Isolation and identification of the metabolites of Sudanese medicinal plants and synthesis of flavonoid glycosides

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博　士　論　文

Isolation and identification of the metabolites of Sudanese medicinal plants and synthesis of flavonoid glycosides

（スーダンの薬用植物の代謝物の単離と同定およびフラボノイド配糖体の合成）

令　和　4　年　1　月

神戸大学大学院農学研究科

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ハナ ハッサブ エルラソウル アブデルカリーム モハメド

Contents

Abbreviations

MeOH	Methanol
EtOH	Ethanol
BuOH	Butanol
AcOH	Acetic acid
Ac_2O	Acetic anhydride
EtOAc	Ethyl acetate
Tol	Toluene
Aq.	Aqueous
sat.	saturated
Anh.	Anhydrous
Cat.	catalytic
Temp.	Temperature
rt	Room temperature
°C	Degree Celsius
h	hour
min	minute
s	second
o.n.	overnight
CC	column chromatography
PLC	preparative thin layer chromatography
TLC	Thin Layer Chromatography
IR	Infra-red
UV	Ultra violet
d	Deuterated
GC	Gas chromatography
HPLC	High performance liquid chromatography
DART−MS	Direct analysis in real time mass spectrometry
LC−ESI−MS	Liquid chromatography−electrospray ionization−mass spectroscopy

^{1}H NMR	Proton Nuclear magnetic resonance
^{13}C NMR	Carbon 13 Nuclear magnetic resonance
^{1}H-^{1}H COSY	hydrogen-hydrogen Correlation Spectroscopy
DEPT	Distortion less Enhancement by Polarization Transfer
HMQC	Heteronuclear Multiple Quantum Coherence
HMBC	Heteronuclear Multiple-bond Correlation spectroscopy
δ	Chemical shift
ppm	Part per million
p-TsOH	Para Toluene sulfonic acid
All	Allyl group
DMDO	Dimethyl dioxirane
NaOMe	Sodium methoxide
BnBr	Benzyl bromide
BzCl	Benzoyl chloride
Me_2SO_4	Dimethyl sulfate
DMSO	Dimethyl sulfoxide
DMF	Dimethylformamide
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
4 Å MS	Molecular sieves
*m*CPBA	*m*-chloroperoxybenzoic acid
AZMBCl	2-Azidomethylbenzoyl chloride
DMAP	4-Dimethylaminopyridine
DBU	1,8-Diazabicyclo [5.4.0] undec-7-ene
TCA	trichloroacetamide
Bu_3P	Tributylphosphine
NaOAc	Sodium acetate
THF	Tetrahydrofuran
TMSI	Trimethylsilyl iodide

TBAB	Tetrabutylammonium bromide
PhSSPh	Diphenyl disulfide
eq.	equivalent
No.	Number
Filt.	Filtration
Conc.	concentration

Chapter 1

General introduction

1.1 General introduction

1.1.1 Isolation and identification of metabolites of the medicinal plants

World Health Organization (WHO) defined the medicinal plants as the plants that have therapeutic properties and are used for the treatment of the diseases. Human being has relied on the medicinal plants as traditional medicines to treat many diseases, particularly infectious diseases since ancient times [1]. The herbal plants are a rich source of the important secondary metabolites that have several biological and pharmaceutical activities such as anti−oxidant, anti−microbial and anti−diabetic activities [2]. The secondary metabolites, whether in a pure form or not, might allow the discovery of new drugs due to their chemical diversity [3]. Tedious processes are always required for the preparation of the plant metabolites. Collection and authentication of the medicinal plants are the first step, then grinding the selected parts follows after drying. The extraction and fractionation are operated to purify and isolate the bioactive compounds. Finally, the isolated metabolites are subjected to qualitative and quantitative analyses to identify their structure and to determine their quantity in the plant [4]. Selection of the suitable solvent is important for the extraction of the desired compounds. Properties of the solvent might affect selective extraction of active compounds from plants. The solvents should not react with the extracted compounds and not interfere the bioassay, and must enable removal easily by evaporation at low temperature. In addition, it is ideal that the solvents are low toxic and cost [4,

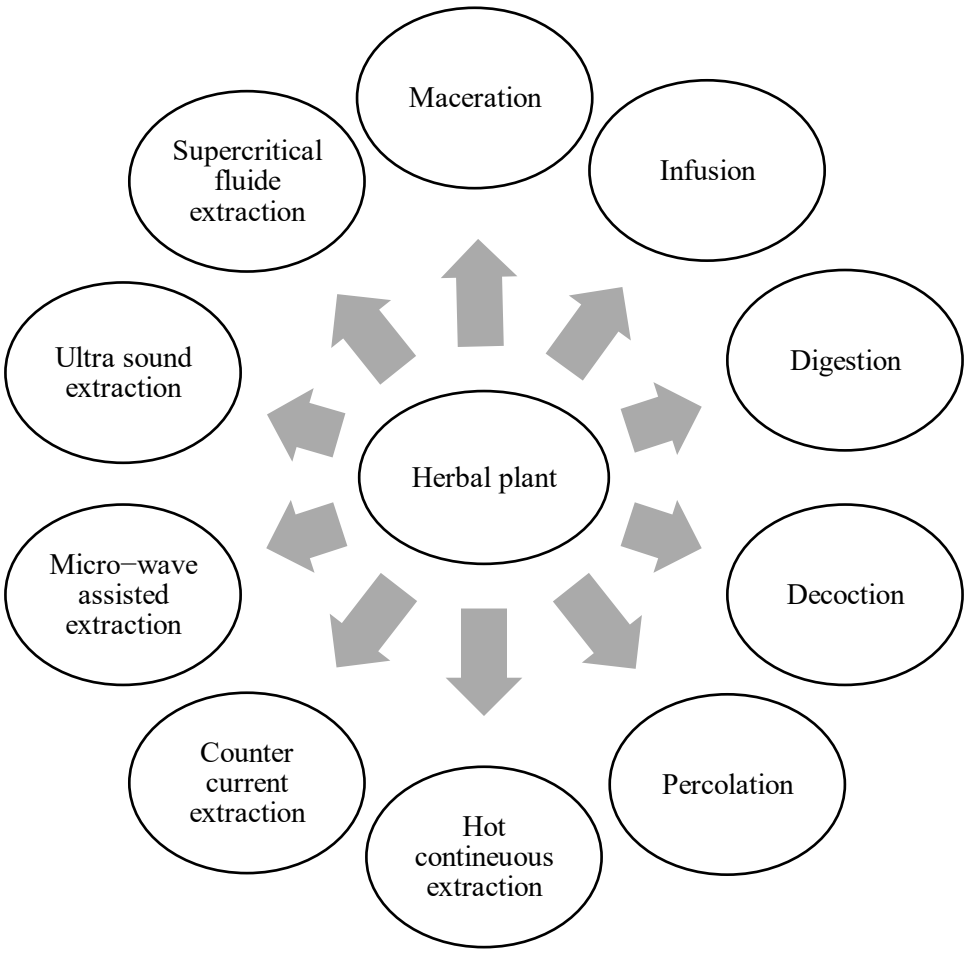
5]. The extraction techniques are described in scheme 1 for the extraction of the metabolites. Variations in the extraction methods are based on the stability of plant materials, nature of the solvent used, and duration of the extraction period. Maceration is a suitable method for the extraction from plants when prolonged time is required. Microwave is recently employed to reduce the extraction period. Attention must also be paid to prepare the required final volumes which satisfy the intended usage. Currently, maceration is an ideal method for human consumption. On the other hands, the other extraction techniques are used for the experimental purposes [4, 6].

Separation of the metabolites from the crude extracts still faces some difficulties in determination of the chemical structures. The separation and isolation of the metabolites are achieved by physical and chemical methods. The physical separation techniques utilize the separation funnels to operate fractional distillation, and this technique is employed for the extraction of the hydrocarbons from oils. In addition, fractional crystallization is used for the large volume of metabolites in a plant-scale operation. This separation method is conducted by generation of crystalline through the concentration of crude extracts. As another method, fractional liberation is utilized for the separation of cinnamon alkaloids. This method is achieved by converting the molecules to salt form then separated by the precipitation of the compounds from the crude

mixture. The sublimation technique is another separation method for the metabolites. This method is utilized for the separation of volatile oils by converting the solid materials to gas state without passing through liquid state. Various chromatographic methods (TLC, CC, PLC, PC, HPLC, and GC) are other separation methods. The chromatographic separation utilizes charge, shape and size of the separating molecules. The method requires mobile phase and the stationary phase. Different mechanism is utilized for the separation of metabolites with chromatography. Compounds are adsorbed on the support material in the presence of stationary phase. The separation relies on the affinity of the compounds to the stationary phase, and on partition the compounds between two or more immiscible solvents. Separation by ion exchange techniques is helpful for polar metabolites according to their global charge. The size exclusion method is the separation based on the molecular size of the compounds [7].

After these separations are operated, the isolated compounds are analyzed by the combination of the various spectroscopic techniques (UV−Vis, FT−IR, NMR, and MS) [8]. UV−visible method is utilized for the identification of different compounds that have absorption at UV region. The quantitative analysis of the metabolites is possible by using the compound-specific wave length. IR is the suitable for identification of the functional groups of the metabolites with high sensitivity. NMR techniques are utilized for the identification of the type of protons and carbons as well as

how they arrange in the molecules. MS analysis provides the molecular weight of the compounds by measuring the mass to charge ratio of ionized molecules. The fragmentation of ions gives information of fragments of the molecule [4, 8, 9].



Scheme 1 Extraction techniques

1.1.2 Synthesis of flavonoid glycoside

Flavonoids and their glycosides are explored as a fundamental component in different fields such as medicinal, pharmaceutical and bioceutical field. Flavonoids have significant biological

activities such as anti-mutagenic and anti-inflammatory as well as anti-oxidant and anti-carcinogenic activities. [10, 11]. The synthesis of such compounds is playing an important role to illustrate the biological activities and to determine the construction of the complicated molecules. [12]. Flavonoid glycosides are constituted from the aglycone flavonoid and sugar moiety connected with *C* or *O*−glycosidic bond. Three protocols are generally utilized for the synthesis flavonoid *O*−glycosides. As the first protocol, Zemplen−Faraks protocol employs strong bases in homogeneous medium [13]. The protocol is not widely utilized because of their low glycosylation yield, since some flavonoids like isoflavone are unstable under basic conditions. Partial hydrolysis of the glycosyl donor occurs under the condition. The second protocol is Koenigs−Knorr protocol, which is reported in 1901 for the glycosylation of alkyl hydroxyl groups by using silver salt as a promotor [14]. Various flavonoids have been successfully synthesized by using this protocol, and the method was applied for the glycosylation of several flavonoid and glycosyl bromide donors. Although the protocol provides good yields of flavonoid glycoside, the usage of many amounts of silver salt might cause severe environmental problems when the reaction runs on a large scale. The last protocol is phase−transfer catalyzed (PTC) protocol. This protocol was developed to overcome the limitations of the two protocols; Zemplen−Farkas protocol (strong basic conditions) and Koenigs−Knorr protocol (excess amount of sliver salt). The glycosylation of the aglycone flavonoid with the sugar moiety employs a mixture of the $CHCl_3$ and aq. KOH and benzyl

triethylammonium bromide as a promoter to afford low to moderate yields of flavonoid glycosides. The limitation of this protocol is the formation of undesired side products, that raised from the hydrolysis and β-elimination of the donors. Carbonate salt such as sodium and potassium carbonate along with potassium chloride were added to reduce the formation of the unwanted side products and enhanced the glycosylation yields [16]. Furthermore, the hydroxyl group at C−3 of the flavonol is unreactive because the formation of the hydrogen bond with C−4 carbonyl group makes its direct glycosylation to be difficult. Two strategies would be utilized for the synthesis of 3−*O*−glycoside flavonols. The first one is the direct glycosylation of aglycone flavonoid and sugar moiety. The second one is the connection of the flavonoid after formation of the glycosidic bond. The second method is employed when the direct glycosylation is not feasible [13]. Flavonols (3-hydroxyflavones) are synthesize by oxidation of flavone at C−3. When the C−2 and C−3 double bond of the flavone is connected to electron donating group, the oxidation will work well. On the other hand, electron accepting groups might be unreactive substrate toward electrophilic and nucleophilic oxidizing agents. Thus, the epoxidation of flavones with the classical oxidants such as *m*CPBA, alkaline H_2O_2 have failed [17]. Hypervalent iodine enables the 3−hydroxylation of flavone; the formation 3−hydroxy flavones via acid catalysis hydrolysis of the corresponding intermediate formed by iodobenzene diacetate [18]. A well-known oxygen transfer agent dimethyldioxirane (DMDO) converts the flavones at subambient temperature to the

corresponding epoxide, which rearranges to 3−hydroxyflavones on standing at rt [17]. Algar−Flynn−Oyamada (AFO) reaction is another synthetic method for the flavonols from 2′−hydroxyacetophenone and benzaldehyde in two steps; aldol condensation (formation of chalcone) and oxidative cyclization with alkaline H_2O_2 [19]. In 2020, Wang and the coworkers developed a one-pot aerobic oxidative synthesis of flavonols from 2′-hydroxyl-acetophenone and benzaldehyde in the presence of pyrrolidine in water under the atmospheric condition [20].

1.2 Objectives

1.2.1 General objectives

1.2.1.1 To isolate and identify the metabolites of the Sudanese medicinal plants

1.2.1.2 To synthesize flavonoid −3−*O*−glycoside

1.2.2 Specific objectives

1.2.2.1 To extract the desired material of the whole plant of the *Striga hermonthica* with methanol using hot continuous extraction (Soxhlet extractor, 50 °C) and cold extraction using maceration extraction with aq. MeOH (80%), separate the flavonoids with liquid−liquid fractionation, purify with the chromatographic techniques (CC and PLC) and identify with NMR spectroscopy.

1.2.2.2 To extract the desired crude material of the *Solenostemma argel* leaves with 80% aqueous

ethanol using maceration method, separate the metabolites with liquid−liquid funnel separation, purify with CC and PLC and identify the structure of isolated compound with IR and NMR spectroscopy.

1.2.2.3 To synthesize the flavonol 3−*O*−disaccharide, particularly kaempferol 3−*O*−neohesperidoside using phase−transfer catalyzed (PTC) protocol.

Chapter 2

Striga hermonthica

2.1 Introduction

Striga hermonthica belongs to Orobanchaceae family, and is widely distributed in Africa. The plant has different vernacular names; it is known as Al-Buda in Sudan, and several names are utilized as Wuta wuta, Kudiji, Dodon dawa and Makasar dawa (guinea corn killer) in Nigeria [21]. Although *S. hermonthica* is chlorophyllus plant, it is annual hemi parasitic plant thus needs a host to complete its life cycle. The plant is deemed to be the most serious biotic constraint on subsistence agriculture [22], where it may completely decimate cereal yields of the host plants by competing host sinks for carbon and nutrients. *S. hermonthica* is ubiquitous parasitic weed of several major crops such as maize (*Zea mays* L.), rice (*Oryza sativa* L.) millet (*Pennisetum glaucum* L. leeke) and sorghum (*Sorghum bicolor* L. Moench) [23]. The pot experiments showed that *S. hermonthica* parasitized on cowpea, dolichos bean and soya bean to cause a loss in yield of these beans [24]. Though *S. hermonthica* cause a serious problem on agriculture, it has some useful activity for people. It was reported that the plant had insecticidal activity against *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae), which is affecting the ovicidal, larvicidal with an emerging adult rate [25]. And *S. hermonthica* has less anthelmintic activity against free living Rhabditid anematoide, *Cuenorhabditis elegans* organisms [26]

2.1.1 Description

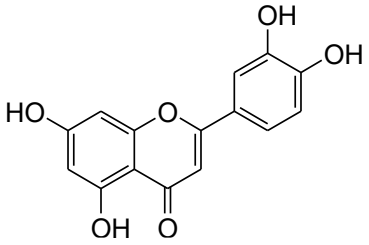
S. hermonthica has pink to red, yellow or white flowers with hard, fibrous and hairy stem (Fig. 1). The plant is 80 cm high and produce about 40,000 tiny seeds per plant [27].

Fig. 1 *Striga hermonthica*

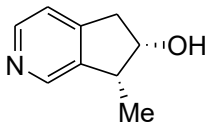
2.1.2 Phytochemistry

The phytochemical studies of the plant [28] and the extracted oil [22] of *S. hermonthica* showed the presence of many secondary metabolites such as alkaloids, flavonoids and steroids along with terpenes, tannins, coumarins, cardiac glycosides, and saponins. From ethyl acetate fraction of acetone crude extract, luteolin was isolated in pure form [29] as well as the alkaloid venoterpine, which has been isolated from the 10% HCl extract of the whole plant or from the alkaline $CHCl_3$ exact with sodium carbonate, washed with petroleum ether, chromatographed with alumina and $CHCl_3$ as eluted solvent and identified with spectroscopic methods (UV, IR, ^{1}H NMR and MS) [30]. Beside luteolin and venoterpine, other compounds; apigenin, chrysoeriol, coumaric acid, and β−sitosterol have been identified using DART−MS and LC−ESI−MS from the dry powder and methanolic extract, respectively, [31]. Also, the HPLC analysis of the glacial acetic acid

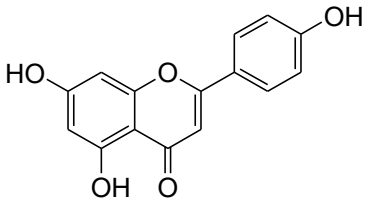
extract showed the presence of salicylic acid, gallic acid, quercetin**,** chlorogenic acid, phenol**,** resorcinol, rutin**,** catechin, vanillin and *p*−hydroxy benzoic acid [32] and baicalin, luteolin 7−*O*−glucuronide, mannitol, aucubin, methyl pheophorbide b, lophophorine, and skimmin [33].



