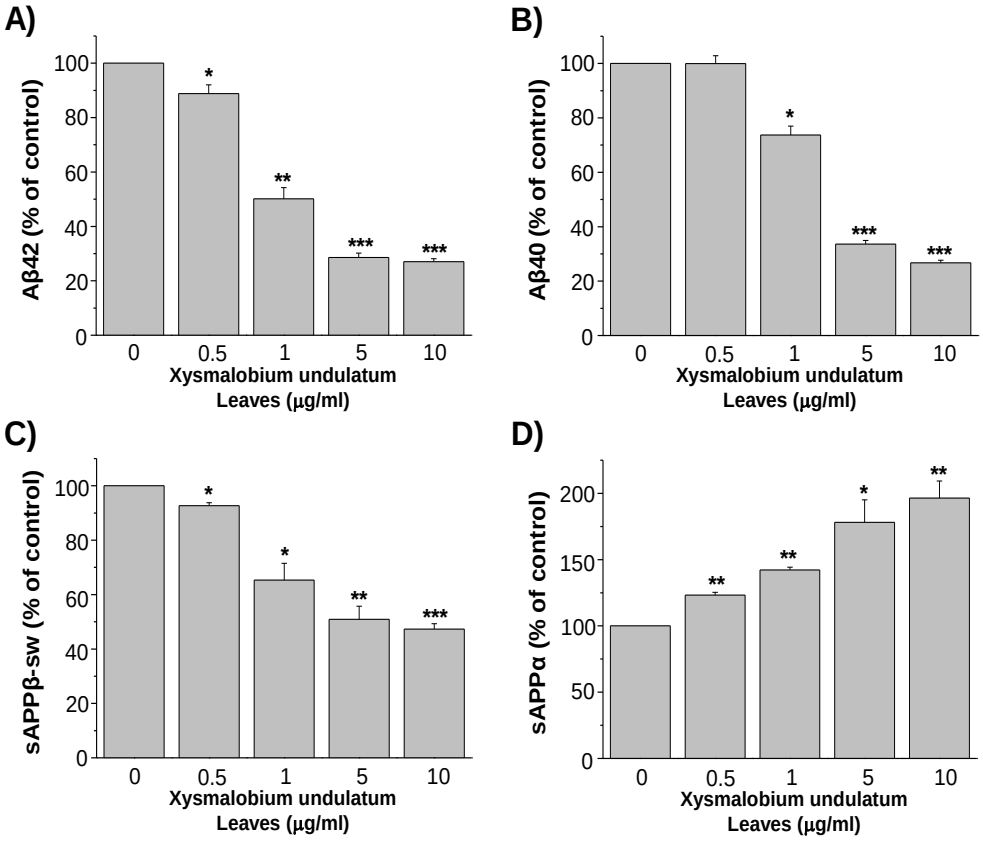


Fig. 3. Change in the levels Aβ, sAPPβ-sw, sAPPα by extract of leaves of *X.undulatum*.

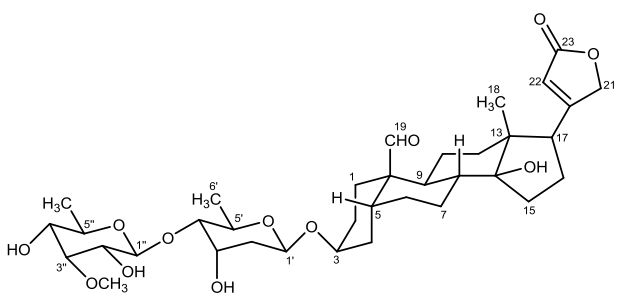
Cells were incubated with indicated concentrations of extract of leaves of *X.undulatum*for 8 h, the levels of Aβ, sAPPβ-sw, and sAPPα were measured from the conditioned media by using specific ELISA methods. (A-C) The levels of Aβ42 (A, n = 4), Aβ40 (B, n = 4), and sAPPβ-sw (C, n = 4) were decreased by the extract. (D) The level of sAPPα was increased by the extract (n = 4). **, P<0.01; ***, P<0.001.



