INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

ISOLATION OF STIGMASTEROL FROM 80% AQUEOUS ETHANOL ROOT EXTRACT OF *BRIDELIA DUVIGNEAUDII* J.LEON AND ITS HYPOGLYCAEMIC ACTIVITY ON ORAL GLUCOSE LOADED WHITE ALBINO MICE

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ABSTRACT

Aim: In this study, 80% aqueous ethanol root extract of *Bridelia duvigneaudii* J.Leon was subjected to isolation of phytochemicals. The study led to the isolation of stigmasterol[10] in pure state. The hypoglycaemic activity of stigmasterol[10] was determined. **Methods:** Isolation of phytochemicals from the 80% aqueous ethanol root extract of *Bridelia duvigneaudii* J.Leon was done using column chromatography packed with silica gel and eluted by solvents ranging from non-polar, medium polar and polar. The isolated pure compound (stigmasterol) was identified based on Nuclear Magnetic Resonance data (^{1}H NMR, ^{13}C NMR, COSY, HSQC and HMBC) and by comparing the spectral characteristics reported in the literature. Evaluation for hypoglycaemic activity of stigmasterol was conducted in normal albino mice by using Oral Glucose Tolerance Test (OGTT). The statistical analysis of results was carried out using Student *t*-test followed by one way Analysis of variance (ANOVA) and Tukey's multiple comparisons at probability value ($p < 0.05$). **Results:** Column chromatographic separation led to isolation of ubiquitous steroid compound, namely, stigmasterol[10]. At doses of 50, 100 and 200 mg/kg, stigmasterol exhibited hypoglycaemic activity by lowering blood glucose in treated white albino mice statistically different from that of untreated group at 0.5, 1, 2, 3 and 4 hours after administration, $p < 0.05$. **Conclusions:** The present work revealed that, stigmasterol has good hypoglycaemic activity, thus, should be considered as a potential lead compound for developing hypoglycaemic agent (s) in future. However, further research is still needed to investigate the compound especially its various biological effects in this species.

Keywords: *Bridelia duvigneaudii*, Hypoglycaemic activity and Oral Glucose Tolerance Test.

1.0 INTRODUCTION

Diabetes Mellitus also known as diabetes is a chronic disease which is stated by an increase in blood glucose level in the body (hyperglycemia) for a prolonged period due to relative or absolute the pancreas not producing insulin or the cells of the body not responding properly to the insulin produced[1–3]. Long-term complications of diabetes which are the major causes of morbidity and death in diabetic patients include kidney failure, heart disease, stroke, foot ulcers, and damage to the eye [2,4].

Due to severe dangerous and life threatening complications, diabetes is currently a major health problem causing reduced life quality and death[2]. It is reported that, in 2015, over 400 people in the world were living with diabetes and this number is projected to increase to over 600 million by the year 2040[5].

Modern medicines currently used for diabetes management which include insulin injections and oral hypoglycemic agents have not been able to cure the disease at all[4]. Additionally, the available modern medicines cause adverse side effects and complications to the patients after prolonged use [6]. For example; insulin allergy, altered metabolic control, insulin antibodies, placental transfer of insulin antibodies and autoimmunity are the side effects of insulin after prolonged use[6] whereas, hypoglycaemia, weight gain, oedema and mild anemia are side effects of oral hypoglycaemic agents after prolonged use[7,8]. Therefore, diabetes is still a serious healthcare problem worldwide that needs solution.

Hence, an alternative therapy for diabetes is needed, the trend is now on the use of medicinal plants[4,6.]

Basing on traditional medicine information, diabetes has been treated with herbs and medicinal plants for a long time[9]. However, limited studies have attempted to assess the outcome from the patients who have used these herbal remedies. More than 400 medicinal plants traditionally claimed as antidiabetics have been recorded worldwide. However, few of them have undergone scientific and medical evaluation to assess their efficacy and safety[10]. Thus, World Health Organization (WHO) have recommended for assessment of the traditionally claimed antidiabetic medicinal plants in order to reveal their efficacy and safety in order to be standardized for wider use[9].

1.1 Some of the reported plant crude extracts with hypoglycemic activity

Several plant crude extracts have shown antidiabetic activity when scientifically assessed using presently available experimental techniques[10,11]. Some of the antidiabetic potential plant crude extracts are given in Table 1.