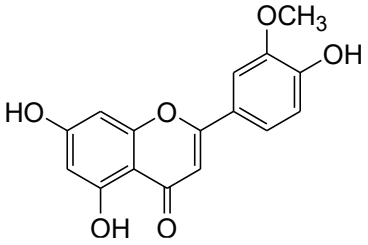
Luteolin



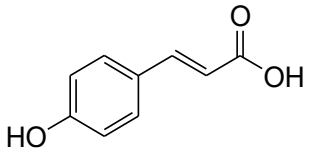
Venoterpine



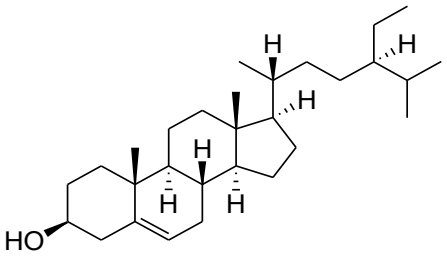
Apigenin



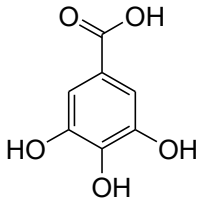
Chrysoeriol



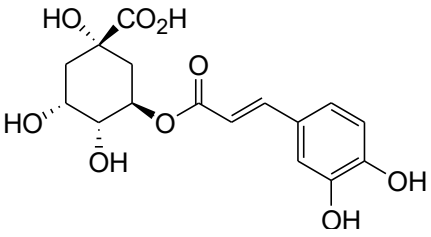
p−Coumaric acid



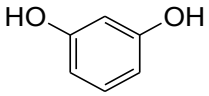
β−Sitosterol

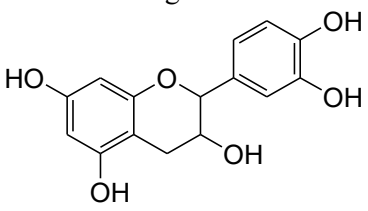


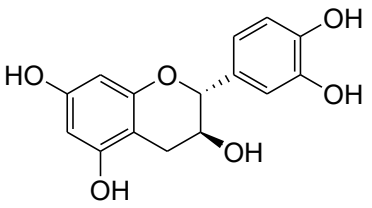
Gallic acid



Chlorogenic acid

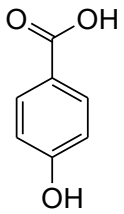


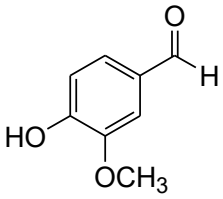


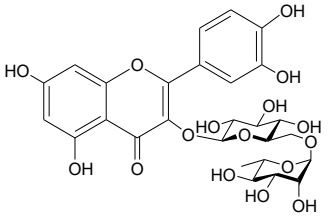


Resorcinol

Catechin



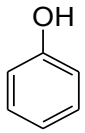


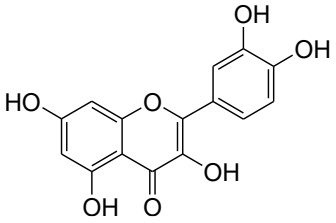


p–hydroxy benzoic acid

Vanillin

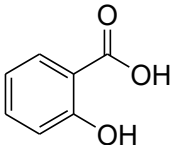
Rutin

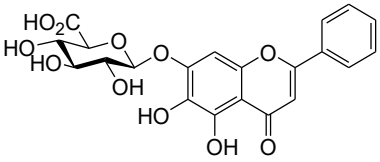




Phenol

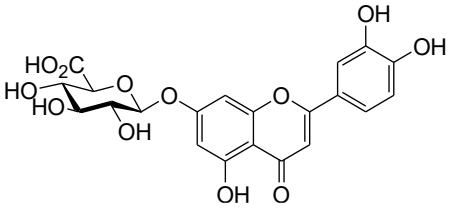
Quercetin

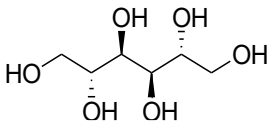




Salicylic acid

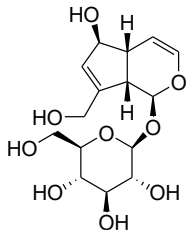
Baicalin



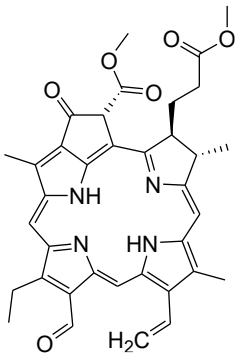


luteolin 7–*O*–glucuronide

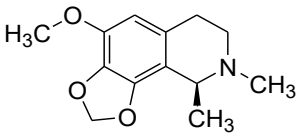
Mannitol



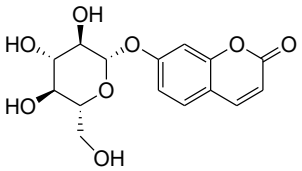
Aucubin



Methyl pheophorbide b



Lophophorine



Skimmin

Fig. 2 Structures of the metabolites of the *Striga hermonthica*

2.1.3 Pharmacological activity

S. hermonthica is known as a medicinal plant in some parts of Africa and in India. The decoction of the plant has been used traditionally for the treating pneumonia in Sudan, particularly, in the western area. *S. hermonthica* is used as a medicinal plant for the diabetes, as well as the leprosy ulcers in the eastern region of Africa, where the decoction of the roots is utilized as abortifacient [34, 35]. Several biological activities of *S. hermonthica* have been reported; the anti−oxidant activity by using DPPH method and acute toxicity of the plant. The crude aqueous acetonitrile extract exhibited a weak cytotoxic activity (IC_{50} of 95.27±2.30 µg/ml) comparing with the isolated luteolin (IC_{50} of 6.80±1.46 µg/ml), and also showed an insignificant acute toxicity (LD_{50}: 1753±44 mg/kg) on mice [29]. It also reported that *S. hermonthica* possessed various anti-

bacterial activities against *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa*, and *Staphylococcus aureus* and showed no anti-fungal activity against *Aspergillus niger* and *Candida albicans* [28, 36]. In addition to these activities, the methanolic extract showed trypanocidal activity with *T.B. brucei* and *T. congolense* [37].

Anti−malarial activity of *S. hermonthica* has been reported that anti-plasmodial test displayed mild to weak activities in vitro (IC_{50} 274.8 µg/ml) by using the parasite lactate dehydrogenase assay, and murine model test exhibited a higher anti−malarial activity in vivo (68.5 % suppression at dose 10 mg/kg weight of mice) comparing with the standard reference drug chloroquine−sensitive *Plasmodium berghei* (ANKA P1) (78.0 %) [38]. Furthermore, Baba *et al.* reported the repellent activity of the extracted oil from the plant against *Anopheles gambiae* and *Culex quinquefasciatu* in 2012. Their results showed that 50% concentration of oil had protection for 180 min against *Anopheles gambiae*, and the oil was more potential against *Culex quinquefasciatus* [22].

2.2 Material and methods:

2.2.1 General

Methanol (MeOH), ethanol (EtOH), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), hexane (Hex) chloroform ($CHCl_3$), and toluene (Tol) were purchased from Kanto Chemical CO., INC. and used without purification. Thin layer chromatography (TLC) and preparative TLC were performed using Merck−KGaA silica gel 60 F_{254} pre−coated plates (0.25 mm) and 0.5 mm, respectively, and silicagel 70 FM TLC plate−wako, and visualized using shortwave UV light, phosphomolybdic acid (PMA) stain and Hanessian's stain with heat. Column chromatography was performed using silica gel 60 N (neutral, sphere, particle size 0.063−0.210 mm). NMR spectra were recorded on JEOL 400 YH instrument and the chemical shifts reported in δ (ppm) relative to deuterated solvent (CD_3OD at 3.31 ppm ^1H NMR and 49.15 ppm ^{13}C NMR. and DMSO−*d6* at 2.5 ^1H NMR ppm and 39.52 ppm ^{13}C NMR). The following abbreviations were used to explain the ^1H NMR data: chemical shift δ (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (*J*, Hz).

2.2.2 Plant material

Striga hermonthica (flowered stage) was collected from Al−Seleit farm−Khartoum−Sudan.

2.2.3 Hot continuous extraction

The extraction was performed following the reported method [6] with some modifications. The whole plant was cleaned, dried in shadow at rt, and disintegrated to coarsely powder. Dried powder was continuously extracted with methanol using Soxhlet extractor at 50 °C until the completion of the extraction process detected by the clearance the of siphoning solvent from the extractor.

2.2.3.1 Solvent acid−base fractionation

Eight grams of dried methanolic extract was dissolved in H_2O and adjusted to pH 3 using citric acid monohydrate (3 %), shaken with hexane in ratio 2:1 (v/v) using separatory funnel, the hexane layer was removed. This method was repeated until hexane soluble materials had extracted. The combined hexane layer was concentrated under vacuum pressure to obtain hexane extract. The pH of the remained aqueous solution was changed to 13 using sodium hydroxide (1 M) then fractionated with $CHCl_3$ and EtOAc to obtain $CHCl_3$ and EtOAc extracts, respectively (Scheme 2).

2.2.3.2 Purification and isolation

The ethyl acetate extract (110 mg) was purified with PLC and eluted with 100 % EtOAc. Colored bands with PMA stain were collected and labeled as F 1 and F 2. F 2 corresponded to compound **3** and F 1 was subjected to further purification using Tol: $CHCl_3$: MeOH (4:4:1) followed by CH_2Cl_2: MeOH 15:1, as eluted solvents, yielding two subfractions F 1.1 and F 1.2. They corresponded to compound **1** and compound **2,** respectively. The characterization of the compounds was achieved by ^{1}H NMR spectroscopy and comparison with reported data (Scheme 2).

2.2.4 Cold extraction

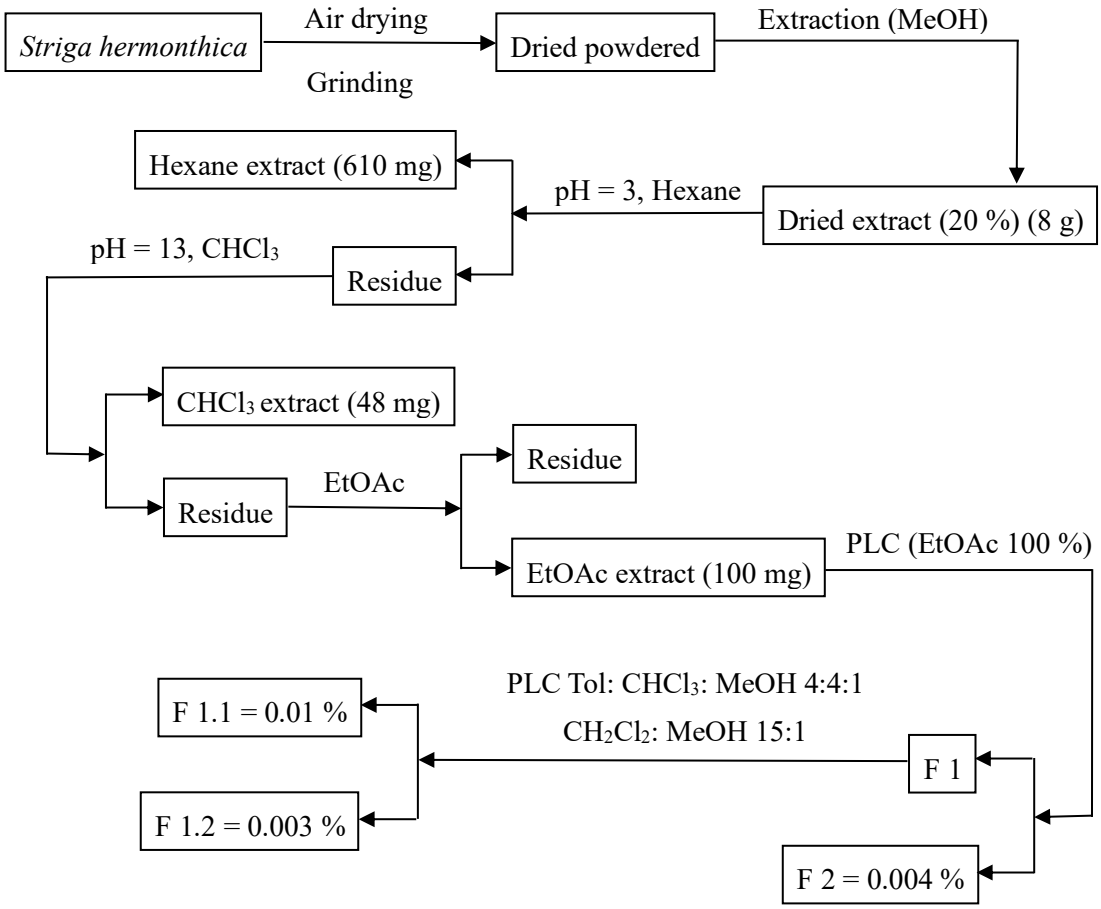
The coarsely powdered *S. hermonthica* plant (100 g) was soaking in aqueous methanol (80%) for three days at room temperature. The mixture was stirred periodically. Then the marc was filtered off and macerated again with aqueous methanol 80% for 4 days with daily filtration and evaporation. The filtrates were concentrated under vacuum pressure using rotary evaporator to obtain the methanolic extract (21 g) [6].

2.2.4.1 Liquid−liquid fractionation

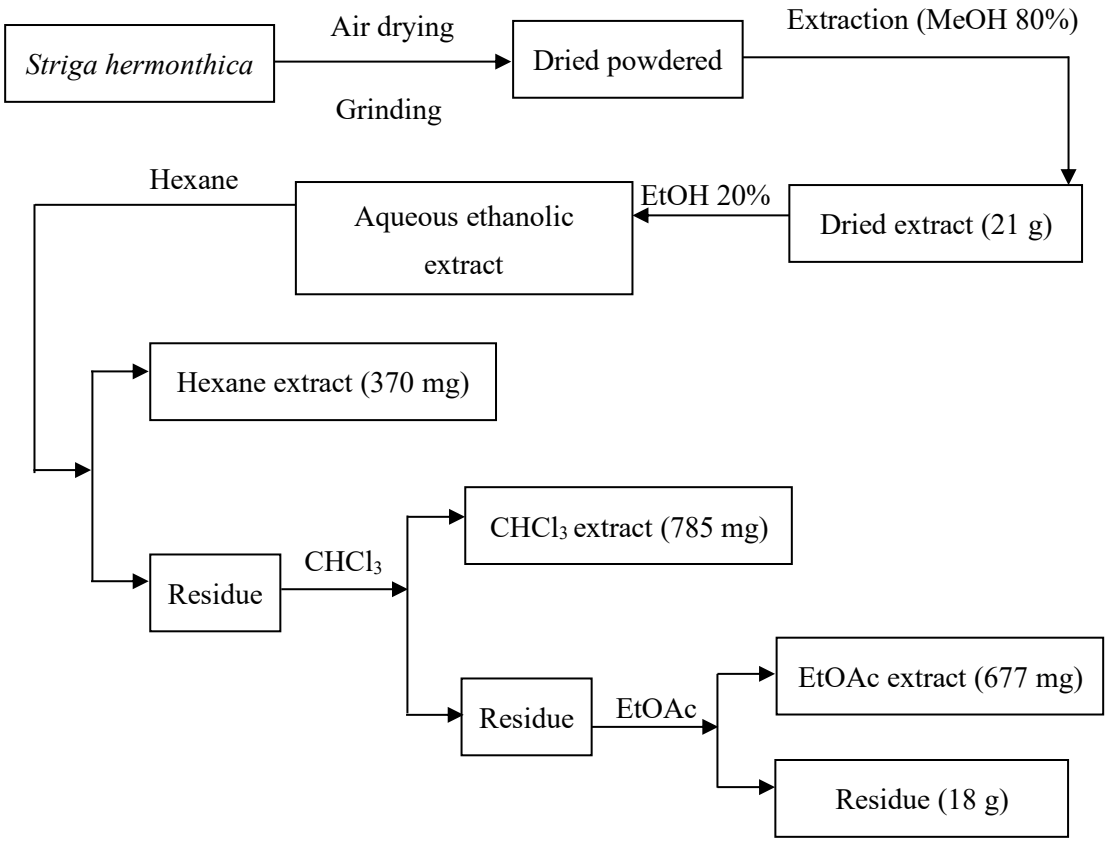
The dried extract (21 g) was dissolved in aqueous ethanol (20%) and fractionated with hexane (600 ml) using separating funnel (1 L). Hexane layer was separated and concentrated under vacuo, then the aqueous ethanol layer was again fractionated 10 times with the recovered hexane until the extraction of the soluble material had completed. The dried hexane fractions were combined to afford the hexane extract (370 mg). By following the same procedure, the remaining aqueous layer was shaken first with $CHCl_3$ (600 ml × 7) and then with EtOAc (600 ml × 10) to obtain $CHCl_3$ (785 mg) and EtOAc (677 mg) extract, respectively. Then the residue of aqueous ethanol was concentrated using rotary evaporator at 40 °C and 2.0 mbar pump to afford 18 g of the residue extract [92] (scheme 3).

2.2.4.2 Purification and isolation

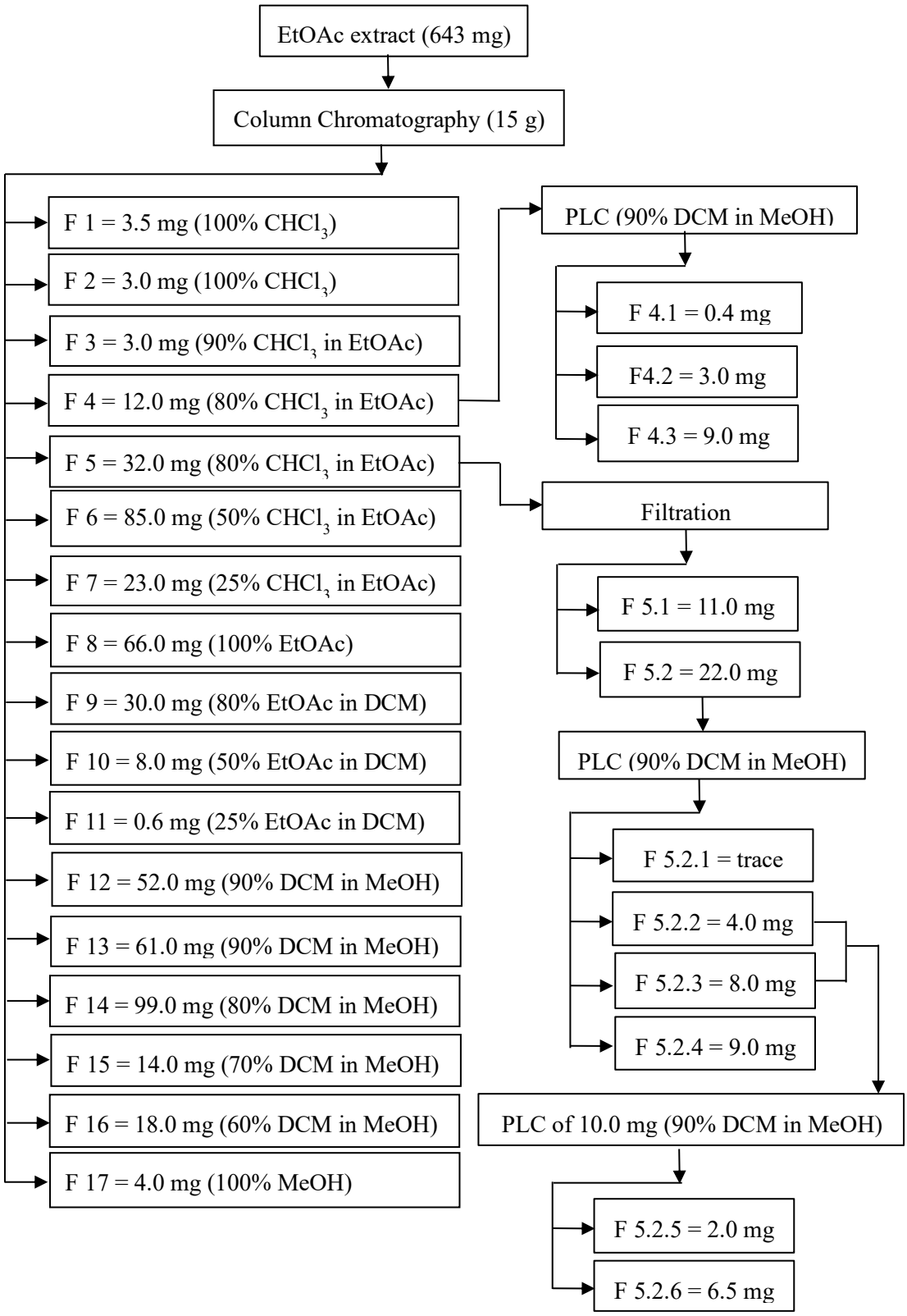
The EtOAc extract (643 mg) was chromatographed over silica gel (15 g) column (60 × 2.5 cm) and successively eluted with $CHCl_3$, EtOAc, CH_2Cl_2 and MeOH yielded 17 fractions (F1−F17). F4−F7 were dissolved in MeOH and applied to PLC using 90% CH_2Cl_2 in MeOH as eluted solvent. Similarly F12 was purified with PLC and 80% CH_2Cl_2 in MeOH. Then the obtained fractions (F 4.1, F5.1, F5.2, F5.3, F6.1, F6.2, F7.1, F7.2 and F12.2) were subjected to further purification using PLC with 90% CH_2Cl_2 in MeOH and 80% CH_2Cl_2 in MeOH as solvents (scheme 4,5, and 6). Four compounds were obtained and identified based on the analysis of NMR spectra as well as through comparison with the reported data.