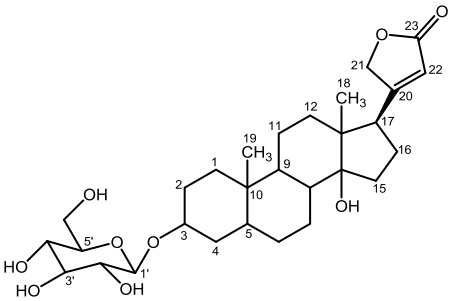
3.4. Cardenolide glycosides isolated from DCM:MeOH extract of X. undulatum through bioguided fractionation

15 fractions (AT-1-49A to AT-1-49O) were collected from the silica gel column and then were tested the effect on Aβ42 production (Supplementary Fig. S2). Cells were incubated with 10 μg/ml fractions for 8 h, and the level of Aβ42 was measured from the conditioned media by specific ELISA. Two fractions, AT-1-49N and AT-1-49O, potently decreased the Aβ42 secretion. Two pure compounds belong to the cardenolide glycosides , Crotoxigenin-3-*O-β*-digitalopyranosyl-(1-4)-*O-β-digitoxopyanoside* [A] and desglucouzarin [B] were isolated from the two active fractions using the preparative HPLC which were found to be active ingredients after bioassay screening for the reduction of Aβ42. The negative mode of ESI-MS revealed a pseudomolecular ion *m/z* 723.3600 $[M+HCOO]^-$, corresponding to a molecular formula of $C_{36}H_{54}O_{12}$ identified as crotoxigenin-3-*O-β*-digitalopyranosyl-(1-4)-*O-β-digitoxopyanoside* [A] as the first report of this cardenolide isolated from *X. undulatum.* The second active compound gave a pseudomolecular ion peak at *m/z* 581.2974 $[M+HCOO]^-$ with

a molecular formula $C_{29}O_9H_{44}$ corresponding to desglucouzarin [B] (Supplementary Fig. S3 and Fig. S4). The ^{1}H and ^{13}C NMR data of compound [A] (Table S1) corresponds to literature data (Gohar et al., 2000) and of compound [B] (Table S2) corresponds to published information (Rakotondramanga et al., 2016). These compounds are the first reports for the reduction of Aβ42.



Crotoxigenin-3-O-β-digitalopyranosyl-(1-4)-O-β-digitoxopyanosid [A]



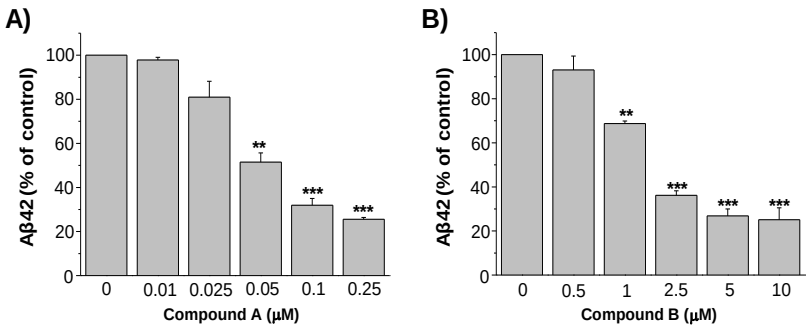
Desglucouzarin [B]

3.5. The effect of the compounds on Aβ42 *production*

Two compounds, [A] and [B], were treated with various concentrations in APPsw-transfected HeLa cells for 8 h. The level of Aβ42 from the conditioned media was measured using specific ELISA kit. The level of Aβ42 was potently decreased by compound A in a dose-dependent manner (Fig. 4A). At 0.1 and 0.25 μM, compound A significantly decreased the levels of Aβ42 by 68.1 ± 3.1% and 74.5 ± 0.9%, respectively. In addition compound [B] significantly decreased the level of Aβ42 in a dose-dependent manner (Fig. 4B). The level of Aβ42 was significantly decreased by 73.2 ± 3.2% and 74.9 ± 5.4% at 5 and 10 μM of compound B, respectively. We next tested the cell viability of both compounds [A] and [B] (Supplementary Fig. S5). When cells were treated with compound A and B at a high concentration of 10 μM for 8 h, they induced cytotoxicity to a slight degree. These data indicated that compound A and B are the active compounds, responsible for the effect of Aβ42 production in *X. undulatum* extract.

Fig. 4. Change in the level Aβ42 by compounds of *X.undulatum* extract.

Cells were incubated with indicated concentrations of compound A and B for 8 h. The level of Aβ42 was measured from the conditioned media by using specific ELISA. (A) The level of Aβ42 was decreased by compound A (n = 4). (B) The level of Aβ42 was decreased by compound B (n = 4). **, P<0.01; ***, P<0.001.