1.2 Some of the reported plants and isolated pure compounds with antidiabetic and hypoglycemic activity

Medicinal plants have been reported to show hypoglycemic and antidiabetic potential due to the presence of useful active phytochemical compounds which include terpenoids, alkaloids, phenolics, flavonoids, saponins, and cardiac glycosides[17].

Some of the isolated compounds with antidiabetic and hypoglycemic activity are given below: Four lanostene-type triterpenoids compounds psidiumlanostenoic acid[1], 12β-Hydroxypsidiumlanostenoic acid[2], Trihydroxypsidiumlanostenoic acid[3] and Psidiumlanostenoic acid glucoside[4] isolated from the leaves of *Psidium guajava* L. (Myrtaceae) exhibited significant antidiabetic activity against streptozotocin-induced diabetic rats[17].

Likewise, an alkaloid berberine chloride**[5]** isolated from methanolic fruit extract of *Helicteres isora* L. (Malvaceae) indicated antidiabetic activity against streptozotocin-induced diabetic rats[18]. Additionally, three compounds, which include loganin**[6]**, morroniside[7] and ursolic acid[8], isolated from dried fruits of *Cornus officinalis* Sieb. et Zucc (Cornaceae) showed hypoglycemic potential in streptozotocin-induced diabetic mice[19]. Similarly, total saponins, lupeol**[9]** and stigmasterol**[10]** isolated from *Strobilanthes cuspidata (*Benth*)* T. Anderson (Acanthaceae) showed antidiabetic potential in alloxan monohydrate induced diabetic rats[20]. Furthermore, the flavanoid called Flavanone-7-*O*-glycoside**[11]** isolated from alcoholic extract of the leaves of *Sauropus androgynus* (L.) Merr. (Phyllanthaceae) showed antidiabetic potential in alloxan monohydrate induced diabetic rats[21].

Basing on ethnobotanical study, *Bridelia duvigneaudii* J.Leon is used traditionally for diabetes management in Urambo district, Tabora-Tanzania[22].

Basing on our previous work, on scientific evaluation of efficacy for antidiabetic potential of 80% aqueous ethanol root extract of *B. duvigneaudii* J.Leon in oral glucose loaded white albino mice, the extract exhibited antidiabetic activity by lowering blood glucose on glucose loaded normal albino mice statistically different from that of untreated group ($p < 0.05$)[23].

Therefore, this study focused on the isolation and identification of phytochemical compound

(s) and determination of their hypoglycaemic activity.

2.0 MATERIALS AND METHODS

2.1 Plant material

The roots of *B. duvigneaudii* J.Leon were collected in Urambo district (5° 4' 39.644" S 32° 4' 10.348" E), Tabora region of Tanzania in February 2017. Authentication of plant species was done by comparison with voucher specimens present in the Herbarium of the University of Dar es Salaam in collaboration with specialist Mr. Haji O. Seleman from the Department of Botany at the University of Dar es Salaam. Voucher herbarium specimen (leaves) was preserved in the Herbarium of the University of Dar es Salaam with voucher number 5006.

2.2 DRUGS AND CHEMICALS

The drug used for this study includes the standard antidiabetic drug (chlorpropamide) (COSMOS Pharmaceutical Limited, Nairobi-Kenya). The chemicals used for this study include organic solvents which are dichloromethane, ethanol, methanol and petroleum ether (CARLO ERBA Reagents SAS, Chaussee du Vexin 27100 Val de Reuil-France) and silica gel 60 (70-230 mesh, 60 angstrom pore size) from Merck KGaA group, Darmstadt, Germany.

2.3 Test animals

Mature male and female white albino mice (20 - 25 gm) were obtained from the Animal house of ITM, MUHAS. They were kept in aluminium cages and fed on commercial broiler finisher pellets. The animals were accustomed to laboratory conditions for 5 days before experiments. Feed and drinking water were provided *ad libitum* during the whole period of the study except during fasting.