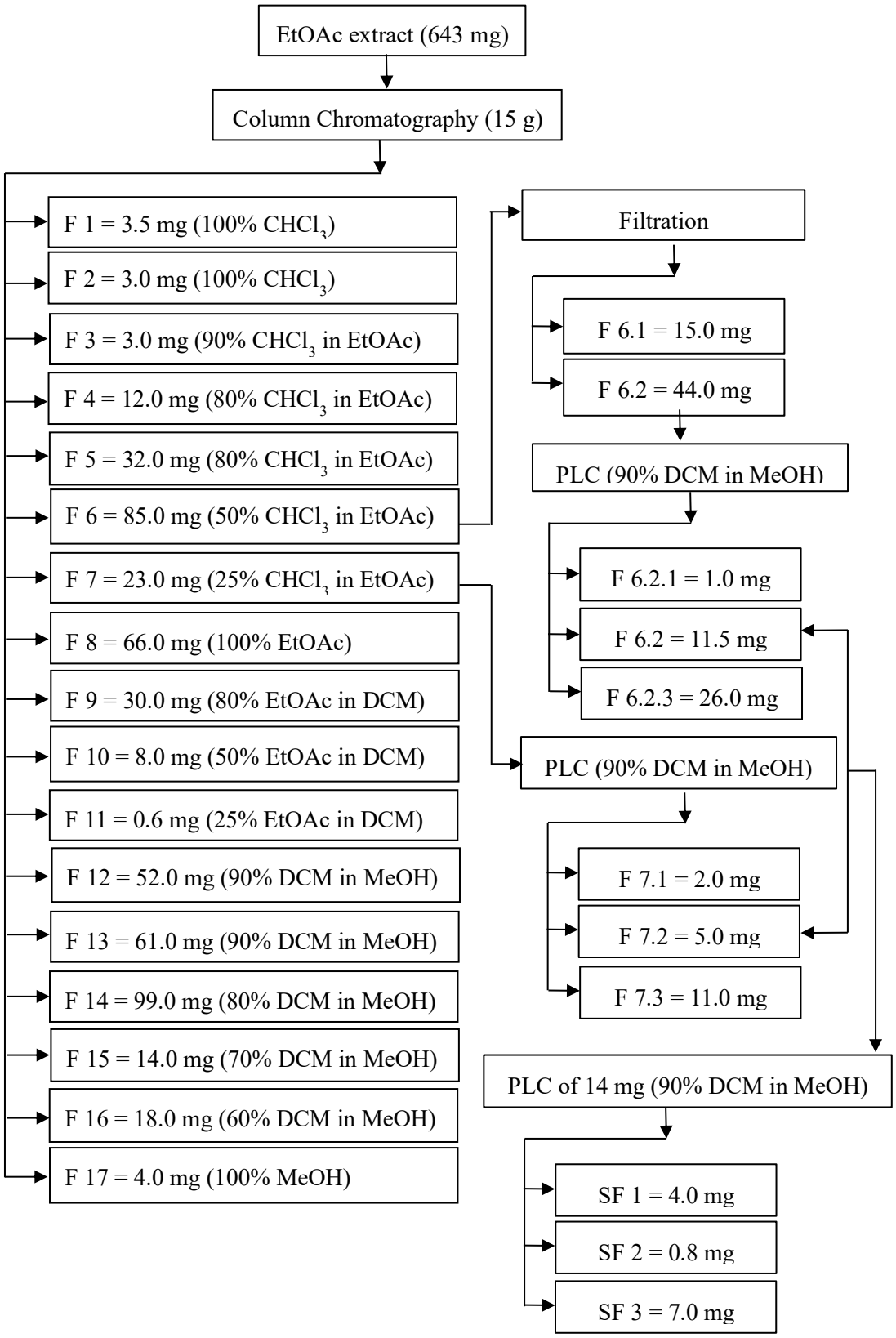
Scheme 2 Hot extraction and solvent acid−base partitioning of *S. hermonthica*



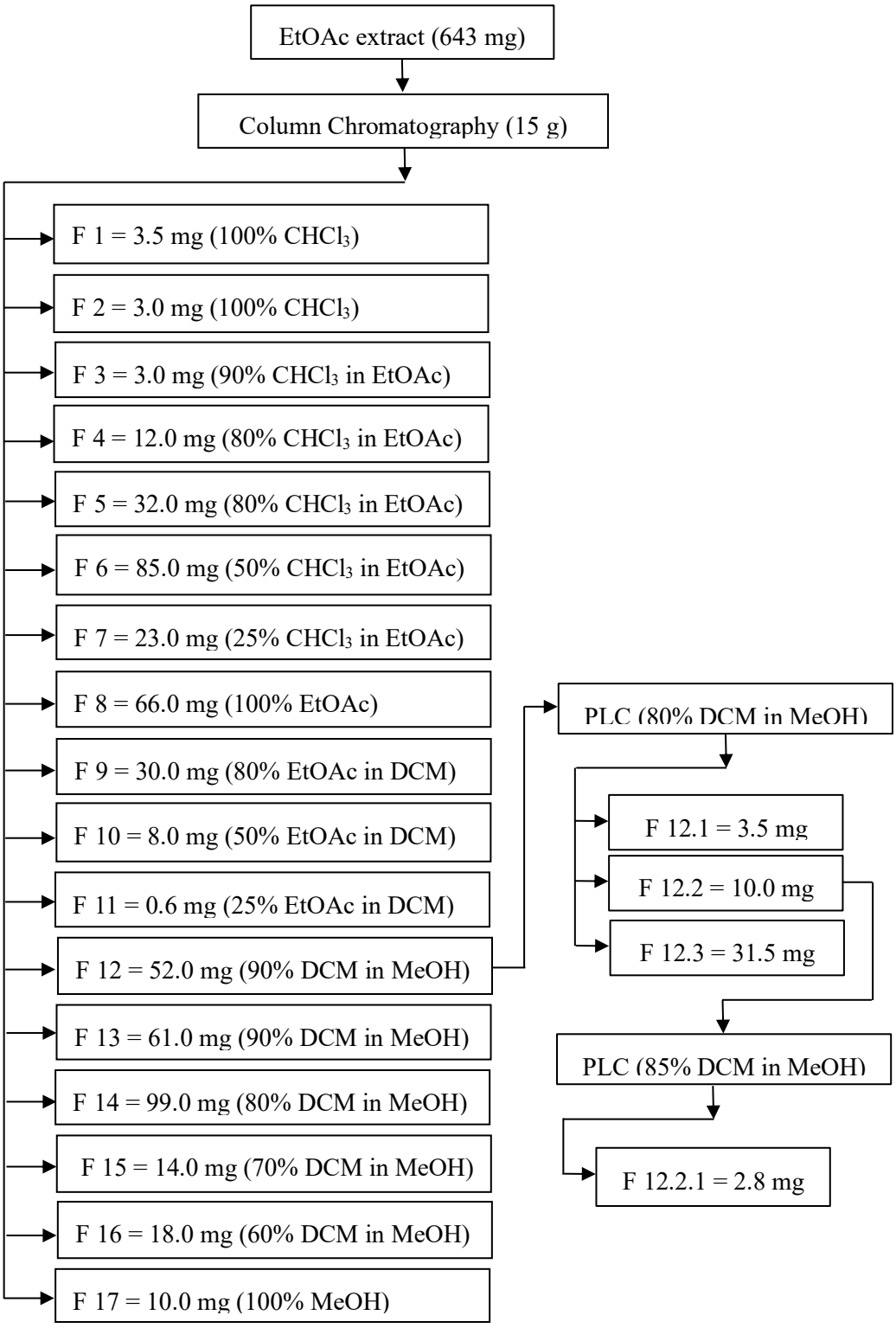
Scheme 3 Cold extraction and liquid−liquid fractionation of *S. hermonthica*



Scheme 4 Purification and isolation of the metabolites of F 4 and F 5 of ethyl acetate extract



Scheme 5 Purification and isolation of the metabolites of F 6 and F 7 of ethyl acetate extract



Scheme 6 Purification and isolation of the metabolites of F 12 of ethyl acetate extract

2.3 Results and discussion

The dried powdered of the whole plant of *S. hermonthica* was extracted with MeOH using Soxhlet extractor at 50 °C, yielded 20% of the crude extract. the dried methanolic extract (8 g). The extract was separated by solvent acid−base fractionation., the purification of EtOAc extract by PLC gave three fractions, which were analyzed by ^{1}NMR spectroscopy and compared with the reported data. Fr.1.1 was identified as chryseoriol [29] and F 1.2 was apigenin. Their concentrations in the dried plant were estimated to be 0.014 and 0.003%, respectively (Scheme 2). On the other hand, the dried whole plant of *S. hermonthica* was extracted with aqueous MeOH (80%) at room temperature to obtain 21% yield of the crude extract. Then the dried methanolic extract was subjected to liquid−liquid partitioning using hexane, $CHCl_3$ and EtOAc as solvents in sequence (Scheme 3). The silica gel column chromatographic separation of EtOAc extract (643 mg) was carried out based on the principle of normal phase chromatography by using silica gel 60 as adsorbent with starting the elution from less polar $CHCl_3$ to more polar MeOH. The method led to separate 17 different polarity fractions (F1−F17). Then the obtained fractions have been purified with plate coated silica gel (PLC) and eluted with 90% CH_2Cl_2 in MeOH and 80% CH_2Cl_2 in MeOH. The fractions were visualized with Hanessian's−stain and obtained colored spots (orange and yellow color) were subjected to second purification (Scheme 4,5 and 6). And the analysis of the pure fractions led to identified four compounds. F4.1 and F5.2.1 were identified

as chrysoeriol, F5.1, F5.2.5 and Fr.6.1 are apigenin, SF 1 is luteolin and Fr.12.2.1 has been identified as apigenin 7−*O*−glucoside. And their yields in the dried plant were 0.0004, 0.026, 0.004, and 0.003%, respectively.

Chryseoriol was isolated as yellow solid. The TLC chromatogram showed one compound when using 90% CH_2Cl_2 in MeOH (R_f = 0.62) which gives faint yellow color in visible light, pink color with UV light (254 nm), and yellow color with Hanessian's−stains. By using DMSO−*d6*, ^{1}H NMR spectrum displayed signals in the aromatic region; two meta−coupled protons at δ 5.60 and 5.84 ppm with *J* value 2.1 Hz for the protons 6 and 8, respectively. And singlet signal at δ 6.44 ppm attributed to H−3. In addition to three signals at δ 7.35, 6.76 and 7.39 ppm for the protons 2', 5' and 6'. ^{1}H NMR spectrum showed singlet signal at δ 3.83 ppm intensity to three protons attributed to the methoxy group (Table 2). By the comparison with ^{1}H NMR data obtained when using CD_3OD (Table1), the data obtained when using DMSO−*d6* are more consistent with the known compound [125].

Apigenin isolated as faint yellow solid. The TLC chromatogram showed one spot when using 90% CH_2Cl_2 in MeOH (R_f = 0.62) which gives faint yellow color in visible light, pink color with UV light (254 nm), and yellow color with Hanessian's stains. Comparing with acid−baes separation (scheme 2), the liquid−liquid fractionation led to isolate apigenin in high yield (0.026%). Using DMSO−*d6,* the ^{1}H NMR spectrum exhibited signals of the flavone structure.

Two meta-coupled aromatic protons at δ 6.19 and 6.48 ppm (J = 1.5 Hz) for the protons H−6 and H−8, respectively. And four *ortho*-coupled-doublet signals were observed at δ 7.93 and 6.92 ppm (J = 8.6 Hz), for the protons H−2', 6' and H−3', 5', respectively (Table 2). The meta and ortho positions were characterized based on the coupling constants; J_{meta} 1−3 Hz and J_{ortho} 6−10 Hz [41]. In addition, ^{1}H NMR spectrum showed a singlet signal at δ 12.97 ppm for the proton of the hydroxy group C−5 position, whereas the protons of hydroxy groups at C−7 and C−4' were deuterated with deuterium ion of the solvent used (Spectrum 6). On the other hand, using of deuterated methanol led to deuterated all hydroxy protons of the compounds (Spectrum 4). By comparison, the ^{1}H and ^{13}C NMR data obtained when using DMSO−*d6* were compatible with the reported data [42] and different data were obtained when using CD_3OD [43] (Table1).

Luteolin was isolated as yellow solid. The TLC chromatogram showed one compound when using 90% CH_2Cl_2 in MeOH (R_f = 0.42) which gives yellow color in visible light, pink color in UV light (254 nm), and orange color with Hanessian's stains. The ^{1}H and ^{13}C NMR spectra obtained in DMSO−*d6* exhibited the flavone structure, the obtained data are slightly different from the reported data [42] (Table 2). In addition, ^{1}H NMR analysis using CD_3OD showed H−6' signal was overlapped with H−2' at δ 7.36 ppm, and the spectrum is similar to the reported spectrum [44].

Apigenin 7−*O*−β−glucoside also obtained as yellow solid. One TLC spot was observed when using 80% CH_2Cl_2 in MeOH (R_f = 0.42) and gives yellow color in visible light, pink color with

UV light (254 nm), and orange color with Hanessian's stains. The compound has been characterized by the combination of NMR spectra obtained in DMSO−*d6* and CD_3OD. NMR spectra using CD_3OD showed characteristic pattern of apigenin unit with one anomeric proton signal at 5.08 ppm (H−1"), with coupling constant (*J*) 8.0 Hz, which is correlated with C−1" in HMQC exhibited the β−sugar. Also, the ^1H NMR spectrum revealed two geminal protons at δ 3.91 and 3.71 ppm for H−6"a and H−6"b correlated with C−6" at δ 60.8 ppm in HMQC (Spectrum 14) corresponding to methylene group which is confirmed by DEPT 135 spectrum (Spectrum 13). Also, NOESY spectrum showed correlations between H−6 and H−8 of apigenin with H−1"of the sugar moiety which exhibited the linkage point between the flavone and the sugar unit at C−7 (Spectrum 15). On the other hand, for the comparison with the literature data, the compound has been analyzed with NMR spectroscopy using DMSO−*d6*; the spectra exhibited slightly different from the literature value [45].

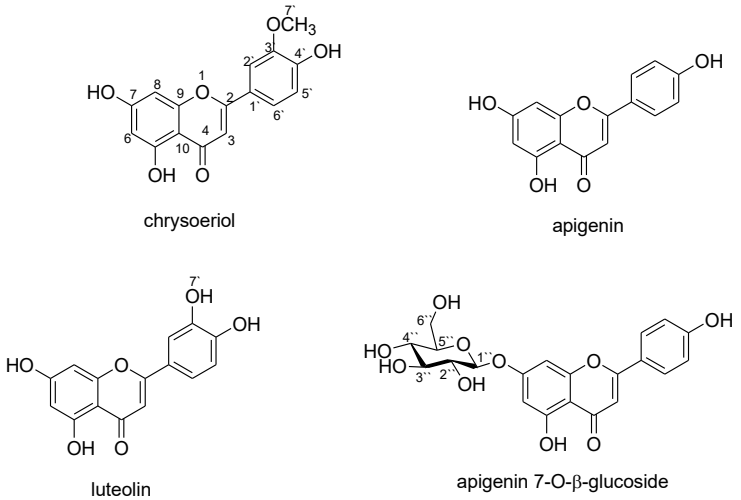


Fig. 3 Structures of flavones isolated from *S. hermonthica*

Table 1 ^{1}H NMR of the isolated flavones from *S. hermonthica* (400 MHz, CD_3OD, rt)

position	chry.	apig.	lute.	apig 7−*O*−glu.
	δ (H, m, *J* in Hz)	δ (H, m, *J* in Hz)	δ (H, m, *J* in Hz)	δ (H, m, *J* in Hz)
2	–	–	–	–
3	6.45 (s, 1H)	6.51 (s, 1H)	6.48 (s, 1H)	6.59 (s, 1H)
4	–	–	–	–
5	–	–	–	–
6	6.02 (d, 1H, 2.1)	6.11 (d, 1H, 2.0)	6.13 (d, 1H, 2.0)	6.48 (d, 1H, 2.0)
7				
8	6.22 (d, 1H, 2.1)	6.34 (d, 1H, 2.0)	6.35 (d, 1H, 2.0)	6.83 (d, 1H, 2.0)
9	–	–	–	–
10	–	–	–	–
1′	–	–	–	–
2′	7.43 (d, 1H, 1.4)	7.83 (d, 2H, 8.8)	7.36 (d, 2H, 8.8)	7.83 (d, 2H, 8.8)
3′	–	6.92 (d, 2H, 8.8)	–	6.83 (d, 2H, 8.8)
4′	–	–	–	–
5′	6.88 (d, 1H, 8.4)	6.92 (d, 2H, 8.8)	6.89 (d, 2H, 8.8)	6.83 (d, 2H, 8.8)
6′	7.47(dd, 1H, 8.5; 1.7)	7.83 (d, 2H, 8.8)	7.36 (d, 2H, 8.8)	7.83 (d, 2H, 8.8)
	3.94 (s, 3H)	–	–	–
1″	–	–	–	5.08 (d, 1H, 8.0)
2″	–	–	–	3.97-3.92 (m, 1H)
3″, 5``	–	–	–	3.50 (m, 2H)
4″	–	–	–	3.42 (d,1H, 9.5)
6‴a	–	–	–	3.92-3.85 (m, 1H)
6‴b	–	–	–	3.72 (d, 1H, 17.9)

chry.: chrysoeriol, api.: apigenin, lute.: luteolin, apig 7−*O*−glu.: apigenin 7−*O*−glucoside

Table 2 [1]H NMR of the isolated flavones from *S. hermonthica* (400 MHz, DMSO−*d6*, rt)

position	chry.	apig.	lute.	apig 7−*O*−glu
	δ (H, m, *J* in Hz)	δ (H, m, *J* in Hz)	δ (H, m, *J* in Hz)	δ (H, m, *J* in Hz)
2	–	–	–	–
3	6.44 (s, 1H)	6.79 (s, 1H)	6.51 (s, 1H)	*
4	–	–	–	–
5	–	–	–	–
6	5.60 (d, 1H, 2.1)	6.19 (d, 1H, 1.5)	6.02 (d, 1H, 1.9)	6.38 (d, 1H, 2.0)
7				
8	5.84 (d, 1H, 2.1)	6.48 (d, 1H, 1.5)	6.29 (d, 1H, 1.9)	*
9	–	–	–	–
10	–	–	–	–
1′	–	–	–	–
2′	7.35 (d, 1H, 1.3)	7.93 (d, 2H, 8.6)	7.31 (d, 1H, 2.2)	7.84 (t, 2H, 9.2)
3′	–	6.92 (d, 2H, 8.6)	–	*
4′	–	–	–	–
5′	6.76(d, 1H, 8.8)	6.92 (d, 2H, 8.6)	6.75 (d, 1H, 8.3)	*
6′	7.39(dd, 1H, 8.4; 1.9)	7.93 (d, 2H, 8.6)	7.34 (d, 1H, 8.3, 2.3)	7.84 (t, 2H, 9.2)
7′	3.83 (s, 3H)	12.97 (s, 1H)	–	
1″	–	–	–	5.05 (d, 1H, 7.5)
2″−6″	–	–	–	3.79−3.15 (m)