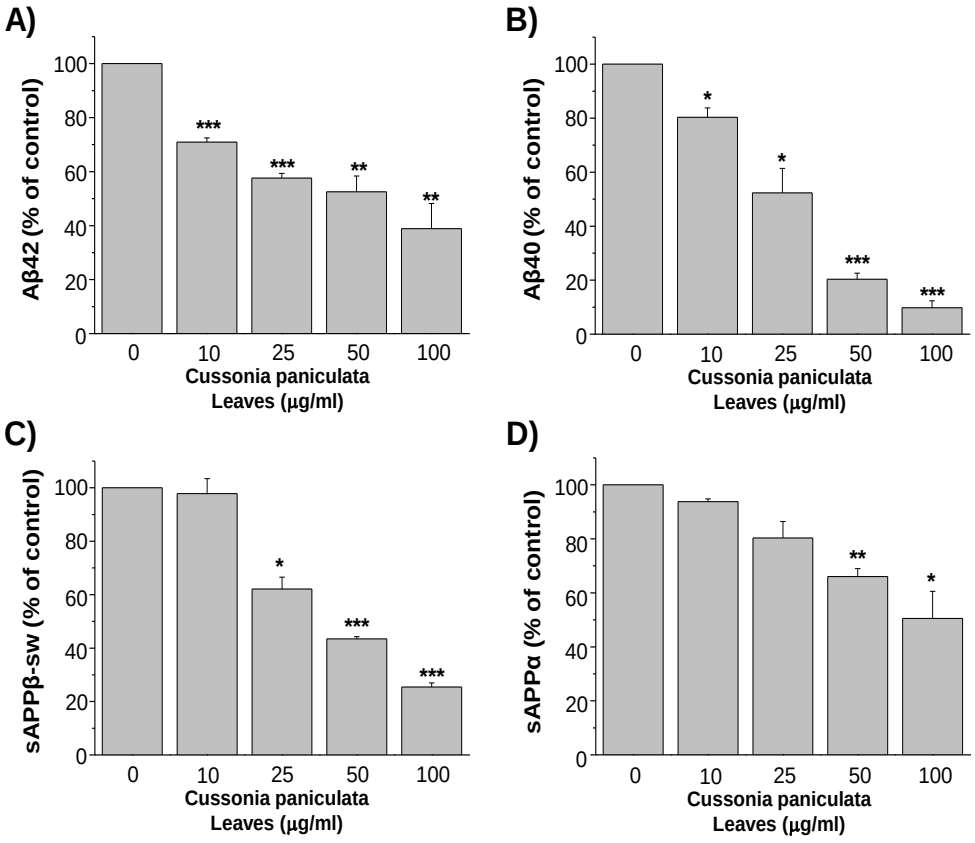


3.6. The effect of Cussonia paniculata on APP processing and cytotoxicity

To observe the effect of extract of leaves of *C. paniculata* on Aβ42 production in a dose-dependent manner, cells were incubated with 10, 25, 50, and 100 μg/ml extract for 8 h. The level of Aβ42 was measured from the conditioned media by specific ELISA kit. The extract of leaves of *C. paniculata* significantly reduced Aβ42 production in a dose-dependent manner (Fig. 5A). We next tested the levels of APP proteolytic products from the conditioned media using specific ELISA kits for Aβ40, sAPPβ-sw and sAPPα. The level of Aβ40 was significantly decreased by 47.7 ± 9.1% and 79.6 ± 2.2% at25 and 50μg/ml of extract, respectively (Fig. 5B). The level of sAPPβ-sw was also significantly decreased by 37.9 ± 4.4% and 56.6 ± 0.8% at 25 and 50μg/ml of extract, respectively (Fig. 5C). In addition, this extract significantly reduced sAPPα production in a dose-dependent manner (Fig. 5D).Extracts of leaves of *C. paniculata* (Fig. 2B) did not influence cell viability but at a high concentration of 100 μg/ml, it induced cytotoxicity. These results indicate that the extract of leaves of *C. paniculata* effect APP processing at non-toxic concentrations, thereby decreasing Aβ42 production.

Fig. 5. Change in the levels Aβ, sAPPβ-sw, sAPPα by extract of leaves of *C.paniculata.*

Cells were incubated with indicated concentrations of extract of leaves of *C.paniculata* for 8 h, the levels of Aβ, sAPPβ-sw, and sAPPα were measured from the conditioned media by using specific ELISA methods. The levels of Aβ42 (A, n = 3), Aβ40 (B, n = 3), sAPPβ-sw (C, n = 3), and sAPPα (D, n = 3) were decreased by the extract. *, *P*<0.05; **, *P*<0.01; ***, P<0.001.