2.4 Preparation and extraction of plant material

Plant sample was air-dried and milled into coarse powder by using a milling machine type Y (Hangyu®, China) available at ITM, MUHAS. About 500 gm of ground plant material was extracted with 80% aqueous ethanol by percolation method at 25-33^{o}C and after 24 hours filtered through whatman number 1 filter paper. The procedure was repeated twice to ensure comprehensive extraction of the plant material in which extracts were then pooled together. The filtrate was evaporated under vacuum using rotary evaporator (Büchi Labortechnik, Flawil, Switzerland) at 40°C. The extract was freeze dried at -80^{0}C using the Edwards freeze drier (Edwards High Vacuum International Crawley, Sussex, England) to yield 76 gm.

2.5 Isolation of phytochemical compounds

The 80% aqueous ethanol *B. duvigneaudii* roots extract was subjected to isolation under column chromatography (CC) packed with silica gel (stationary phase) and eluted by solvent systems (mobile phases) of varying polarities starting with low polar solvent, petroleum ether (PE) followed by medium polar solvent, dichloromethane (DCM) and finally to high polar solvent, methanol (MeOH). The crude extract initially analyzed in thin layer chromatography (TLC) to establish the solvent system for use in the isolation using column chromatography.

The crude extract of *B. duvigneaudii* root (35 g) was packed in column (5 cm x 60 cm) over silica gel (300 gm) then, eluted and fractions each of 10 ml were collected.

By using TLC to monitor separations, the column was eluted by 100% PE to give 9 fractions (f_{1-9}) followed by increasing polarity to 75%:25% PE-DCM to afford 13 fractions (f_{10-22}). Polarity of the solvent system further increased to 50%:50% PE-DCM to yield 14 fractions (f_{23-36}) followed by increasing polarity to 75%:25% DCM-PE to give 18 fractions (f_{37-54}). Furthermore, the polarity of the solvent system increased to 100% DCM giving 8 fractions (f_{55-62}) followed by increasing polarity to 5%:95% MeOH-DCM yielding 15 fractions (f_{63-77}). The polarity of the solvent system increased to 10%:90% MeOH-DCM affording 20 fractions (f_{78-97}) finally, the polarity of the solvent system increased to 20%:80% MeOH-DCM giving 15 fractions (f_{98-112}).

According to TLC profiles, the fractions pooled together according to the similarity in their chemical profile as follows: Fractions f_{1-9}, f_{10-22}, f_{23-36}, f_{37-44}, f_{45-54}, f_{55-62}, f_{63-77}, f_{78-97} and f_{98-112} as shown in **figure 11.**

The following fractions were few in amount, first (0.52 mg), second (0.47) third (0.32 mg), fifth (0.73), sixth (0.21 mg), seventh (0.38 mg), eighth (0.25 mg) and ninth (0.67 mg) hence, not subjected to further purification.

The fourth fraction indicated a single spot on TLC, so it was purified by precipitation in 100% methanol; the powder was filtered out and washed with 100% methanol to give 16.2 mg of pure compound labeled[10]. Collected compound was white powder soluble in dichloromethane.

Therefore, pure compound labeled[10] was subjected to NMR analysis. Some of its amount (0.473 g) was used in hypoglycaemic activity test in white albino mice.

2.6 Spectroscopic analysis of isolated pure compound[10]

The column chromatographic separation of the 80% aqueous ethanol *Bridelia duvigineaudii* roots extract led to the isolation of steroid, namely, stigmasterol[10].

Spectroscopic experiment for of isolated pure compound was conducted at the Institute of Chemistry, University of Potsdam in German.

NMR data used to elucidate the structure of isolated compound**[10]** (^{1}H NMR, ^{13}C NMR, COSY, HSQC and HMBC) were recorded on a Bruker Avance DRX-500 (500 HZ) NMR spectrometer. Sample was dissolved in dichloromethane (CD_2Cl_2) and chemical shifts were expressed relative to the solvent (CD_2Cl_2; 5.32 ppm).

2.7 Determination of hypoglycaemic activity of isolated stigmasterol[10]

Hypoglycaemic activity of stigmasterol[10] was evaluated at a dose of 50, 100 and 200 mg/kgb via *in vivo* test (using albino mice) in oral glucose loading animal model by OGTT[23–25].

2.7.1 Oral glucose administration

White albino mice acclimatized for 5 days were fasted for 18 hours before the beginning of the experiment and were then orally loaded by gavage with freshly prepared glucose (1 gm/kg) 30 minutes after solvent/stigmasterol/chlorpropamide administration[23].