*[1]H NMR overlapped signals between 6.76−6.66 ppm for H−3, H−6 and H−3′,5′

Table 3 ^{13}C NMR of the isolated flavones from *S. hermonthica* (100 MHz, CD_3OD, rt)

carbon	chry.	apig.	lute.	apig 7−*O*−glu
2	165.75	165.77	169.9	164.78
3	101.99	103.22	103.5	102.93
4	183.42	183.34	183.6	184.06
5	163.08	163.32	163.	159.06
6	96.50	101.72	101.5	101.17
7	174.71	180.32	166.1	167.76
8	88.74	96.25	96.0	96.11
9	159.95	162.92	159.8	162.97
10	103.66	103.85	104.3	107.10
1′	123.6	123.06	123.6	125.21
2′	110.5	129.27	114.0	129.85
3′	149.9	117.22	147.4	118.80
4′	153	159.76	151.7	136.84
5′	117.1	117.22	117.0	118.80
6′	121.8	129.27	120.3	129.85
7′	57.0	-	-	-
1″	-	-	-	101.74
2″	-	-	-	71.39
3″	-	-	-	77.97
4″	-	-	-	64.5
5″	-	-	-	74.9
6″	-	-	-	62.6

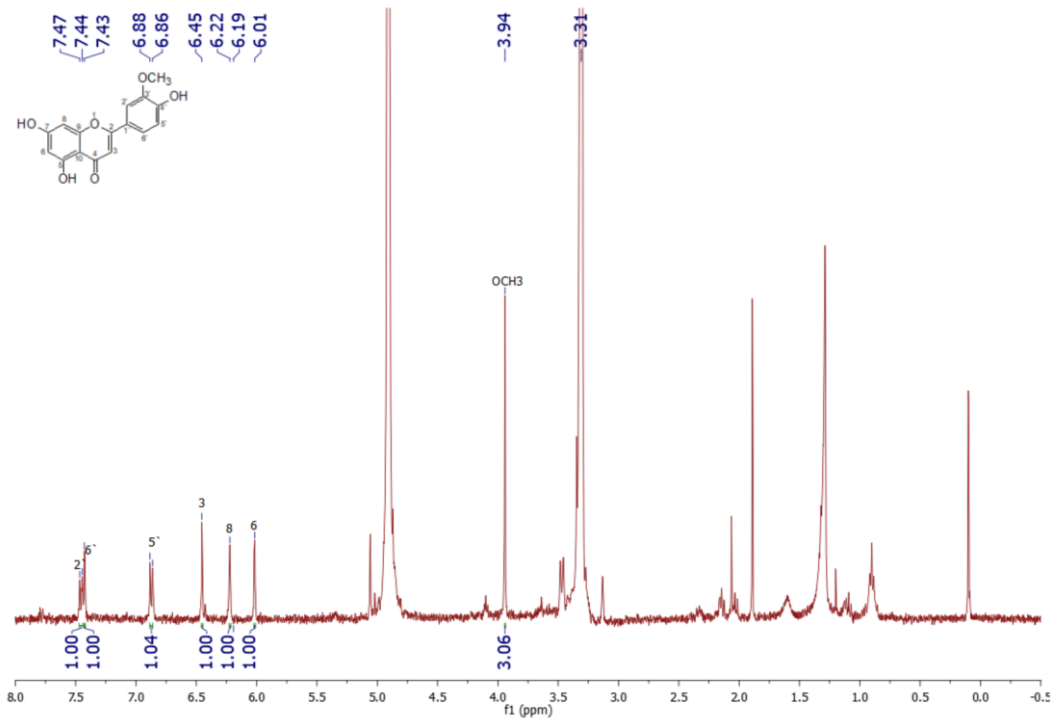
Table 4 ^{13}C NMR of the isolated flavones from *S. hermonthica* (100 MHz, DMSO−*d6*, rt)

carbon	apig.	lute.	apig 7−*O*−glu
2	163.8	163.7	162.6
3	102.9	102.3	n.d.
4	181.8	181.0	181.5
5	161.5	161.4	156.6
6	98.9	99.6	99.9
7	164.2	167.6	165.0
8	94.0	94.3	97.0
9	157.3	157.5	n.d.
10	103.7	105.4	105.2
1′	121.2	119.5	122.3
2′	128.5	112.2	128.7
3′	116.0	146.6	117.3
4′	161.2	152.5	161.1
5′	116.0	115.6	117.3
6′	128.5	119.0	128.7
7′	-	-	-
1″	-	-	99.9
2″	-	-	73.1
3″	-	-	77.2
4″	-	-	69.6
5″	-	-	76.4
6″	-	-	63.2

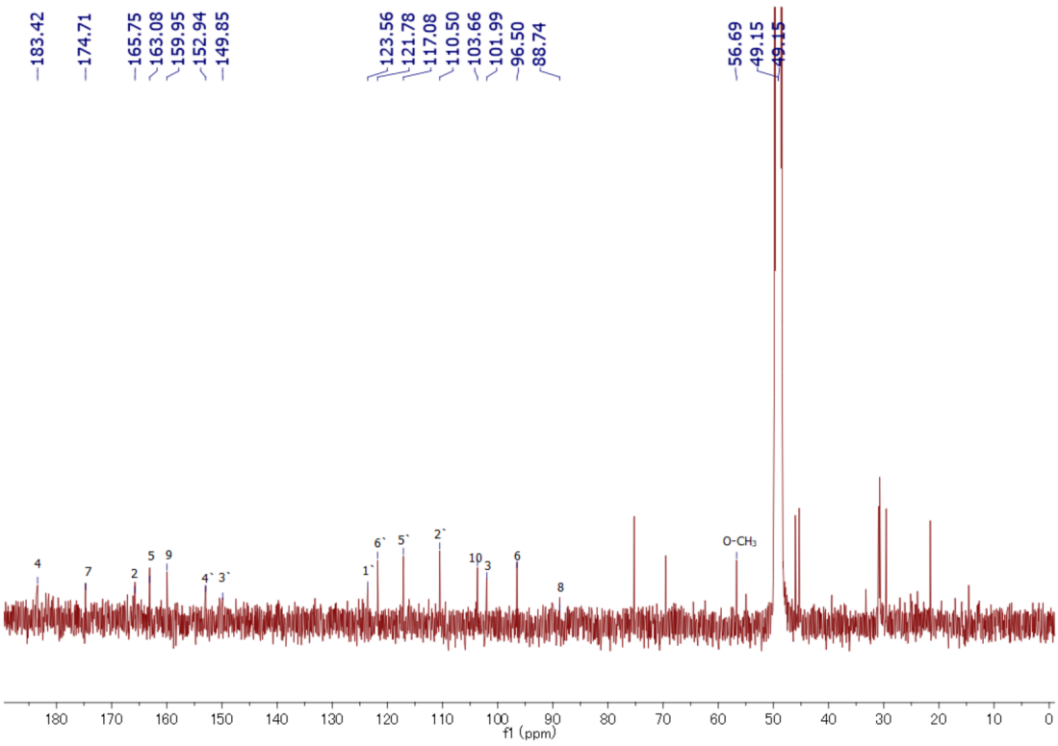
n.d.: not detected

2.4 Conclusion

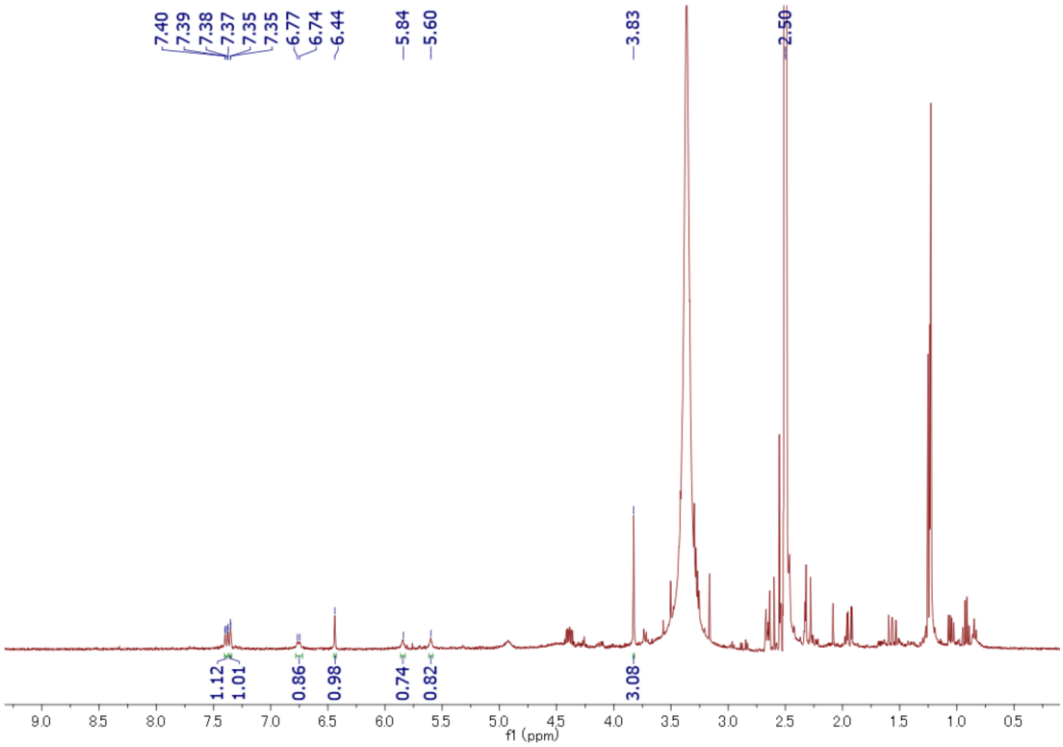
The dried whole plant of *S. hermonthica* was extracted with two different extraction procedures. Both methods yielded about 20% of the crude extract. Four flavones were purified and isolated with liquid−liquid separation and chromatographic techniques, whereas only two flavones were separated when using solvent acid−base fractionation. The flavones were identified as chrysoeriol, apigenin, luteolin and apigenin 7−*O*−β−glucoside. And the concentrations of the isolated flavones were calculated. These flavones were reported to have biological activities, for instance apigenin and luteolin were reported to have anti−mutagenic activity [42] as well as anti−cancer activity of apigenin which has significant potential in the prevention and therapy of the cancer diseases [46] beside anti−proliferative and anti-oxidant activity of apigenin 7−*O*−β−glucoside [47]. Accordingly, *S. hermonthica* could be useful in treating diseases and used as a resource of pharmaceutical manufacture to provide valuable medicine.



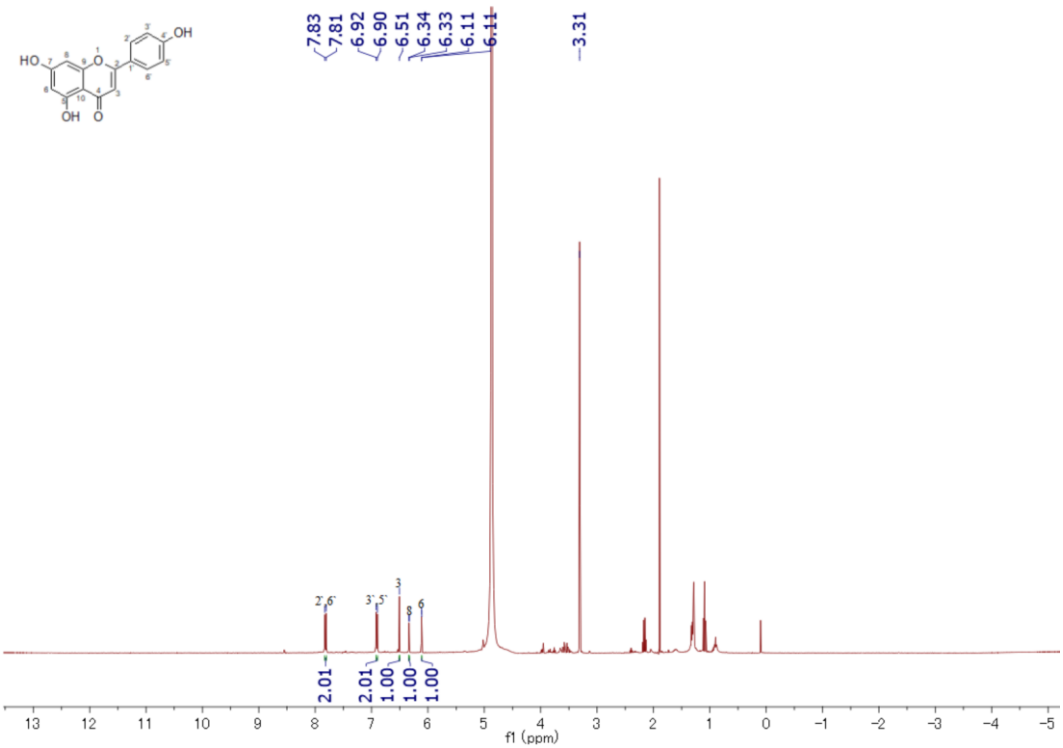
Spectrum 1 ^{1}H NMR of chrysoeriol (CD_3OD)



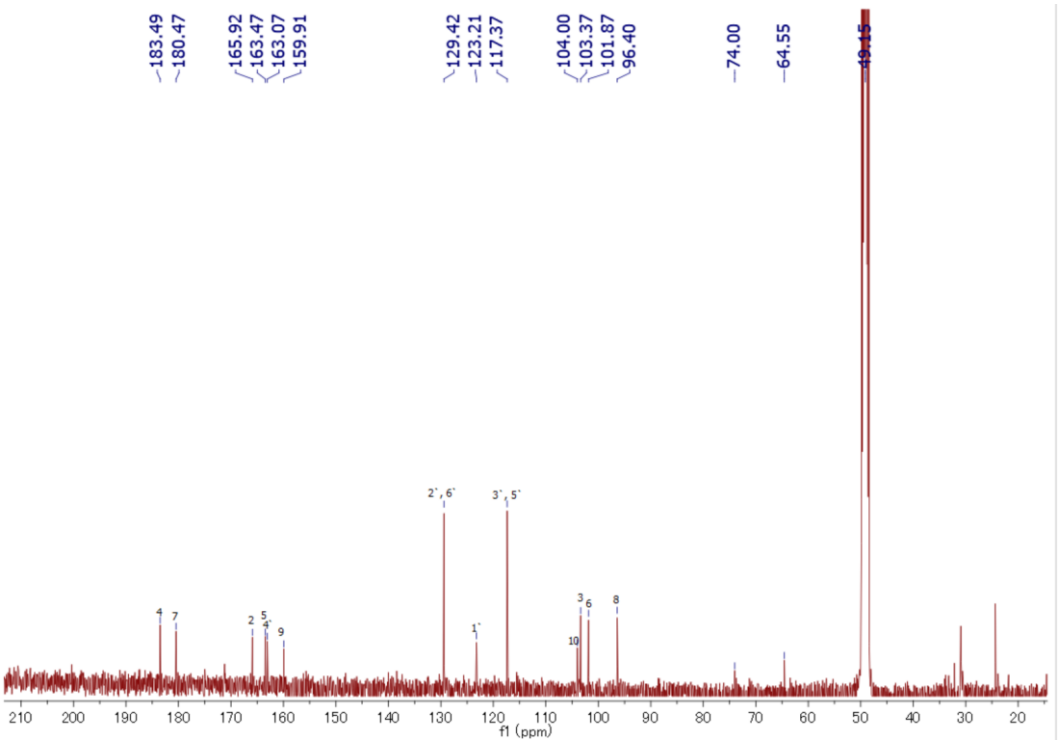
Spectrum 2 ^{13}C NMR of chrysoeriol (CD_3OD)



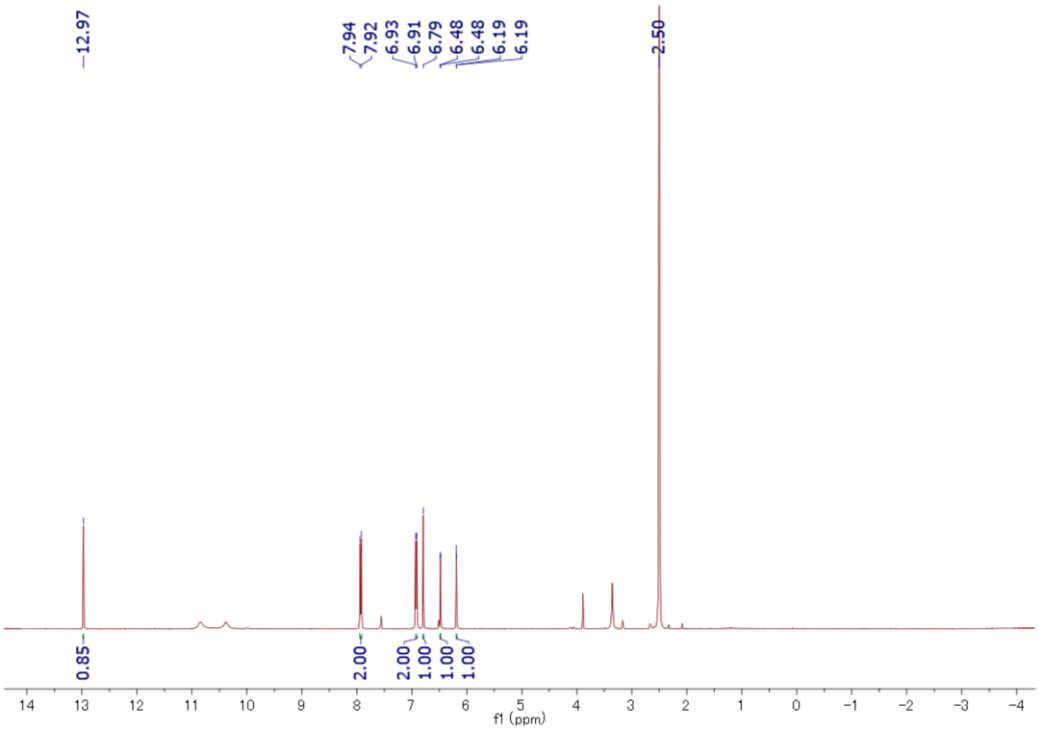
Spectrum 3 ^{1}H NMR of chrysoeriol (DMSO−*d6*)