3.7. Chemical characterization of DCM:MeOH extract of Cussonia paniculata

Using ESI negative mode, a total of four compounds were tentatively identified which included one flavonoid and three triterpenoid saponins (Fig. 7) (Table S3). Peak 1′ with *m/z* 609.1453 $[M-H]^-$ corresponding to molecular formula $C_{27}H_{30}O_{16}$, fragmented to produce a base peak ion at *m/z* 301.0340 [M-glucose unit]$^-$. Comparison of the accurate mass, retention time and the fragmentation pattern of peak 1′ and the pure standard confirmed the compound to be rutin (Supplementary Fig. S6). Triterpene glycosides is also one of the major class isolated from *C. paniculata* (Adedapo et al., 2008). From this extract we tentatively identified three triterpene glycosides based on their accurate mass generated from MassLynx V 4.1 peak 2′ identified as clethroidoside B with *m/z* 987.5145 $[M-H]^-$ and molecular formula $C_{49}H_{80}O_{20}$; peak 3′identified as pseudoprostodioscin with *m/z* 1029.5261 $[M-H]^-$ and molecular formula $C_{51}H_{82}O_{21}$ and peak 4′ identified as spinasaponin A with *m/z* 793.4369 $[M-H]^-$ with molecular formula $C_{42}H_{66}O_{14}$.

Fig. 6. Change in the levels Aβ, sAPPβ-sw, sAPPα by extract of leaves of S.*brachypetala.*

Cells were incubated with indicated concentrations of extract of leaves of *S. brachypetala* for 8 h, the levels of Aβ, sAPPβ-sw, and sAPPα were measured from the conditioned media by using specific ELISA methods. (A-C) The levels of Aβ42 (A, n = 4), Aβ40 (B, n = 4), and sAPPβ-sw (C, n = 4) were decreased by the extract. (D) The level of sAPPα was increased by the extract (n = 4). *, *P*<0.05; **, *P*<0.01; ***, P<0.001.