2.7.2 Experimental design

Body weight to the nearest gram and fasting blood glucose levels of the animals were determined at the beginning of the experiment. Five groups of white albino mice, six albino mice (n = 6) in each received the following treatment schedule:

Group I: Negative control (distilled water, 5 ml/kg orally)
Group II: Positive control (chlorpropamide, 100 mg/kg orally)
Group III: Stigmasterol**[10]** (50 mg/kg orally)
Group IV: Stigmasterol**[10]** (100 mg/kg orally)
Group V: Stigmasterol**[10]** (200 mg/kg orally)

2.7.3 Blood glucose determination

Blood glucose level in blood collected from each mouse by partial tail amputation procedure from the tail vein was measured after glucose loading at 0.5, 1, 2, 3 and 4 hours by commercially available glucose kit based on a glucose oxidase enzymatic assay and determined by a glucose meter known as ACCU-CHEK® Active (Roche Diabetes care GmbH, Mannheim –Germany) [23,26,27].

2.8 Data and statistical analysis

2.8.1 NMR data

NMR data used to elucidate the structure of isolated compound[10] (^{1}H NMR, ^{13}C NMR, COSY, HSQC and HMBC) NMR data were processed using Top Spin software.

2.8.2 Hypoglycaemic activity

The results of blood glucose levels were expressed as mean ± Standard Error of the Mean (SEM) with sample size (n = 6). The statistical analysis of results was carried out using Student *t*-test followed by one way Analysis of variance (ANOVA) and Tukey's multiple comparisons probability value ($p < 0.05$).

2.9Ethical approval

During the study, the following issues were taken into consideration: Few mice were kept in each cage to enable mice to express their normal behavior. Clean water and appropriate feed was given and mice were kept at the temperature of 22^{o}C (±3^{o}C) and the relative humidity was 55 %. The Director of Research and Publications of MUHAS gave an ethical clearance of the protocol for this study.

3.0 RESULTS AND DISCUSSIONS

3.1 RESULTS

3.1.1 Isolation of pure compound labelled[10]

The column chromatographic separation of the constituents of the 80% aqueous ethanol root extract of *B. duvigneaudii* J.Leon led to the isolation of steroid, namely, stigmasterol**[10]**.

3.1.2 Structure elucidation of isolated compound labelled**[10]**

The structure of the isolated compound**[10]** was identified on basis of NMR data (^{1}H NMR and ^{13}C NMR, COSY, HSQC and HMBC) and by comparing their spectral characteristics reported in the literature as shown in figure**[10]**.

The ^{1}H and ^{13}C NMR values for all the protons and carbons were assigned based on COSY, HSQC and HMBC correlations as shown in Table 2 and Table 3.

The ^{13}C NMR spectra of stigmasterol**[10]** indicated 29 carbon signals at chemical shifts, δ_C in ppm as follows:

37.7 (C-1), 32.1 (C-2), 72.0 (C-3), 42.7 (C-4), 141.3 (C-5), 121.8 (C-6), 32.3 (C-7), 32.3 (C-8), 50.6 (C-9), 36.9 (C-10), 21.5 (C-11), 40.1 (C-12), 42.6 (C-13), 57.3 (C-14), 24.7 (C-15), 29.4 (C-16), 56.4 (C-17), 12.2 (C-18), 19.6 (C-19), 138.8 (C-20), 129.6 (C-21), 40.9 (C-22), 21.4 (C-23), 51.7 (C-24), 32.3 (C-25), 19.2 (C-26), 21.3 (C-27), 25.8 (C-28) and 12.5 (C-29).

The ^{1}H NMR spectra of stigmasterol**[10]** exhibited the presence of two methyl singlets at δ 0.68 (3H, *s*, CH_3-29) and 1.00 (3H, *s*,

CH_3-28); three methyl doublets that appeared at δ 0.83 (3H, *d*, *J* = 6.4 Hz, CH_3-26), 0.85 (3H, *d*, *J* = 6.4 Hz, CH_3-27) and 0.92 (3H, *d*, *J* = 6.5 Hz CH_3-19); and a methyl triplet at δ 0.80 (3H, *t*, *J* = 7.2 Hz, CH_3-24).