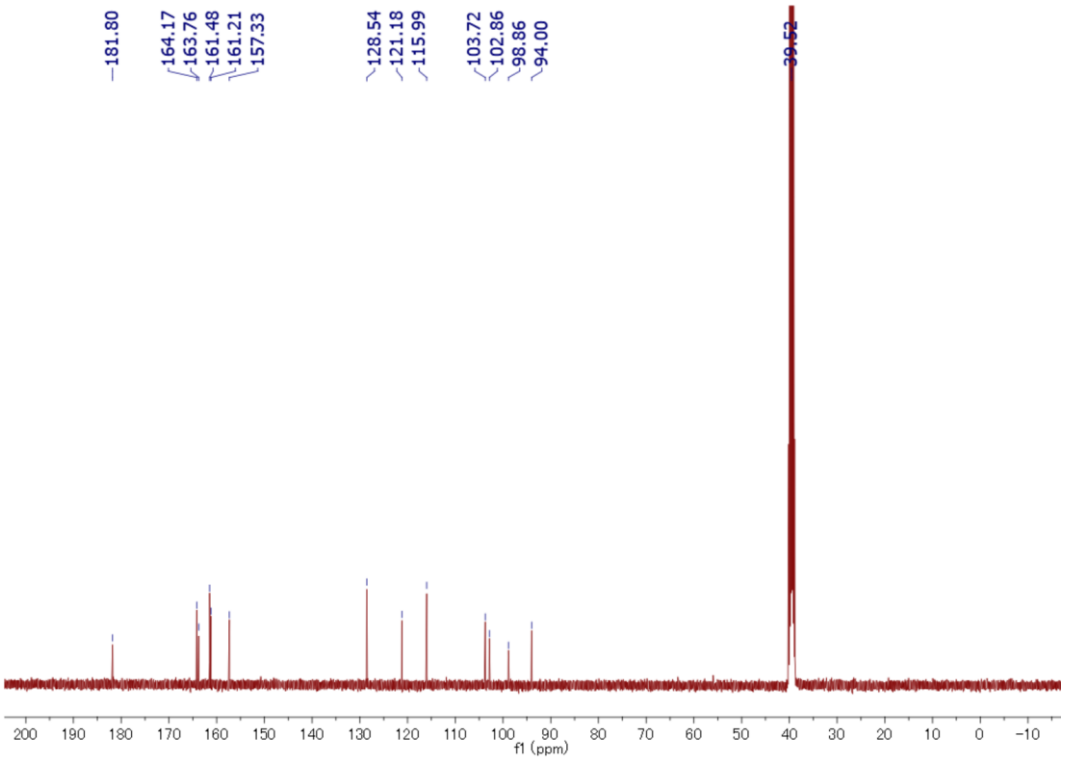
Spectrum 4 ^{1}H NMR of apigenin (CD_3OD)



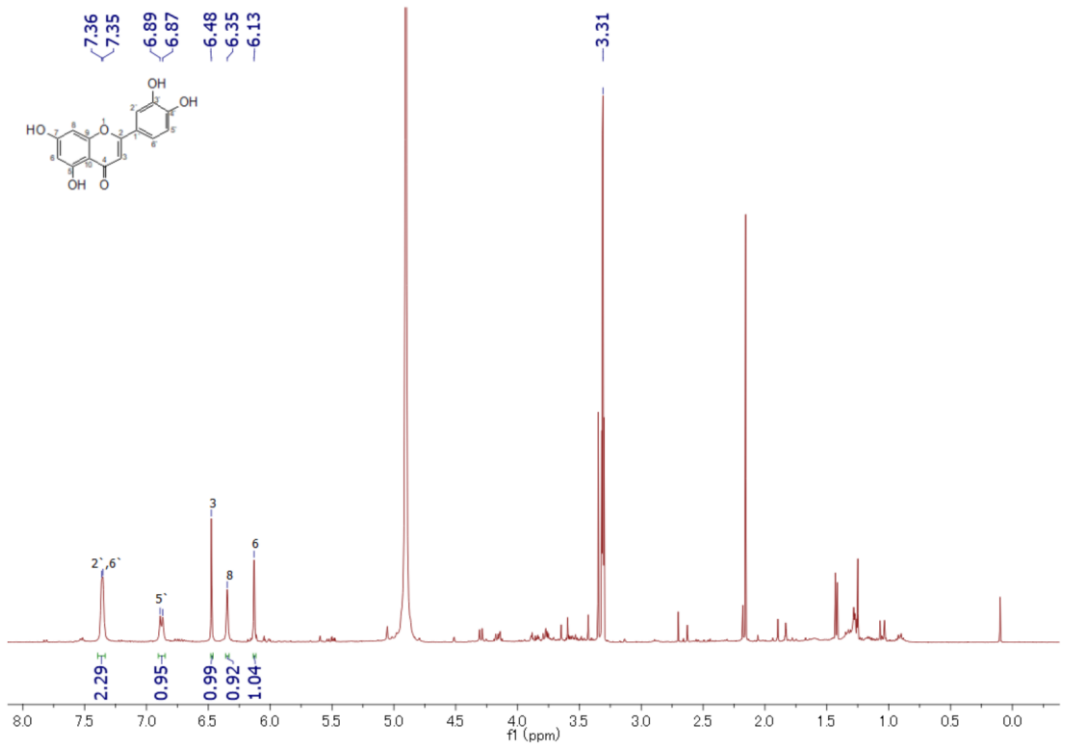
Spectrum 5 ^{13}C NMR of apigenin (CD_3OD)



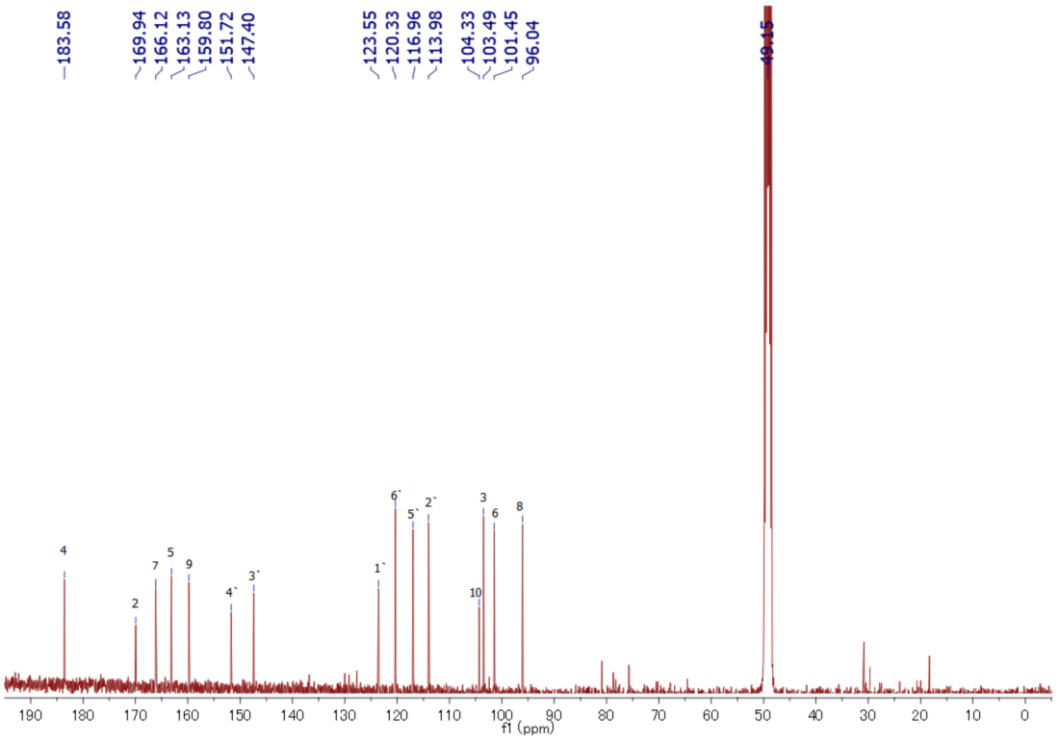
Spectrum 6 ^{1}H NMR of apigenin (DMSO−*d6*)



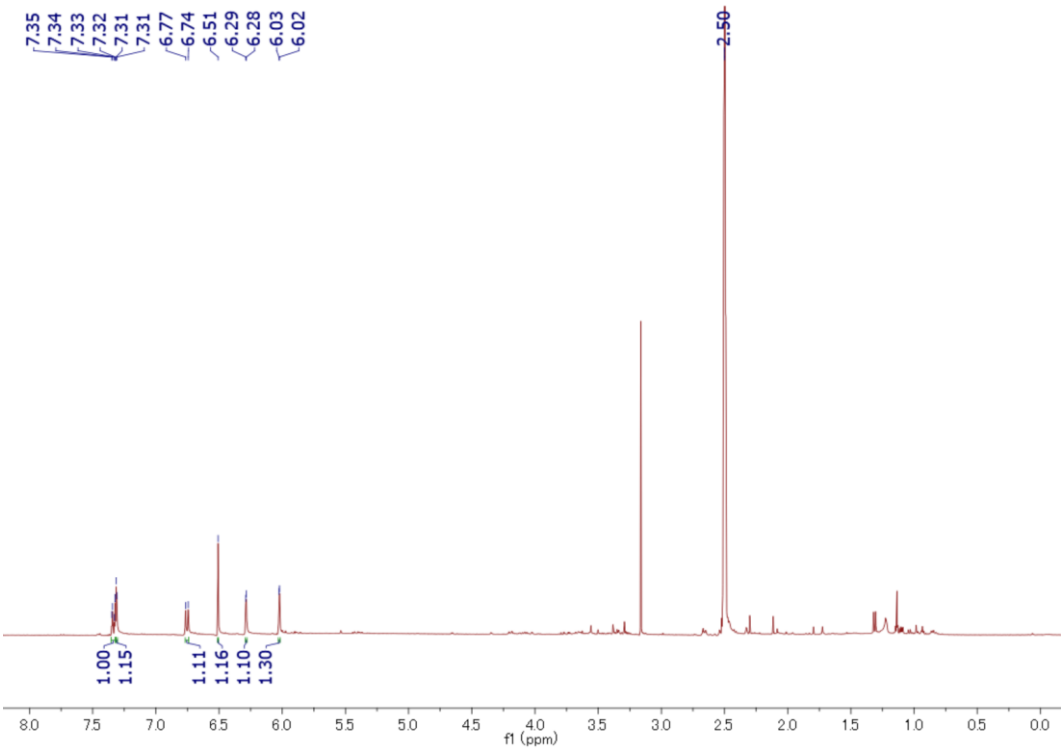
Spectrum 7 ^{13}C NMR of apigenin (DMSO−*d6*)



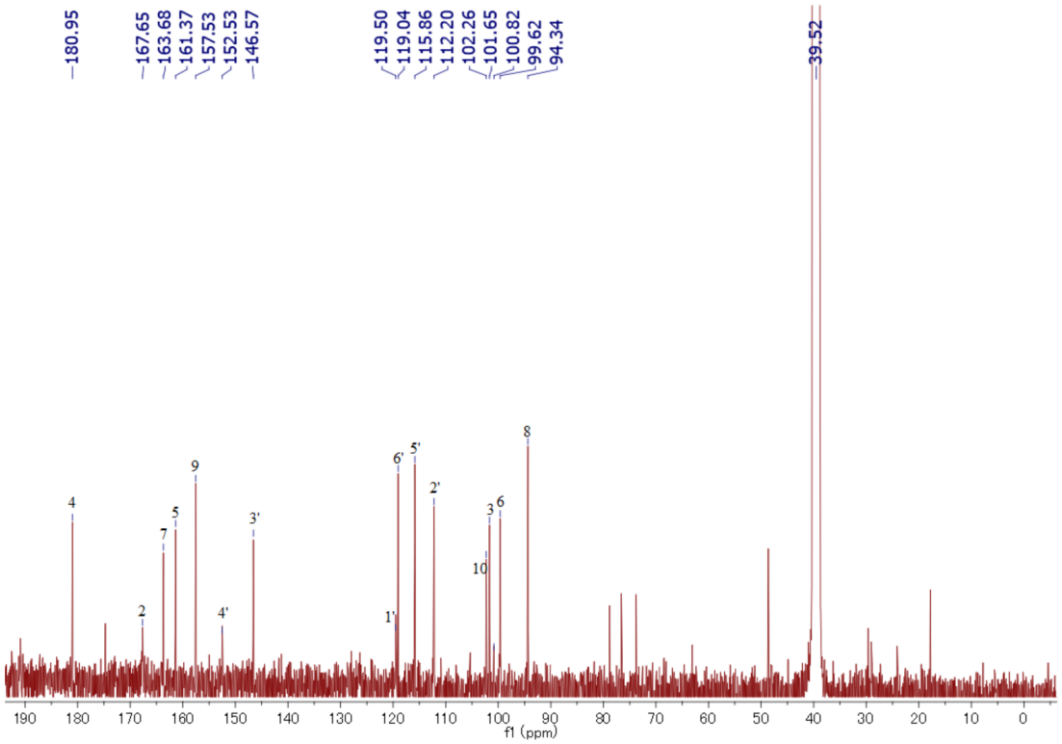
Spectrum 8 ^{1}H NMR of luteolin (CD_3OD)



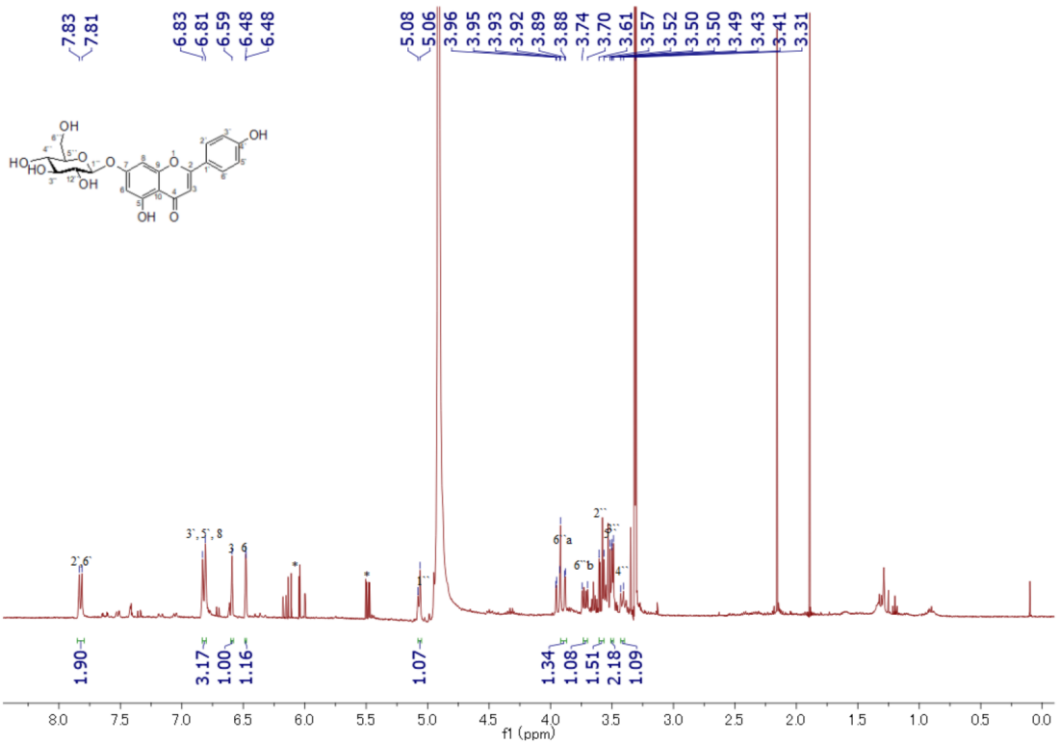
Spectrum 9 ^{13}C NMR of luteolin (CD_3OD)



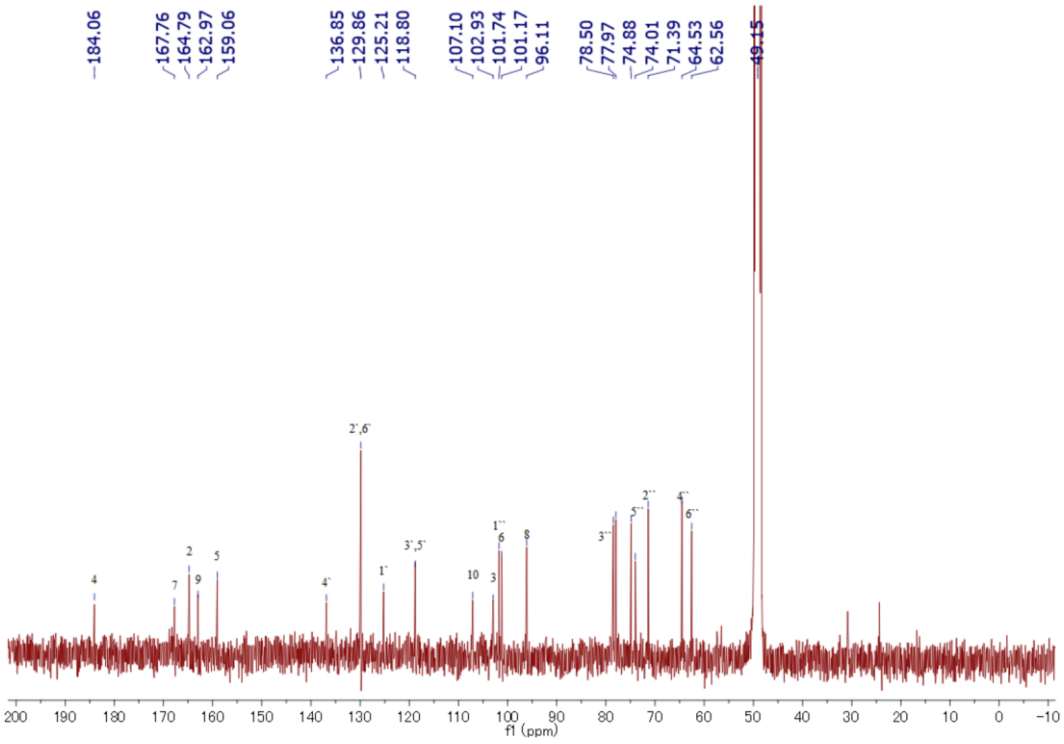
Spectrum 10 ^{1}H NMR of luteolin (DMSO−*d6*)



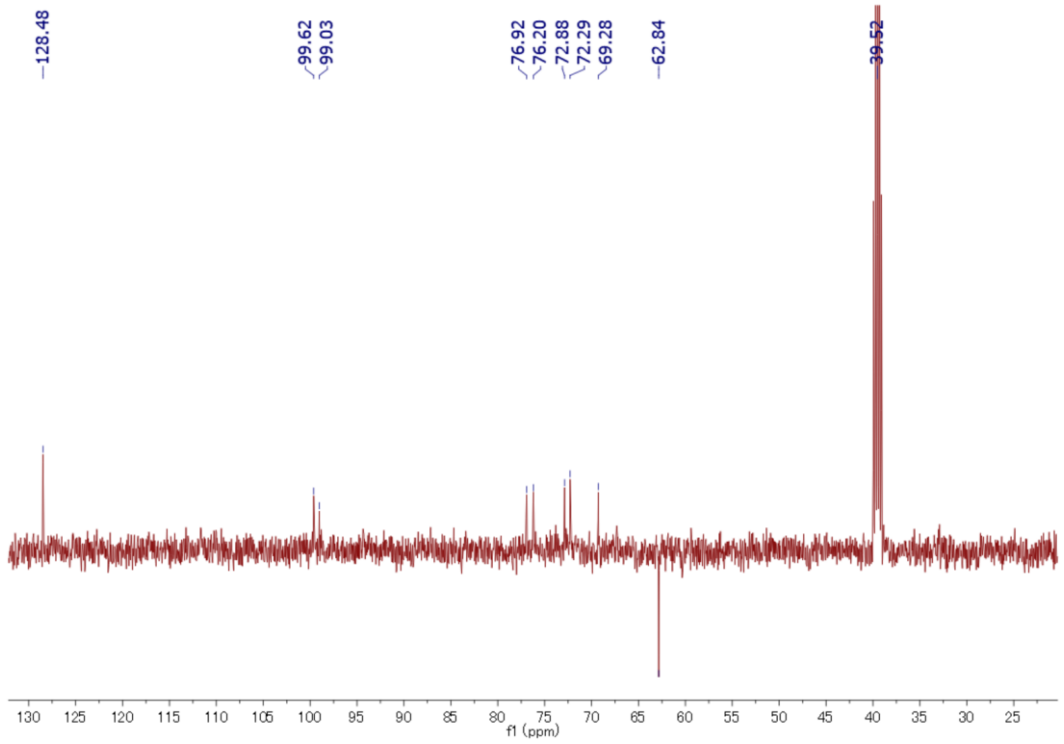
Spectrum 11 ^{13}C NMR of luteolin (DMSO−*d6*)