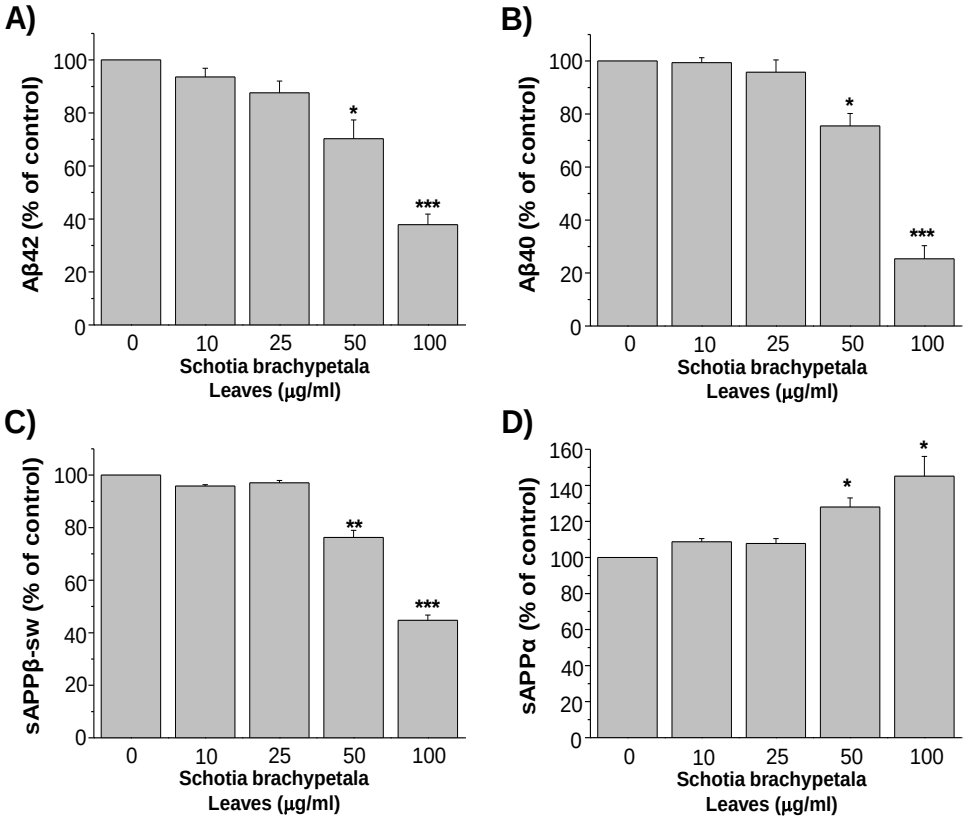
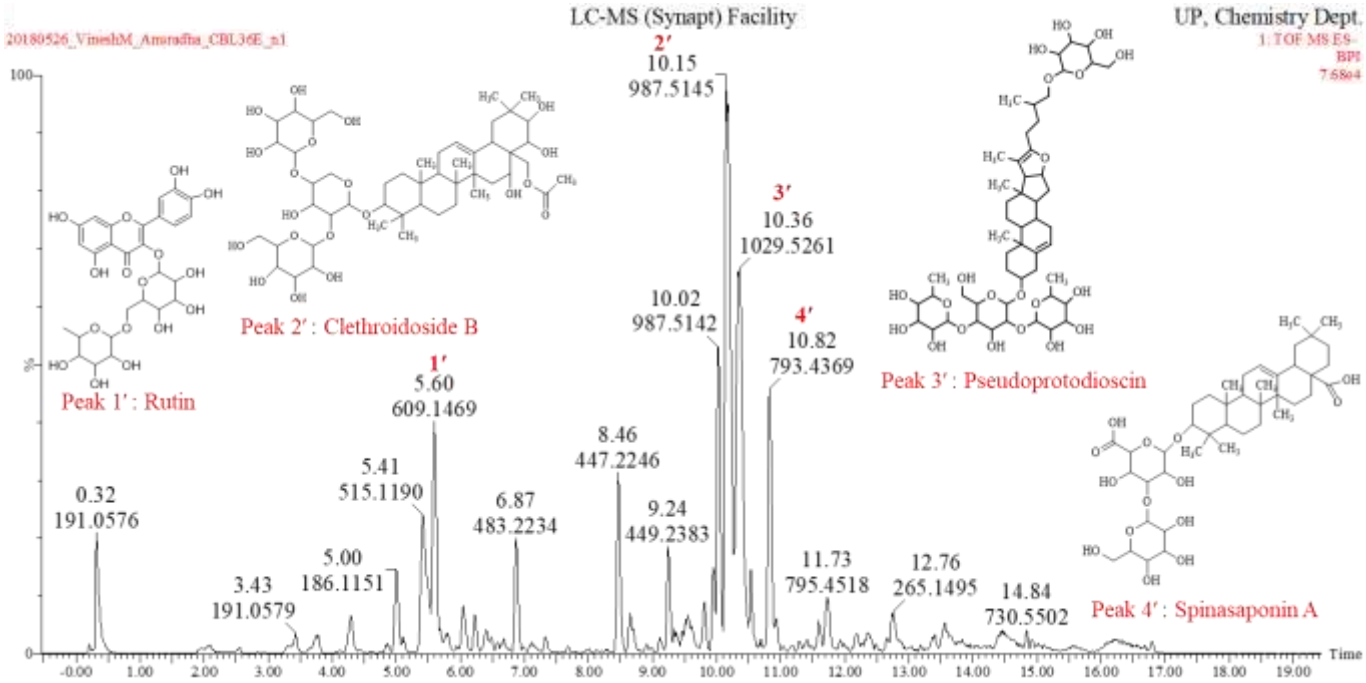


Fig. 7. ESI negative mode BPI chromatogram of *C. paniculate* leaf extract.

A total of four compounds were tentatively identified Rutin (peak 1′), clethroidoside B (peak 2′), pseudoprostodioscin (peak 3′), and spinasaponin A (peak 4′). The compound Rutin is confirmed by using pure standard on UPLC-QTOF-MS.