It also indicated protons at δ 5.03 (1H, *dd*, *J* = 15.1 & 8.6 Hz, CH-20), 5.17 (1H, *dd*, *J* = 15.1 & 8.6 Hz CH-21) and 5.34 (1H, *m*, CH-6) implying the presence of three protons corresponding to that of two olefinic bond (three olefinic proton instead of one).

Moreover, the proton corresponding to the C-3 of a steroid moiety appeared as a triplet of doublet of doublets at δ 3.46 (1H, *m*, CH-3).

The above spectral data supported the presence of steroid skeleton having a hydroxyl group at C-3 position with two double bonds at C-5/C-6 and C-20/C-21 and with six methyl groups and these spectral data are consistent to the reported literature values[28,29].

Therefore, basing on the spectra data (^{1}H NMR and ^{13}C NMR) described above and comparing with the literature cited as shown in **Table 2** and **Table 3**, the structure of compound labeled[10] was deduced as the known compound stigmasterol.

3.1.3 Hypoglycaemic activity of stigmasterol[10]

The isolated pure compound, stigmasterol[10] was evaluated for its hypoglycaemic activity at doses of 50, 100 and 200 mg/kg.

At doses of 50, 100 and 200 mg/kg, the compound exhibited hypoglycaemic activity by lowering blood glucose of mice statistically different from that of untreated group, $p < 0.05$ at 0.5, 1, 2, 3 and 4 hours after administration as shown in Table 4.

3.2 DISCUSSIONS

The roots of *Bridelia duvigneaudii* J.Leon is traditionally claimed to have anti-diabetic activity[22]. The previous scientific evaluation of our group on the efficacy for antidiabetic potential of 80% aqueous ethanol root extract of *B. duvigneaudii* J.Leon in oral glucose loaded white albino mice supports the therapeutic claims of the traditional healers[23].

Previous studies reported that, antidiabetic activity of medicinal plants is due to the presence of phytochemical compounds which are responsible for activity such as terpenoids, alkaloids, phenolics, flavonoids, saponins, and glycosides[17,23.] Previous preliminary phytochemical analysis of our group on 80% aqueous ethanol root extract of *B. duvigneaudii* J.Leon revealed the presence of terpenoids, phenolics, saponins and cardiac glycosides[23].

The column chromatographic separation of the constituents of the 80% aqueous ethanol root extract of *B. duvigneaudii* J.Leon led to the isolation of steroid, namely, stigmasterol[10].

Its structure was identified on basis of NMR data (^{1}H NMR and ^{13}C NMR, COSY, HSQC and HMBC) and by comparing their spectral characteristics reported in the literature.

When its hypoglycaemic activity was evaluated at doses of 50, 100 and 200 mg/kg, stigmastero[l10] exhibited hypoglycaemic activity by lowering blood glucose in glucose loaded normal white albino mice statistically different from that of untreated group, $p < 0.05$ at 0.5, 1, 2, 3 and 4 hours after administration.

Chlorprpamide is a synthetic anti-diabetic drug which belongs to the group of sulphonylureas anti-diabetic drugs which cause hypoglycemia by stimulating insulin secretion from the pancreas[23].

The present study reports for the first time the isolation of stigmasterol and its hypoglycaemic potential from *B. duvigneaudii* J.Leon.

Stigmasterol is a steroid compound occurring in many plants. The compound is reported to have a number of biological activities which includes antihypercholesterolemic, antiperoxidative, thyroid inhibition, anti-inflammatory and hypoglycemic properties [21,30,31].

4.0 CONCLUSIONS

Column chromatographic separation of the crude extract of *B. duvigneaudii* roots led to isolation of ubiquitous steroid, namely, stigmasterol[10].

At doses of 50, 100 and 200 mg/kg, stigmasterol exhibited hypoglycaemic activity by lowering blood glucose in treated white albino mice statistically different from that of untreated group at 0.5, 1, 2, 3 and 4 hours after administration, $p < 0.05$.

The present work revealed that, stigmasterol has good hypoglycaemic activity and therefore, should be considered as a potential lead compound for developing hypoglycaemic agent (s) in future.