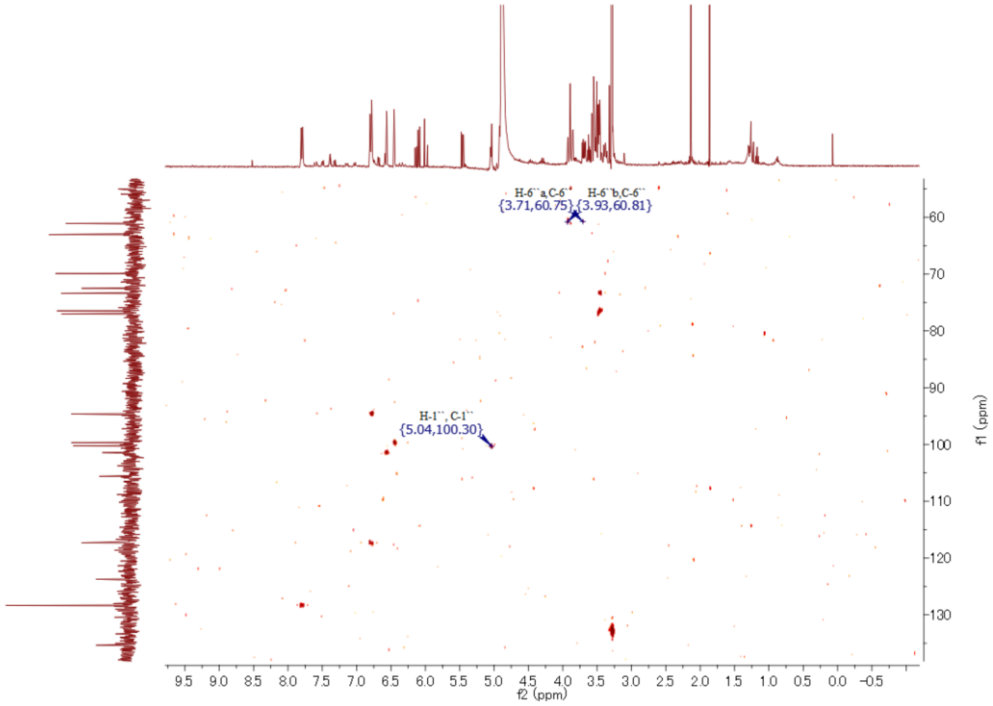
Spectrum 12 ^{1}H NMR of apigenin 7−*O*−β−glucoside (CD_3OD)



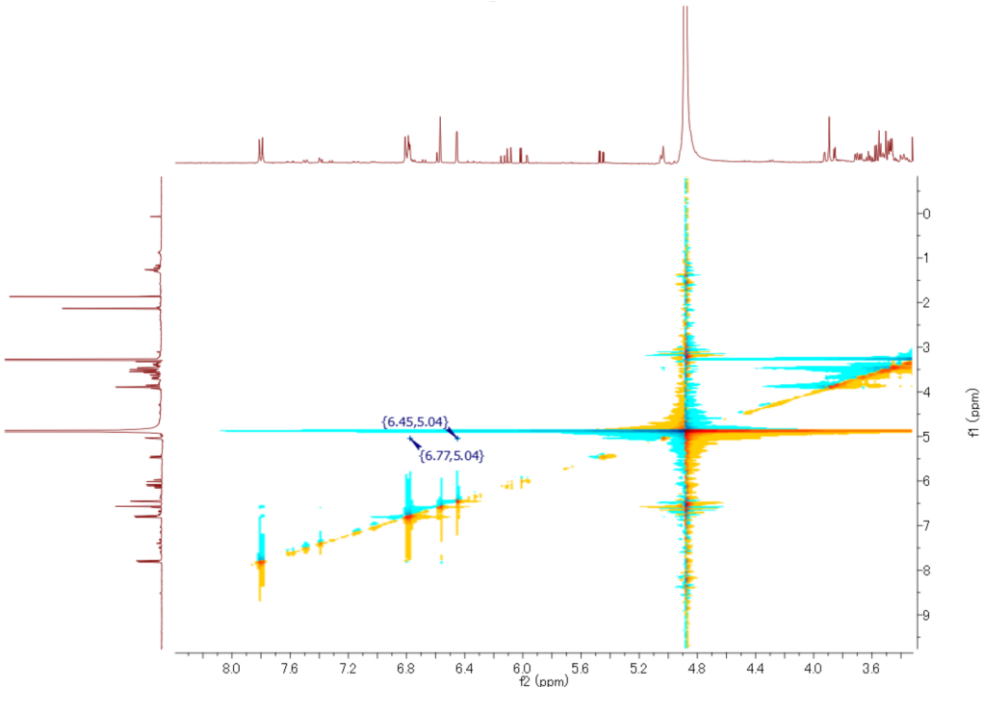
Spectrum 13 ^{13}C NMR of apigenin 7−*O*−β−glucoside (CD_3OD)



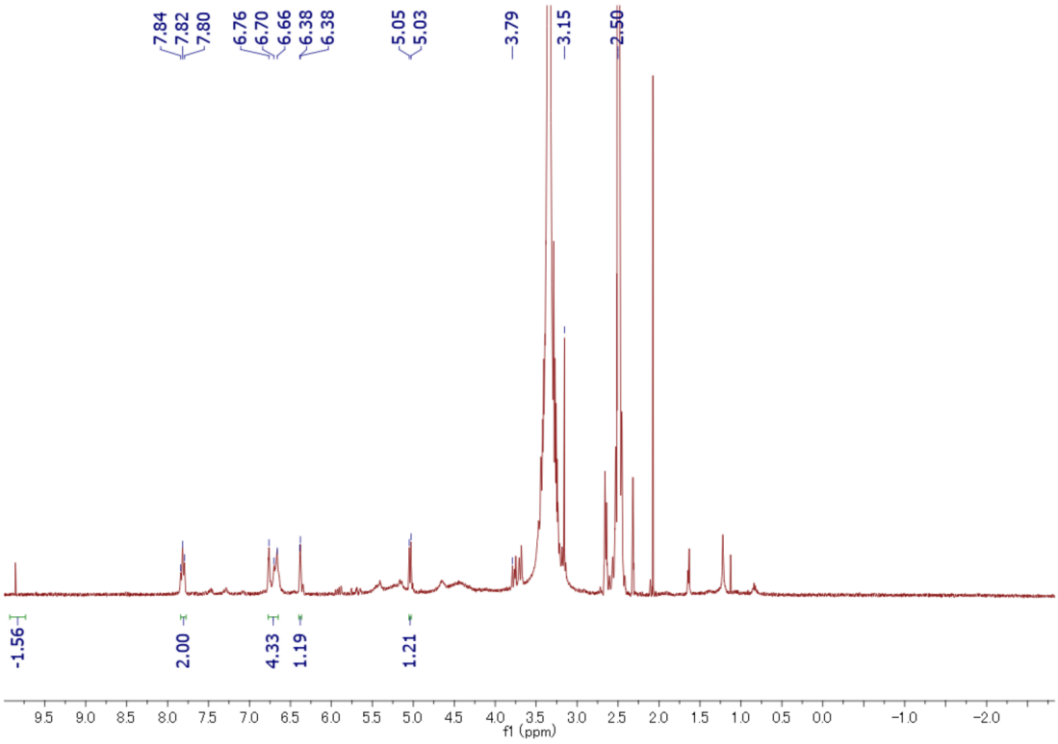
Spectrum 14 DEPT−135 of apigenin 7−*O*−β−glucoside (DMSO−*d6*)



Spectrum 15 HMQC of apigenin 7−*O*−β−glucoside (CD_3OD)



Spectrum 16 NOESY of apigenin 7−*O*−β−glucoside (CD_3OD)



Spectrum 17 ^{1}H NMR of apigenin 7−*O*−β−glucoside (DMSO−*d6*)

Chapter 3

Solenostemma argel

3.1 Introduction

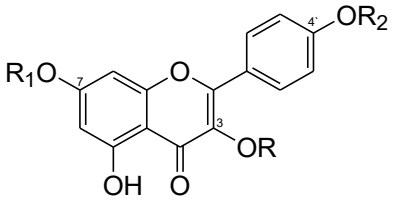
Solenostemma argel (Hayne) is belongs to the Asclepiadaceae family, distributed widely in the north of Africa and Arabian Peninsula (Fig. 4). *S. argel* is available abundantly in the north region of the Sudan, and is locally known as “Al−Hargal” [48]. It is shrub up to 60 cm with several vigorous stems and opposite leaves. The flowering period is from March to June [49].

Fig. 4 *Solenostemma argel* leaves

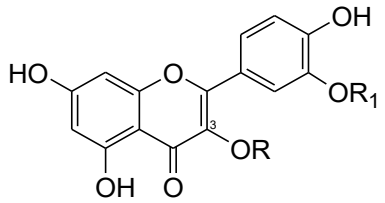
3.1.1 Phytochemicals

The leaves of *S. argel* contain 64% of carbohydrate and 6.5% of the crude fiber. Beside the moisture content (4.4 %), there are ash content (7.7 %), protein (15%), crude oil (1.6 %) phytic acid (3%) and tannin content (0.4%). Moreover, the mineral analysis of the plant leaves revealed the existence of high concentration of minerals as potassium and calcium with a moderate of magnesium and sodium (0.54, 0.06 and 0.03, 0.01 %, respectively). Other minerals are cupper, ferrous, manganese and lead (0.0001, 0.002, 0.002, and 0.001%, respectively). On the other hand, protein fractionation showed the presence of albumins, non−protein nitrogen, prolamine, globulins and glutelin with different concentrations 16.7, 15.3, 11.7, 8.7 and 6.2%, respectively

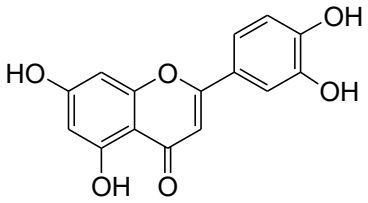
[50]. Furthermore, several classes of secondary metabolites were isolated and identified from different parts *S. argel* plant such as flavonoids and their glycosides [51-57], acylated phenolic glycosides [52, 58], polyhydroxypregnane and their glycosides [52, 53, 55, 59-61] and 15−ketopreganane glycosides namely stemmin C and stemmosides (A-K); stemmosides D-J have a 15 keto, cis CD ring junction are first pregnanes isolated from the plant [62,63,64], where such compounds were isolated only from the marine sponges [65-69]. Furthermore, unusual pregnanes known as argelosides [61] have been isolated from the plant species, the structure of these metabolites has been described in details in Fig. 5 and Fig. 6



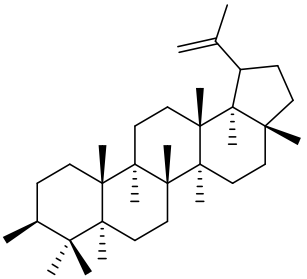
	R	R_1	R_2
Kaempferol	H	H	H
3−*O*−glucopyranoside (astragalin)	Glc	H	H
3−*O*−α−L−arabinoside	Ara	H	H
3−*O*− β−D−xyloside	Xyl	H	H
7−*O*− α−L−rhamnoside	7H	Rha	H
7−*O*− α−L−arabinoside	H	Ara	H
3,7-di-*O*−α−L−glucoside	Glc	Glc	H
3,7−di−*O*−α−L−rhamnoside	Rha	Rha	H
3,4'−di−*O*−β−D−glucoside	Glc	H	Glc
7,4'−di−*O*−β−D−glucoside	H	Glc	Glc
3−*O*−glucuronide	Glu	H	H
3−*O*−glucopyranosyl (1→6)−rhamnopyranose (3−*O*−rutinoside)	Glc(1→6)Rha	H	H
3−*O*−α−rhamnopyranosyl-(1→2)−β−glucopyranoside (3−*O*−neohesperidoside)	Glc(1→2)Rha	H	H
3−*O*−α−D−glucopyranosyl (1→2)−β−D−xylopyranoside	Glc(1→2)Xyl	H	H
3−*O*−α−L−arabinopyranosyl-(1→2)−β−D−galactopyranoside	Glc(1→2)Gal	H	H



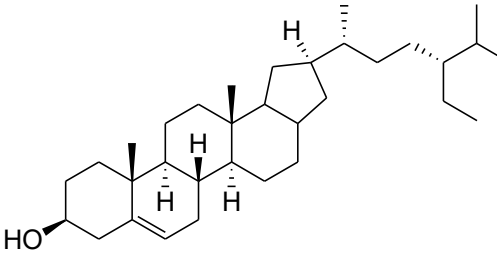
	R	R_1
Quercetin−3−β−D−glucopyranosyl-(1→6)−α−L−rhamnopyranoside (rutin)	Glc(1→6)Rha	H
Quercetin−3−*O*−α−rhamnopyranosyl-(1→2)−β−glucopyranoside (Quercetin 3−*O*−neohesperidoside)	Glc(1→2)Rha	H
Isorhamnetin−3−*O*−β−D−glucoside	Glc	CH_3
Quercetin −3−*O*−D−glucopyranoside	Glc	H



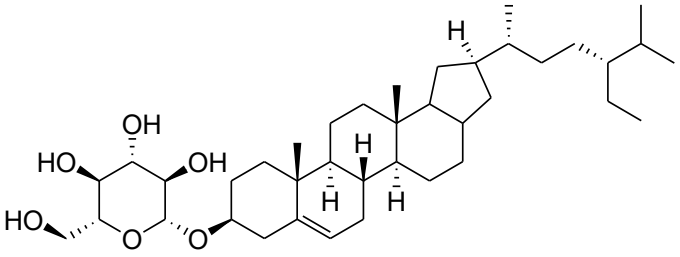
Luteolin



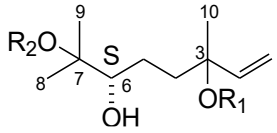
Lupeol



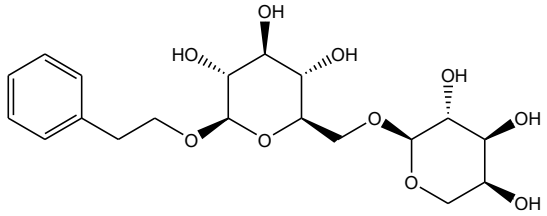
(-)−β−Sitosterol



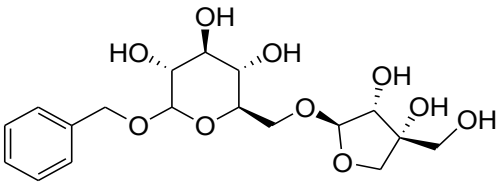
Daucosterol



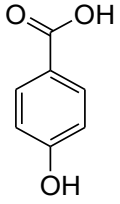
	R	**R_1**
Aglycon 6,7−dihydroxy−dihydrolinalool	H	H
6,7 dihydroxydidydrolinalool 7−*O*−β−glucopyranoside	H	β-Glc
6,7 dihydroxydidydrolinalool−3−*O*−β−glucopyranoside	β-Glc	H



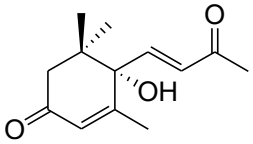
2−phenylethyl
O−α−arabinopyranosyl−(1→6)−β−glucopyranosid
e



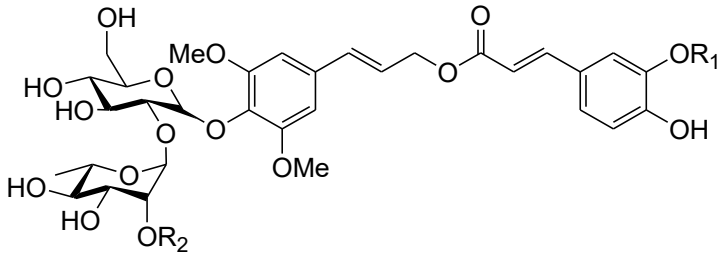
benzyl alcohol *O*−β−apio
furanosyl−(1→6)−β−glucopyranoside



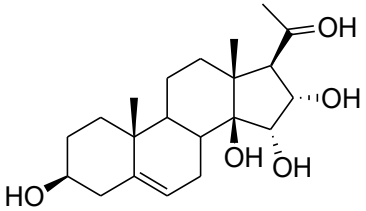
p−hydroxybenzoic acid



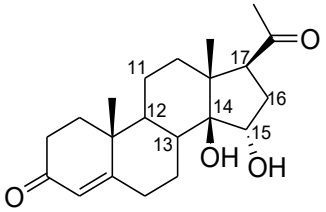
Dehydrovomifoliol



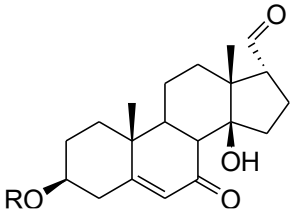
	R	R_1
solargin I	Me	H
solargin II	H	H
solargin III	Me	α-L-Rha
solargin IV	H	α-L-Rha



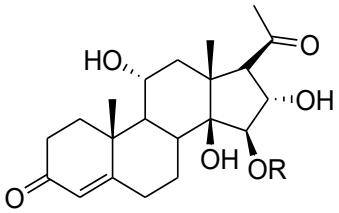
Pregnan−5−ene−20−one



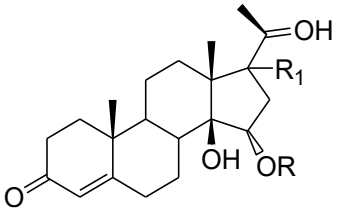
14β,15α−dihydroxy−17αH−pregn−4−ene−3,20−dione

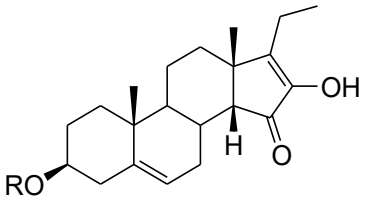


	R
Pregn−5−ene−3,14−β−dihydroxy−7,20−dione−3−*O*−β−glucopyranoside	Glc
Pregn−5−ene−3, 14−β−dihydroxy−7, 20−dione derivative	A

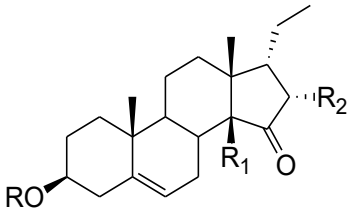


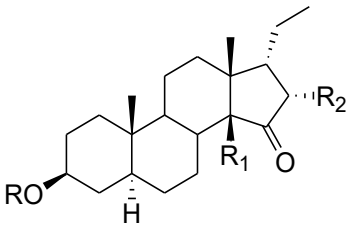
	R
14β,15β,16α,11α−tetrahydroxy−17αH−pregn−4−ene−3,20−dione	H
14β,16α,11α−trihydroxy,15β−(β−D−Xylopyranosyl(1→2)−β−D−Xylopyranoside)−17αH−pregn−4−ene−3,20−dione (solenoside A)	β−D−Xyl(1→2) β−D−Xyl