3.8. The effect of Schotia brachypetala on APP processing and cytotoxicity

In our study, we confirmed the dose-dependent effect of extract of leaves of *S. brachypetala* on Aβ42 production. Cells were incubated with 10, 25, 50, and 100 μg/ml extract for 8 h, and then the Aβ42 level from the conditioned media was measured by specific ELISA kit. The secreted level of Aβ42 was decreased by the extract of leaves of *S. brachypetala* in a dose-dependent manner (Fig. 6A). Next, we tested the levels of APP proteolytic products from the conditioned media using specific ELISA kits for Aβ40, sAPPβ-sw and sAPPα. The secreted level of Aβ40 was significantly decreased by the extract (Fig. 6B). In addition, this extract induced the decrease of sAPPβ-sw level (Fig. 6C) and the increase of sAPPα level (Fig. 6D). At 50 μg/ml extract, the sAPPβ-sw level was reduced by 23.7 ± 2.7%, while the sAPPα level was increased by 28 ± 5%. Extracts of leaves of *S. brachypetala* (Fig. 2C) did not influence cell viability but high concentration of 100 μg/ml induced cytotoxicity. These results suggest that the extract of leaves of *S. brachypetala* decreased Aβ42 production at non-toxic concentrations through decreasing amyloidogenic processing of APP and increasing the non-amyloidogenic processing of APP.

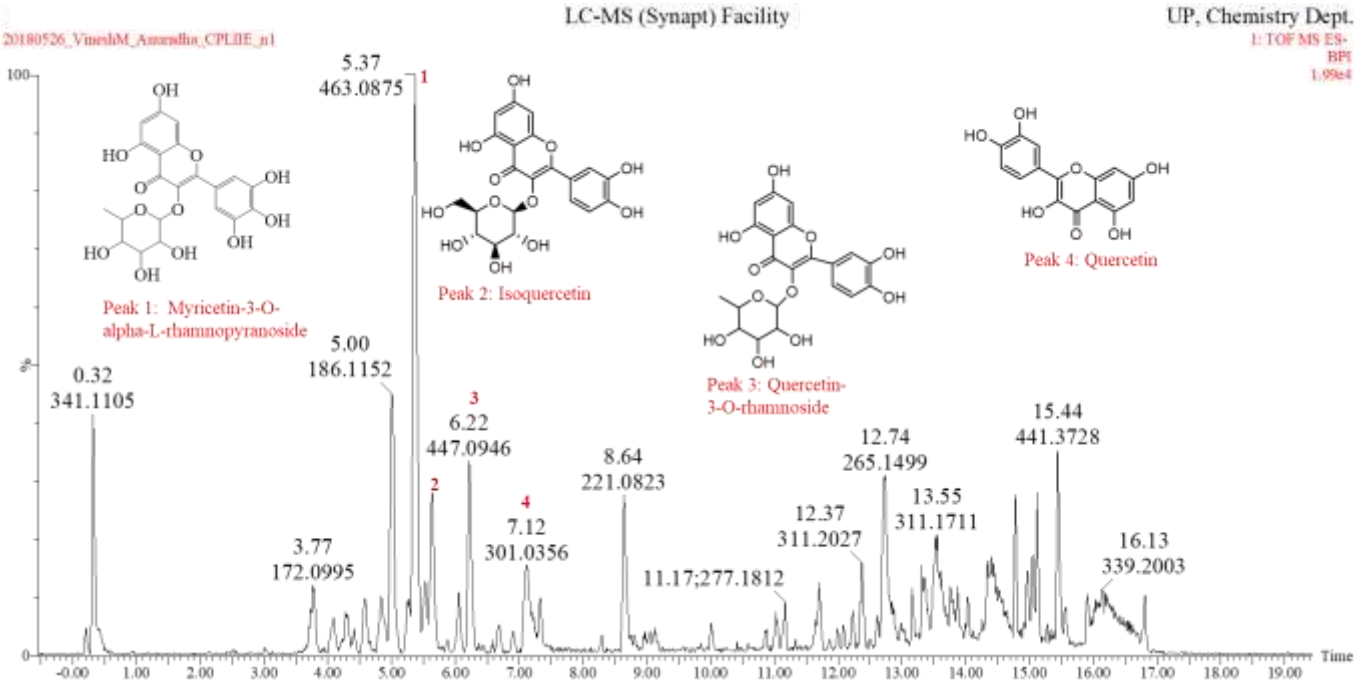
3.9. Chemical characterization of DCM:MeOH extract of Schotia brachypetala

Using ESI negative mode, a total of four compounds were tentatively identified belonging to the class of flavonoids: peak 1 identified as myricetin-3-O-alpha-L-rhamnopyranoside with *m/z* 463.0875 $[M-H]^-$; peak 2 identified as isoquercetin with *m/z* 463.0872 $[M-H]^-$; peak 3 identified as quercetin-3-o-rhamnoside with *m/z* 447.0946 $[M-H]^-$ and peak 4 identified as quercetin with *m/z* 301.0356 $[M-H]^-$ (Fig. 8) (Table S4). Two compounds, isoquercetin and quercetin were confirmed by using pure standards

(Supplementary Fig. S7 and Fig. S8) and the other two compounds myricetin-3-O-alpha-L-rhamnopyranoside (Saldanha et al., 2013) and quercetin-3-o-rhamnoside (Sánchez- Rabaneda et al., 2003) were tentatively identified by comparing the fragmentation patterns with that from literature. The flavonoid quercetin-3-O-rhamnoside was previously reported to be active for the reduction of Aβ42 from the previous findings (Hassaan et al., 2014).