5.0 ACKNOWLEDGEMENTS

The authors acknowledge the German Academic Exchange Service (DAAD) for sponsoring the study. They also wish to thank Mr. Haji O. Suleiman of the Botany Department, University of Dar es Salaam, Tanzania for collection and authentication of the plant material.

Table 1: Some plant crude extracts having antidiabetic activity

Plant species (family)	Part used	Type of extract	Reference
Achyranthes aspera L. (Amaranthaceae)	Whole plant	Aqueous and methanolic	[12]
Allium cepa L. (Liliaceae)	Fresh bulbs	Ether	[10]
Allium sativum L. (Liliaceae)	Fresh bulbs	Ethanolic	[13]
Aloe vera (L) Burm.(Asphodelaceae)	Leaf pulp	Aqueous	[14]
Azadirachta indica A. Juss. (Meliaceae)	Root bark	Alcoholic	[15]
Caesalpinia bonducella (L) Roxb. (Caesalpinaceae)	Root bark	Methanolic	[10]
Dioscorea dumetorum (Kunth) Pax. (Dioscoreaceae)	Tubers	Methanolic	[10]
Mangifera indica L.(Anacardiaceae)	Leaf	Aqueous	[10]
Psidium guajava L. (Myrtaceae)	Fruits	Methanolic	[16]
Tamarindus indica L. (Caesalpinaceae)	Seed	Aqueous	[10]

Table 2: ^{13}C NMR chemical shifts (CD_2Cl_2, δ_C in ppm) for stigmasterol [10]

Position	Observed δ	Reported δ*	Position	Observed δ	Reported δ*
1	37.7	37.6	16	29.4	29.3
2	32.1	32.1	17	56.4	56.2
3	72.0	72.1	18	12.2	12.1
4	42.7	42.4	19	19.6	19.4
5	141.3	141.1	20	138.8	138.7
6	121.8	121.8	21	129.6	129.6
7	32.3	31.8	22	40.9	40.5
8	32.3	31.8	23	21.4	21.2
9	50.6	50.2	24	51.7	51.2
10	36.9	36.6	25	32.3	31.9
11	21.5	21.5	26	19.2	19.0
12	40.1	39.9	27	21.3	21.2
13	42.6	42.4	28	25.8	25.4
14	57.3	56.8	29	12.5	12.2
15	24.7	24.4			

Key: *: [28,29]

Table 3: ^{1}H NMR chemical shifts (CD_2Cl_2, δ_H in ppm) for stigmasterol [10]

Position	Splitting pattern, number of hydrogen	Observed δ	Reported δ*
C-3	*m,* 1H	3.46	3.53
C-6	*m*, 1H	5.34	5.38
C-19	*d*, *J* = 6.5 Hz, 3H	0.92	0.91
C-20	*dd*, *J* = 15.1 & 8.6 Hz, 1H	5.03	5.00
C-21	*dd*, *J* = 15.1 & 8.6 Hz, 1H	5.17	5.25
C-24	*t*, *J* = 7.2 Hz, 3H	0.80	0.81
C-26	*d*, J = 6.4 Hz, 3H	0.83	0.83
C-27	*d*, *J* = 6.4 Hz, 3H	0.85	0.85
C-28	*s*, 3H	1.00	1.00
C-29	*s*, 3H	0.68	0.68

Key: *: [28,29]

Table 4: Mean blood glucose level of mice (mmol/L) recorded at 0, 0.5, 1, 2, 3 and 4 hours after administration with various doses of stigmasterol [10]

T (h)	Mean blood glucose level in mice (mmol/L) (p – value)				
	Distilled water (5 ml/kg)	Chlorpropamide (100 mg/kg)	stigmasterol [10] (200 mg/kg)	stigmasterol [10] (100 mg/kg)	stigmasterol [10] (50 mg/kg)
0	3.83±0.23	3.83±0.28 (0.24)	3.8±0.31 (0.24)	4.0±0.38 (0.28)	3.8±0.17 (0.66)
0.5	6.53±0.38	5.79±0.19 (0.007)	5.24±0.58 (0.002)	4.95±0.26 (0.008)	4.48±0.3 (0.001)
1	5.18±0.29	3.23±0.14 (0.002)	3.02±0.19 (0.002)	2.98±0.35 (0.002)	3.22±0.44 (0.005)
2	4.34±0.26	2.69±0.25 (0.01)	2.43±0.19 (0.02)	2.52±0.21 (0.01)	2.58±0.24 (0.03)
3	3.92±0.26	2.38±0.29 (0.02)	2.08±0.17 (0.02)	2.1±0.12 (0.02)	2.37±0.22 (0.05)
4	3.48±0.14	1.78±0.17 (0.005)	1.97±0.12 (0.005)	2.0±0.10 (0.005)	2.08±0.19 (0.04)