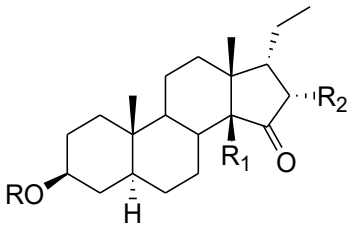


	R	R_1		R
stemmoside A	β−D−Glc	α-H	stemmoside E	B
stemmoside B	β−D−Glc	β-H	stemmoside F	C





	R	R_1	R_2		R	R_1	R_2
stemmoside C	C	OH	H	stemmoside I	B	H	OAc
stemmoside D	C	H	OAc	stemmoside J	C	H	OAc
stemmoside G	C	H	OH	stemmoside K	C	OH	H
stemmoside H	B	H	OAc				



	R	R_1		R	R_1
argeloside A	E	CH_2OAc	argeloside I	G	Me
argeloside C	D	Me	argeloside J	G	CH_2OAc
argeloside D	D	CH_2OH	argeloside K	C	Me
argeloside E	D	CH_2OAc	argeloside L	C	CH_2OAc
argeloside F	E	Me	argeloside M	F	CH_2OH
argeloside G	F	Me	argeloside N	I	Me
argeloside H	F	CH_2OAc	argeloside O	J	Me

argeloside B Stemmin C

Fig. 5 Structures of the compounds isolated from *S. argel* from the literature

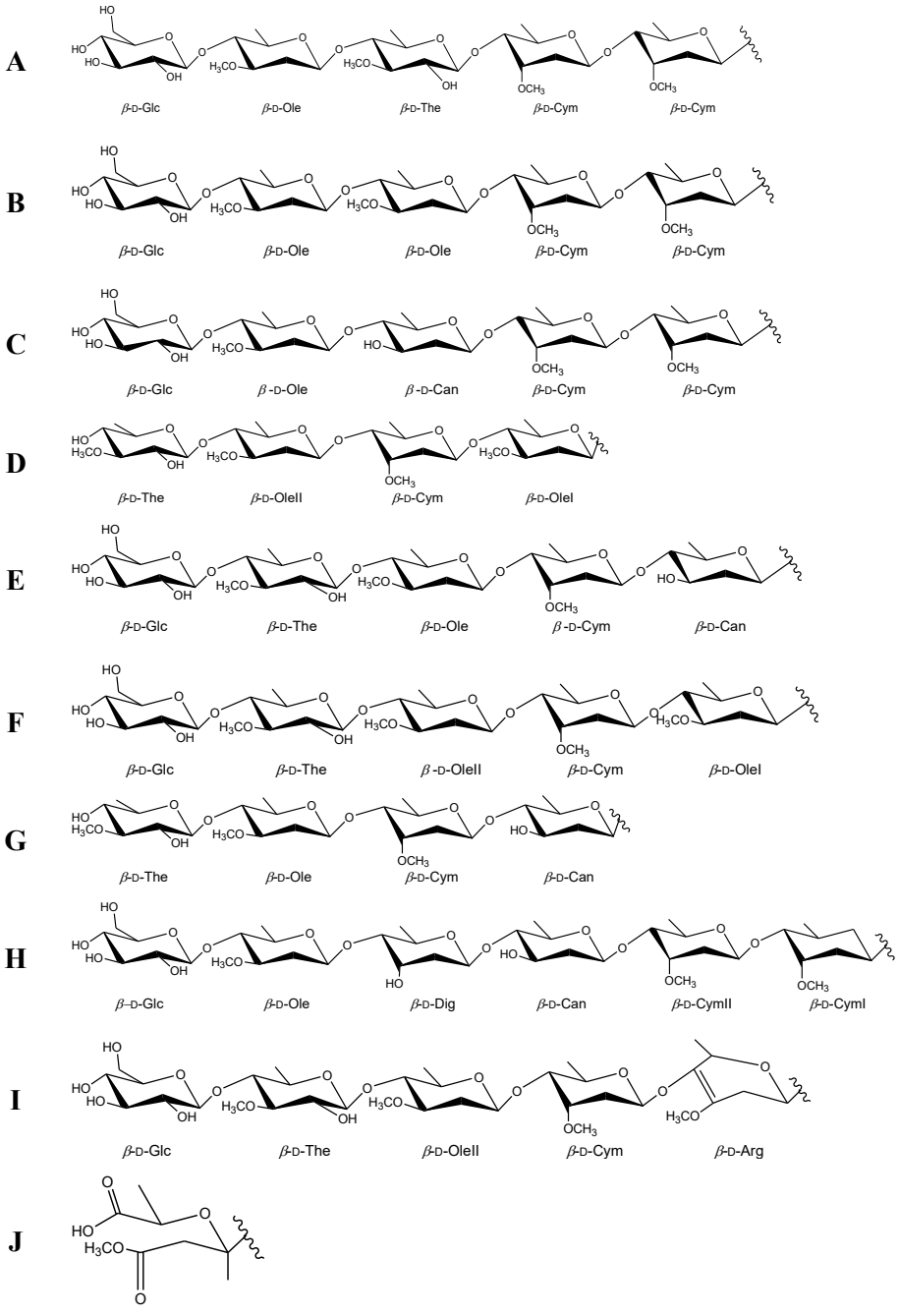


Fig. 6 Structures of the sugar portions of stemmosides

3.1.2 Pharmacological activities

S. argel is a well−known as medicinal plant, traditionally used to treat many diseases; infectious diseases such as cold, coli, urinary tract infections, stomach ache, and gastrointestinal cramps besides expectorant, a remedy for bronchitis, antipyretic, and laxative. Likewise, it is used in the treating of the hypercholesterolemia and the hepatitis A and B [61, 72]. Furthermore, several biological activities have been evaluated for *S. argel* plant. The leaves and stem methanolic and acetonic extracts exhibited anti-oxidant activity [57, 73-77]. Comparing with the methanolic extract, the isolated kaempferol−3−*O*−α−D−glucopyranosyl (1→2)β−D− xylopyranoside and kaempferol−3−*O*−α−L−arabinopyranosyl(1→2)β−D−galactopyranoside showed a moderate radical scavenging [57]. The reported studies were proved the antimicrobial activity of the aqueous extract of the leaves and methanol/water and chloroform/water extracts of the aerial parts [72, 78-80]. In addition, the plant extract showed anti−inflammatory activity [76], along with the isolated compounds (solenoside A, kaempferol 3−*O*−glucoside, kaempferol 3−*O*−rutinoside, argelosides F, L, M and O) [60, 81]. By following a bioassay−guided fractionation procedure, cholinesterase-inhibitory activity of the of *S. argel* leaves was reported. Demmak *et al.* found the isolated kaempferol and kaempferol−3−glucopyranosyl(1→6)rhamnopyranose were active on both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The kaempferol displayed the highest inhibitory effect [52]. Furthermore, several studies reported the antitumor activity of

the plant extract [82, 83]. Isolated pregnanes and their glycosides were reported to reduce the VEGF−induced KS cell proliferation [61, 64, 71]. Beside the activities mentioned before, the polar metabolites of the plant were reported to have anti−arthritic activity [84] and analgesic activity [74, 85]. In addition to the activities, anaesthetic activity [86], anti−spasmodic activity, and significant gastroprotective effects might be a promising therapy for ulcer [86-88]. The plant is rich source of nutrients and is shown to reduce the hypercholesterolemia as well as the anemia [89, 90]. The plant has also a good hypoglycemic potency [73]. Elsareh *et al.* reported in 2016 that the plant could be used in the control of the schistosomiasis, because it has molluscicide properties by affecting the egg masses and neonates of *B. pfeifferi* snails [91].

3.2 Material and methods:

3.2.1 General

Methanol (MeOH), ethanol (EtOH), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), hexane (Hex) chloroform ($CHCl_3$), acetic acid (AcOH), and butanol (BuOH) were purchased from Kanto Chemical CO., INC. and were used without purification. Thin layer chromatography (TLC) and preparative TLC were performed using Merck-KGaA silica gel 60 F_{254} pre-coated plates (0.25 mm) and 0.5 mm, respectively, and visualized using short-wave UV light, phosphomolybdic acid (PMA) stain and Hanessian's stain with heat. Column chromatography was performed using silica gel 60 N (neutral, sphere, particle size 0.063-0.210 mm). NMR spectra were recorded on JEOL 400 YH instrument and the chemical shifts reported in δ (ppm) relative to deuterated solvent (CD_3OD at 3.31 ppm ^1H NMR and 49.15 ppm ^{13}C NMR and D_2O at 4.79 ppm ^1H NMR). The following abbreviations were used to explain the ^1H-NMR data: chemical shift δ (ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad), coupling constant (*J*, Hz). Infrared (IR) spectra were recorded on Thermo fisher scientific spectrometer- Nicolet iS5 and the data were reported.

3.2.2 Plant material:

Solenostemma argel was collected from Abu-Hamad, North of Sudan-Sudan, and authenticated at the Herbarium of the Department of Phytochemistry and Taxonomy, Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), National Centre for Research, Sudan.

3.2.3 Extraction

The extraction was performed following the reported method [6] with some modifications. Leaves of the collected plant was cleaned, dried in shadow at rt, and disintegrated to coarsely powder. The dried powdered was placed in stoppered conical flask with aqueous ethanol (80%) for 3 days at room temperature with frequent agitation. The obtained mixture was then filtered and evaporated, and the marc was again macerated in aqueous ethanol 80% for 4 days at rt with frequent agitation. This daily filtration and evaporation were repeated until the soluble matter had dissolved. The combined extracts were exposed to air to complete the dryness.

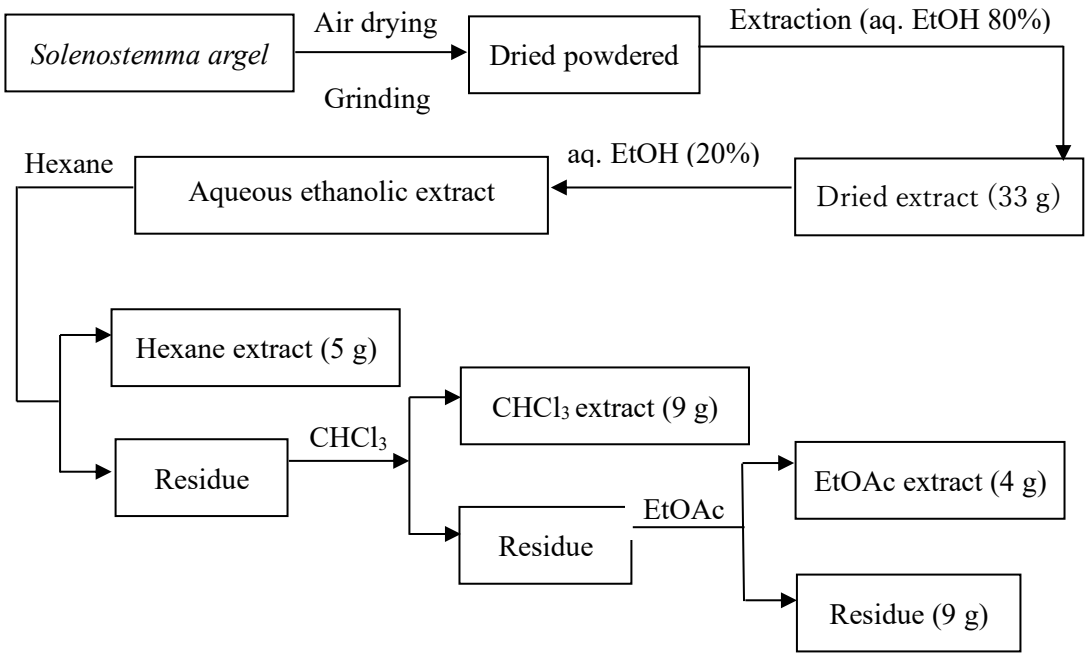
3.2.4 Liquid−Liquid fractionation

The fractionation was conducted as described in the literature [92] with slight modification. The dried ethanolic extract (33 g) was dissolved in aqueous ethanol (20%), then, hexane was added to the aqueous ethanol in the ratio 2:1 (v/v). The resulting solution was shaken in a separating funnel. The hexane layer was removed, and then hexane was freshly added to the solution. This extraction with hexane was repeated until hexane soluble materials had extracted. The combined hexane layer was concentrated under vacuum pressure to obtain hexane extract. The remained aqueous ethanol was extracted with $CHCl_3$ and ethyl acetate to obtain respective the $CHCl_3$ extract and the ethyl acetate extract. The ethanol extract was obtained by concentration of the remained aqueous ethanol layer under reduced pressure (Scheme 7).

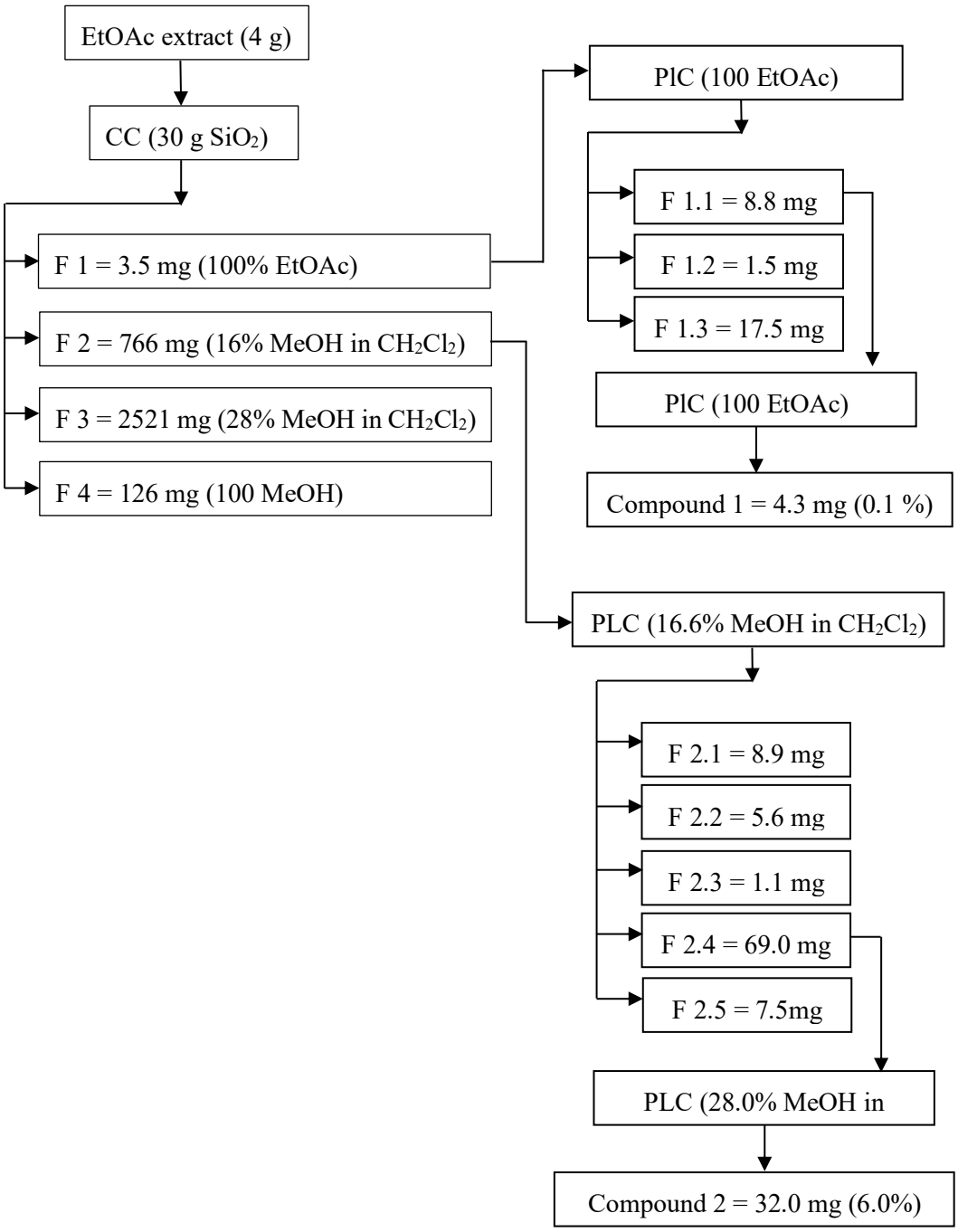
3.2.5 Purification and isolation

The ethyl acetate extract (4 g) was subjected to column chromatography using a column 3×40 cm (diameter × height) packed with silica gel (30 g). Different polarity solvents: 100% EtOAc, 16% MeOH in CH_2Cl_2, 28% MeOH in CH_2Cl_2, 37% MeOH in CH_2Cl_2, and 100% MeOH were used to get fraction 1 (F1), fraction 2 (F2), fraction 3 (F3) and fraction 4 (F4) respectively. F1 (35 mg) was further purified using PLC (10×10 cm) and eluted with 100% EtOAc, the yellow-colored band have collected, dissolved in methanol, filtered through vacuum filtration and concentrated under vacuum pressure and labelled as compound **1**. By using the same procedure, F2 (100 mg) was purified using 16.6% MeOH in CH_2Cl_2 and 28.0% MeOH in CH_2Cl_2 as eluted solvents

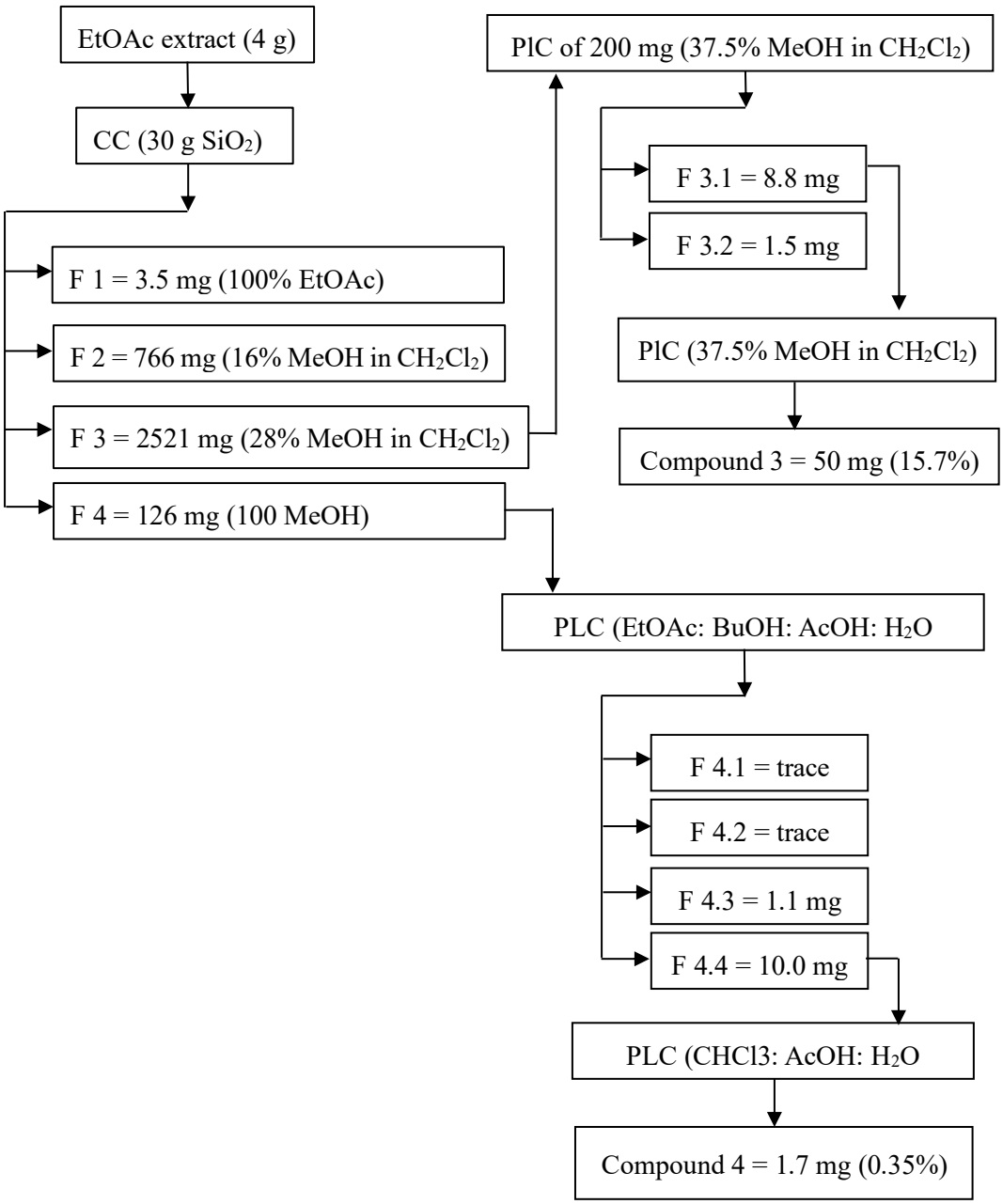
obtained compound **2** (Scheme 8), F3 (200 mg) have eluted with 37.5% MeOH in CH_2Cl_2 yielded compound **3**. Finally, F4 (2 mg) was eluted with EtOAc: BuOH: AcOH: H_2O (16:2:1:1) and $CHCl_3$: AcOH: H_2O (3:3.5:0.5) to get compound **4** (Scheme 9). Compounds (**1**–**4**) were characterized and identified by spectroscopic method including NMR and IR spectroscopy.