Fig. 8. ESI negative mode BPI chromatogram of *S. brachypetala* leaf extract.

A total of four compounds were tentatively identified, two compounds myricetin-3-O-alpha-L-rhamnopyranoside (peak 1) and quercetin-3-O-rhamnoside (peak 3) were tentatively identified. Two compounds isoquercetin (peak 2) and quercetin (peak 4) were confirmed by using pure standards on UPLC-QTOF-MS.



4. Discussion

The leaf extracts of three plants, *X. undulatum*, *S. brachypetala* and *C. paniculata* have shown to significantly decrease the level of Aβ42. To our knowledge there are no prior reports of *X. undulatum* and *C. paniculata* as effective inhibitors against Aβ42, however previous studies have indicated *S. brachypetala* as a potential plant for the treatment of Alzheimer's disease through the reduction of Aβ42.

In this present study, *X. undulatum*, uzara one of the commercialized plants in Europe for the treatment of diarrhea (Helmstädter, 2015) showed potent efficacy towards the reduction of Aβ42. Since *X. undulatum* was showed excellent inhibition of Aβ42 production (ability to inhibit Aβ42 production by 75% more) with limited toxicity based on cell viability, we pursued with further studies to identify the active compounds. However in other studies it was shown that that high concentrations of water extracts of *X. undulatum* lead to cell toxicity (Calitz et al., 2018). This toxicity may be attributed to toxic compounds extracted selectively with the aqueous medium and are not present in the organic extracts used in our study. Other previous studies reported that *X. undulatum* was shown to possess a strong affinity towards serotonin uptake transport protein which plays an important role in the symptoms of

depression and Alzheimer's diseases (Nielsen et al., 2004). Another study has suggested that ethyl acetate root extracts of *X. undulatum* exhibited some acetylcholinesterase inhibitory activity by displaying an IC_{50} value of 0.0005 ± 0.000 mg/ml (Adewusi and Steenkamp, 2011). Traditionally this plant is used in Botswana for headache and abdominal pains (Hutchings and van Staden, 1994) while it has also been recorded in literature for the treatment of diarrhea, dysentery, dysmenorrhea, stomach cramps and indigestion (Van Wyk et al., 2009). Although the plant has been extensively researched and well-documented for its antidepressant effect, acetylcholinesterase inhibitory effects and SSRI activity, there is only limited literature available showing its inhibitory effects for the reduction of Aβ42.

The phytochemistry of *X. undulatum* has been well researched and found that the main class of compounds are the cardiac glycosides (Ghorbani et al., 1990). This class of compound includes the drugs digoxin, digitoxin and ouabain which are used as anti-arrhythmic agents and for the treatment of congestive heart failure (Newman et al., 2008) due to their ability to bind and inhibit the ubiquitous cell surface enzyme Na^+/K^+-APTase (Li et al., 2014). In the current study, two active compounds namely, crotoxigenin-3-*O*-*β*-digitalopyranosyl-(1-4)-*O*-*β*-*digitoxopyanoside* and desglucouzarin were isolated through bioassay guided fractionation and were for the first time reported for their inhibitory activities in the reduction of Aβ42. While the data is promising, it could be challenging to further develop these for the treatment of Alzheimer's disease due to their cardiac toxicity. Crotoxigenin-3-*O*-*β*-digitalopyranosyl-(1-4)-*O*-*β*-*digitoxopyanoside* was isolated and identified for the first time from *X. undulatum*. This compound was previously isolated from leaves of *Alafia* sp., a traditional medicinal antimalarial plant from South of Madagascar (Rakotondramanga et al., 2016). The compound desglucouzarin also isolated from *Asclepias* subulata was found to be one of the compounds responsible for the anti-proliferative activity of the plant with high selectivity towards human cancer cells (Rascón-Valenzuela et al., 2015).