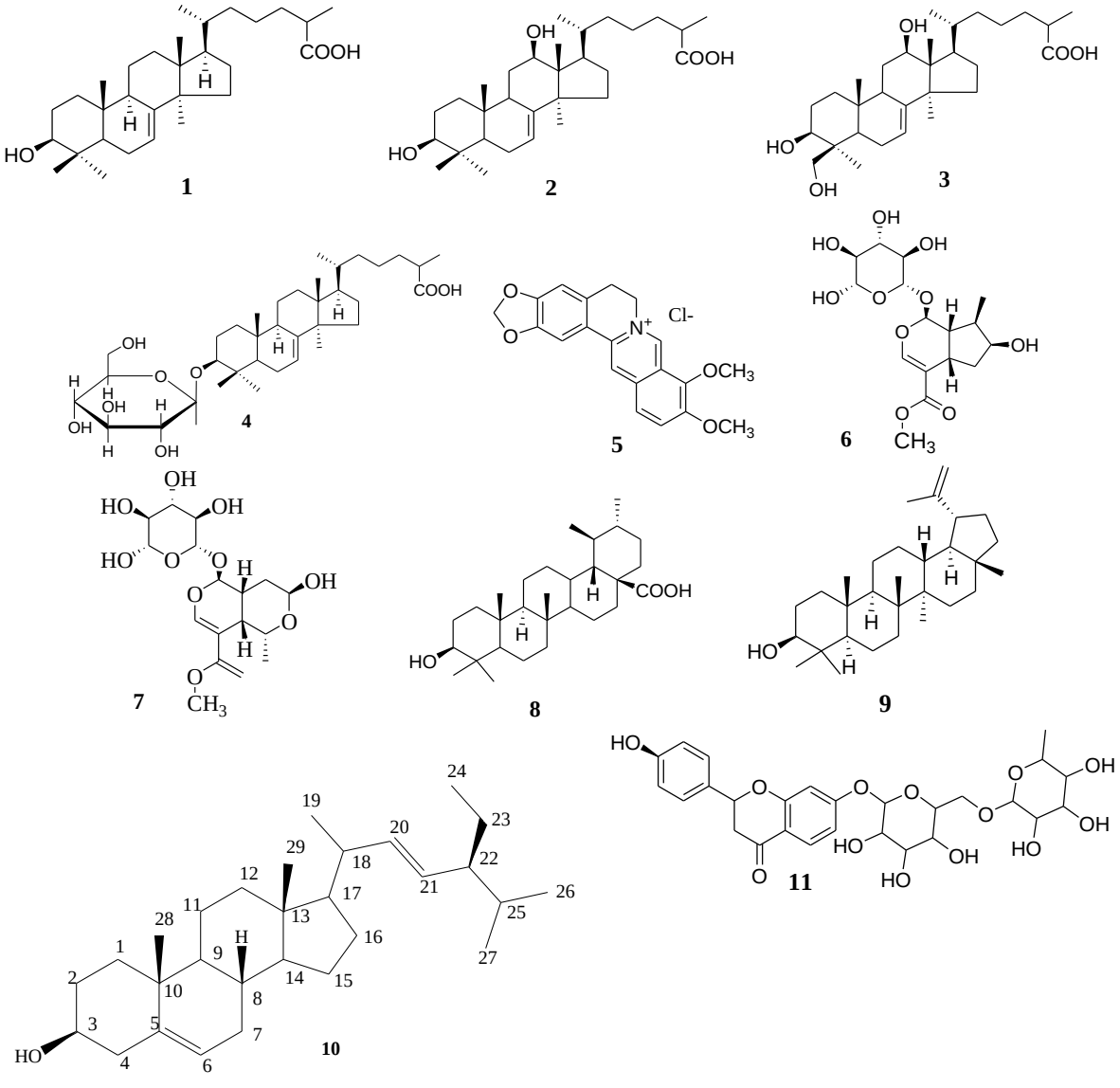

Fig. 1-10: Structures of the pure compounds [1]-[10]