Limited studies of *C. paniculata* were identified which demonstrates the anti-Alzheimer's properties of the plant. The stem bark of *C. paniculata* has demonstrated anti-inflammatory and analgesic activities. The DCM: MeOH leaf extract of *C. paniculata* was reported for their *in vitro* anticancer activity and showed 100% cell growth inhibition at a concentration of 6.25 µg/ml (renal TK10), 15.0 µg/ml (melanoma UACC62) and 55.68 µg/ml (breast MCF7) (Fouché et al., 2008). The chemical profile of the extract of *C. paniculata* using UPLC-QTOF identified rutin as one of the compounds present in the extract which was also confirmed with the use of a standard. Rutin, is one of the major flavonoid glycosides isolated from *Gingko biloba*, which is one of the most investigated plant remedies for Alzheimer's disease, however its efficacy for the treatment of Alzheimer's is still controversial (Canevelli et al., 2014). Previous studies also showed the efficacy of rutin to decrease the Aβ oligomer levels in the brain of AD transgenic mice and has indicated that the compound also exhibited neuro-protective abilities (Xu et al., 2014). One of the major class of compounds in *C. paniculata* is triterpene glycosides (Adedapo et al., 2008). Three triterpene glycosides clethroidoside B, pseudoprostodioscin, spinasaponin A were tentatively identified from the extract. Prior studies have found that saponins exhibit a potent neuroprotective activity including inhibition of tau phosphorylation (A. Sun et al., 2015). The present study support the previous findings of efficacy of rutin for the treatment of Alzheimer's disease and that the triterpene glycosides could be responsible for the reduction of Aβ42.

The DCM:MeOH extracts the roots of *S. brachypetala* has been previously shown to have good antioxidant and acetylcholinesterase inhibitory activities. The acetylcholinesterase inhibitory activity was determined by using Ellman's colorimetric method (Adewusi et al.,

2011). Findings that emanated from work done by (Gary Ivan Stafford et al., 2007) have shown that *S. brachypetala* also exhibited a good MAO-B inhibition activity which is also involved in the treatment of diseases such as Parkinson's disease and Alzheimer's disease. One of the research studies showed that the *S. brachypetala* (leaves and stalks) was found to significantly decrease the Aβ42 peptide by the *in vivo* analysis on the mean mouse Aβ42 concentration using ELISA assay (Hassaan et al., 2014). Four compounds, myricetin-3-O-alpha-L-rhamnopyranoside, isoquercetin, quercetin-3-O-rhamnoside and quercetin were tentatively identified through UPLC-QTOF analysis. This tentatative identification was further supported by comparison of fragmentation patterns from the literature (Sobeh et al., 2016). The flavonoid quercetin-3-O-rhamnoside was previously reported to be active for the reduction of Aβ42 from the previous findings (Hassaan et al., 2014). Our studies confirm the previous findings for the potential of the plant to treat Alzheimer's disease.

5. Conclusion

A total of 33 extracts from different plant parts of 20 plants were screened for the reduction of Aβ42 protein, the hallmark of Alzheimer's disease. Among them, three plant extracts, leaves of *X. undulatum*, leaves of *C. paniculata* and leaves of *S. brachypetala*, potently decreased the levels of Aβ42. The compounds identified from *C. paniculata* and *S. brachypetala* have previously been reported for the reduction of Aβ42 as a result we did not pursue the isolation of these plant extracts. While the phytochemistry of *X. undulatum* is well described, there is no report for the cardenolide glycosides for the reduction of Aβ42. In this study, using bioguided fractionation, we isolated two cardenolide glycosides, Crotoxigenin-3-*O-β*-digitalopyranosyl-(1-4)-*O-β-digitoxopyanoside* and desglucouzarin which showed for the first time strong activity towards the reduction of Aβ42. Further investigation is being conducted to examine the inhibitory effect of active compounds on tau phosphorylation. As the plants are the main source of drugs for the treatment of many diseases, thus our study can also lead to the development of some novel anti-Alzheimer's agents. The research provides some evidence which substantiates the traditional uses of plants.

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Author's contributions

AT collected and extracted different parts (leaves, stems and roots) of 20 plants, developed the chemical profile of the active extracts, carried out bioguided fractionation for the isolation and purification of compounds, did structure elucidation of the active compounds using NMR. YSC carried out the Aβ42 bioassay screening. VJM, HOY and NO supervised this study. All authors read, corrected and approved the final manuscript.

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