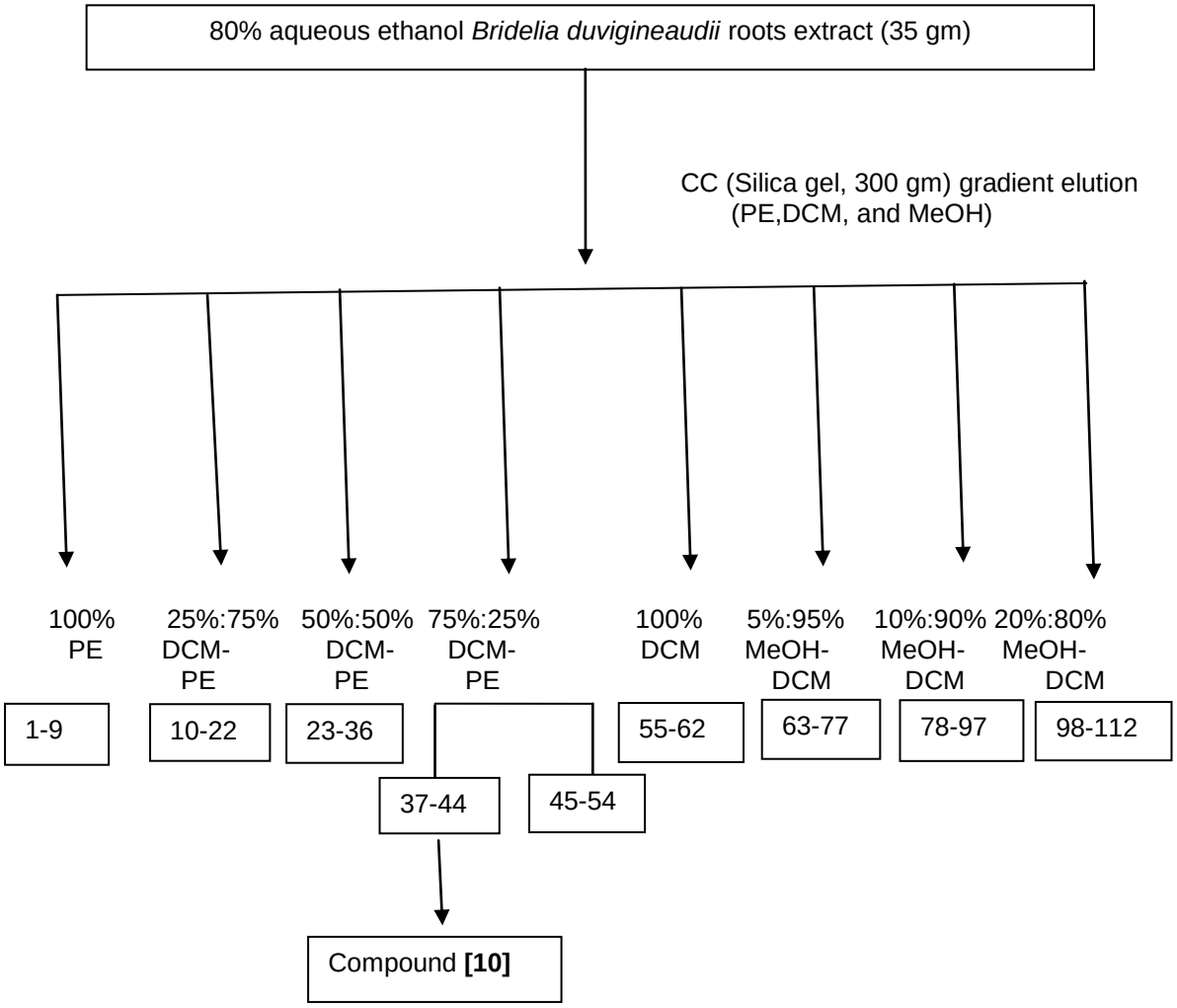

Fig. 11: Scheme of isolation of compounds from 80% aqueous ethanol *B. duvigneaudii* roots extract

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