Scheme 1 Extraction and liquid–liquid separation of the metabolites of *S. argel*



Scheme 8 Purification and isolation of the metabolites of F 1 and F 2 of ethyl acetate extract of *S. argel*



Scheme 9 Purification and isolation of the metabolites of F 3 and F 4 of ethyl acetate extract of *S. argel*

3.3 Results and discussion

The aqueous ethanol extract (33 g) was obtained from the leaves of *S. argel* in 28% yield. By using a liquid−liquid partitioning, the dried extract has been fractionated with hexane, $CHCl_3$ and EtOAc solvent in the sequence. The fatty acids and pigments were removed by hexane and $CHCl_3$ to avoid the interference during instrumental analysis. Compared with other extracts, the EtOAc extract was proved to be lower yield (Scheme 1). The ethyl acetate extract was subjected to column chromatographic separation and preparative TLC (PLC) to yield four compounds (**1**–**4**). By comparison, compound **3** was obtained in high yield (15.7 %) (Scheme 9), whereas compound **1** was yielded in only 0.1% yield in the EtOAc extract (scheme 8). Thus, the dried powdered of the plant leaves contained compound **1** (0.002%), compound **2** (0.2%), compound **3** (0.5 %) and compound **4** (0.001%). The structure of these compounds was determined based on their IR and NMR spectra in comparison with the reported data.

Compound **1** was isolated as faint yellow solid. The TLC chromatogram showed one compound when using CH_2Cl_2/MeOH 5/1 (R_f = 0.79) which gives yellow visible color, pink UV 254 nm, and orange color with PMA, Hanessian's stains with heating. The IR spectrum exhibited absorptions of hydroxyl group (3319 cm^{-1}), α, β-unsaturated ketone (1652 cm^{-1}) and aromatic ring (1510, 1444 cm^{-1}) functions (Table 5). The ^1H NMR spectrum displayed two doublet signals at δ 6.39 and 6.17 ppm for aromatic protons H-8 and H-6, respectively. They are meta to each other

because of the coupling constant values (*J*) is 2.0 Hz, and doublet signals at δ 8.08 and 6.90 ppm showed the ortho position of the protons H-2', 6' and H-3', 5', This is evidenced by the coupling constant values (*J*) 8.8 and 8.9 Hz, respectively. Also, the ^{13}C NMR spectrum indicated the presence of 13 carbon atom (Table 7). Analysis of ^{1}H and ^{13}C NMR spectra and comparison with the values found in the literature [52, 93] led to prove compound **1** as kaempferol. The yield of kaempferol isolated from the stem of *S. argel* was 0.009 % [57], and 0.0086% whole plant [94]. Kaempferol in leaves of *S. argel* was proved to be lower yield (0.002 %).

Compound **2** was obtained as yellow solid and was appeared one spot on the TLC when developed with CH_2Cl_2/MeOH 5/1 (R_f = 0.32). The spot gave pink by irradiation of UV (254 nm), orange color when using PMA, Hanessian's reagents with heating. The IR spectrum exhibited absorptions of hydroxyl group (3323 cm^{-1}), α, β-unsaturated ketone (1653 cm^{-1}) and aromatic ring (1496, 1447 cm^{-1}) functions (Table 5). ^{1}H NMR of compound **2** showed signals corresponding to the kaempferol (Table 6). The NMR spectra exhibited one anomeric proton at 5.22 (H−1") which was correlated with the carbon signal at 104.3 (C−1") in the HMQC (Spectrum 21). The presense of *β*-sugar moiety (*J* = 6.4 Hz) was confirmed with the correlation with C−3 of the kaempferol at 135.0. HMBC analysis established the linkage point of kaempferol and sugar moiety (spectrum 22). Additionally, NMR spectra showed two geminal protons at δ 3.55 (H−6"a) and 3.67 (H−6"b) correlated with one carbon at δ 62.5 (C−6") in HMQC (Spectrum 21). The signals were

corresponded to methylene group, which was also evidenced by DEPT 135 (spectrum 4). Also, HMQC of compound **2** showed that C-4″ correlated with proton at chemical shift regions 3.31–3.34 ppm. This data indicated that H-4″ overlapped with the protons of the solvent used (CD_3OD). Based on the NMR data and the comparison with reported data [52, 95−98], the structure of compound **2** was identified as kaempferol 3-*O*-β-D-glucopyranoside. Furthermore, the yield in the dried powdered of the leaves was found to be (0.2 %), which is higher than kaempferol-3-*O*-β-D-glucopyranoside isolated from the aerial parts of the plant (0.04 %) [55].

Compound **3** was isolated as yellow solid and was appeared one spot on TLC when developed with CH_2Cl_2/MeOH 5/2 (R_f = 0.34). The spot gave pink color when irradiated by UV 254 nm, and gave orange color when using PMA, Hanessian's, stains with heating and no visible color observed. The IR spectrum exhibited absorptions of hydroxyl group (3295.9 cm^{-1}), *α*, *β*-unsaturated ketone (1659.6 cm^{-1}) and aromatic ring (1497.7, 1446.6 cm^{-1}) functions (Table 5). NMR spectra showed signals corresponding to aglycone kaempferol and *β*-glucose (Table 6 and 7) with additional signal characteristic to anomeric proton at δ 5.23 ppm (H-1‴) with coupling constant (*J*) 2.0 Hz indicating the α-correlation of the sugar moiety and anomeric carbon of glucoside at δ =102.8 (C-1‴). Also, ^{1}H NMR showed a doublet signal at δ = 0.95 (3H, H-6‴) correlated with the carbon signal at δ = 17.7 (C-6‴) in HMQC spectrum (Spectrum 25) and correlated with the carbon signal at δ = 70.7 (C-5‴) in HMBC spectrum (Spectrum 26). These

signals were corresponded to methyl group. These data showed the sugar moiety is *α*-rhamnosyl. Finally, the *α*-rhamnosyl anomeric proton H-1‴ (δ = 5.23) correlated with C-2″ (δ = 80.2) of the glucosyl unit in the HMBC spectrum (Spectrum 26). The analysis of NMR data of compound **3** and comparison with the reported data [96, 98] led to the assignment of compound **3** as kaempferol-3-*O*-[*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl] synonymous with kaempferol 3−*O*−neohesperidoside. Furthermore, ^{1}H and ^{13}C NMR of compound **3** were measured with deuterated oxide (D_2O) and compared with NMR spectra obtained in CD_3OD. The results showed the protons 2′,6′ and 3′,5′ signals were shifted up-filed, also the signals of the protons 6 and 8 were extremely disappeared and the signals of sugar protons were overlapped (Spectrum 28) whereas ^{13}C NMR spectrum showed the same signals (Spectrum 29). On the other hand, comparing with the reported data, compound **3** was showed a higher yield (0.5 %) in the dried powdered of the leaves of *S. argel.*

Compound **4** was obtained as crystal. The TLC showed one spot with $CHCl_3$: AcOH: H_2O (3:3.5:0.5) (R_f = 0.26) which give pink with UV (254 nm) and blue color when using PMA and Hanessian's stains with heating. The IR spectrum shown strong peaks at 3314.59 and between 1110.43 and 1049.09 cm^{-1} due to O-H and C-O stretching, respectively. NMR spectra exhibited two sugar moiety, one anomeric proton resonance occur at δ = 5.34 (1H, d, 4.0 Hz, H-1″) (Table 6) as well as signals at δ = 92.1 (C-1″) attributed to the anomeric carbon. In addition, ^{13}C NMR

showed 11 carbon resonance, 3 of which assigned to three methylene carbon, which confirmed by DEPT 135 spectrum (Spectrum 30). Based on the comparison of ^{1}H NMR spectrum of compound **4** with ^{1}H NMR spectrum of authentic sucrose (Spectrum 31) and reported data [99], the compound **4** was characterized as sucrose.

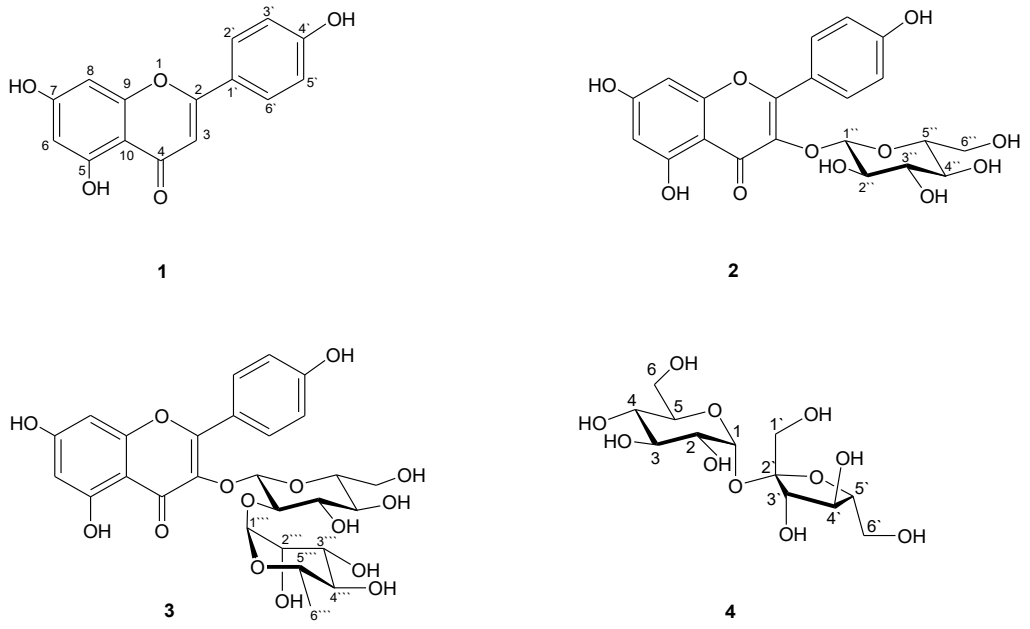


Fig. 7 Structures of compounds isolated from *S. argel*

Table 5 IR data of compound **1**, **2**, **3** and **4** (MeOH, cm^{-1})

NO	1	2	3	4
1	3319.9	3323.07	3295.92	3314.59
2	2925.0	2931.85	2931.28	2926.40
3	1652.8	2360.32	1659.64	1558.17
4	1605.26	1653.26	1607.15	1417.62
5	1564.69	1605.34	1497.65	1260.12
6	1509.59	1496.77	1446.58	1110.43
7	1444.0	1447.08	1358.90	1049.09
8	1367.56	1359.81	1280.48	995.21
9	1312.56	1283.59	1209.52	927.56
10	1252.05	1208.14	1176.46	665.35
11	1222.81	1178.28	1132.34	607.63
12	1167.78	1069.93	1057.24	585.15
13	1116.76	1017.35	1019.06	573.21
14	1087.18	892.27	889.73	561.89
15	1005.20	841.57	837.91	–
16	977.06	641.05	810.05	–
17	882.16	582.11	6238.87	–
18	836.78	–	582.99	–
19	703.49	–	–	–
20	684.27	–	–	–

Table 6 ^{1}H NMR of compound **1**, **2**, **3** and **4** (400 MHz, CD_3OD, rt)

proton	**1**	**2**	**3**	**4**[a]
	δ (H, m, *J* in Hz)	δ (H, m, *J* in Hz)	δ (H, m, *J* in Hz)	δ (H, m, *J* in Hz)
1	–	–	–	5.35 (1, d, 8.4)
2	–	–	–	3.50 (1, dd, 8.0, 4.0)
3	–	–	–	3.71 (1, t, 12.0, 8.0)
4	–	–	–	3.42 (1, t, 8.0, 12.0)
5	–	–	–	3.78 (1, dd, 4.0)
6	6.09 (1, d, 2.0)	6.17 (1, d, 4.0)	6.13 (1, s)	3.75 (4, m)
7	–	–	–	–
8	6.27 (1, d, 2.0)	6.35 (1, d, 2.0)	6.31 (1, s)	–
9	–	–	–	–
10	–	–	–	–
	–	–	–	–
1′	–	–	–	3.60 (2, s)
2′	8.08 (2, d, 8.8)	8.06 (2, d, 8.0)	8.05 (2, d, 8.0)	4.16 (1, d, 8.0)
3′	6.90 (2, d, 8.9)	6.89 (2, d, 8.0)	6.89 (2, d, 8.0)	4.00 (1, t, 8.0)
4′	–	–	–	3.82 (1, dd, 8.0, 4.0)
5′	6.90 (2, d, 8.9)	8.06 (2, d, 8.0)	6.89 (2, d, 8.0)	3.75 (4, m)
6′	8.08 (2, d, 8.8)	6.89 (2, d, 8.0)	8.05 (2, d, 8.0)	–
1″	–	5.22 (1, d, 6.4)	5.74 (1, d, 8.0)	–
2″	–	3.47 (1, t, 8.0)	3.60 (1, m)	–
3″	–	3.39 (1, t, 8.0)	3.55 (1, m)	–
4″	–	-	3.35 (1, brs)	–
5″	–	3.22 (1, ddd, 9.6, 4.0, 2.4)	3.23 (1, m)	–
6‴a	–	3.70 (1, dd, 8.0, 4.0)	3.72 (1, dd, 4.0)	–
6‴b	–	3.55 (1, dd, 8.0, 4.0)	3.51 (1, m)	–
1‴	–	–	5.23 (1, brs)	–
2‴	–	–	4.00 (1, m)	–
3‴	–	–	3.77 (1, dd, 4.0)	–
4‴	–	–	3.33 (1, brs)	–
5‴	–	–	4.03 (1, dd, 8.0)	–
6‴	–	–	0.95 (3, d, 8.0)	–

[a] (400 MHz, D_2O, rt)

Table 7 ^{13}C NMR of compound **1**, **2**, **3** and **4** (100 MHz, CD_3OD, rt)

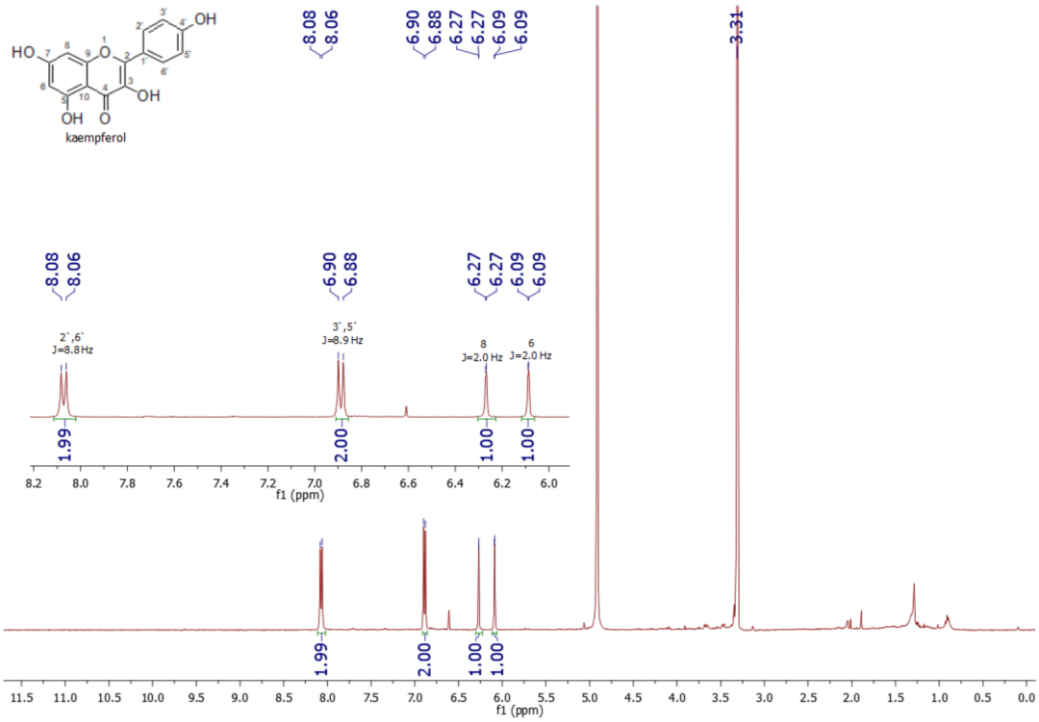
carbon	1	2	3	4[a]
1	–	–	–	92.21
2	148.11	158.80	161.56	71.10
3	137.27	135.51	134.26	72.60
4	177.49	179.44	179.16	69.25
5	158.45	163.13	163.10	72.44
6	99.57	100.66	100.36	60.15
7	166.24	168.09	169.87	–
8	94.70	95.33	95.64	–
9	162.66	158.94	158.05	–
10	104.56	105.31	104.95	–
1′	123.89	122.94	123.29	61.38
2′	130.82	132.40	132.16	103.72
3′	116.45	116.23	116.27	76.44
4′	160.73	161.76	158.84	74.03
5′	116.45	116.23	116.27	81.40
6′	130.82	132.40	132.16	62.39
1″	–	104.32	101.18	–
2″	–	75.86	80.16	–
3″	–	78.18	79.08	–
4″	–	71.44	71.89	–
5″	–	78.57	78.50	–
6″	–	62.72	62.67	–
	–			–
1‴	–	–	102.75	–
2‴	–	–	72.52	–
3‴	–	–	72.38	–
4‴	–	–	74.18	–
5‴	–	–	70.07	–
6‴	–	–	17.67	–

[a] (100 MHz, D_2O, rt)

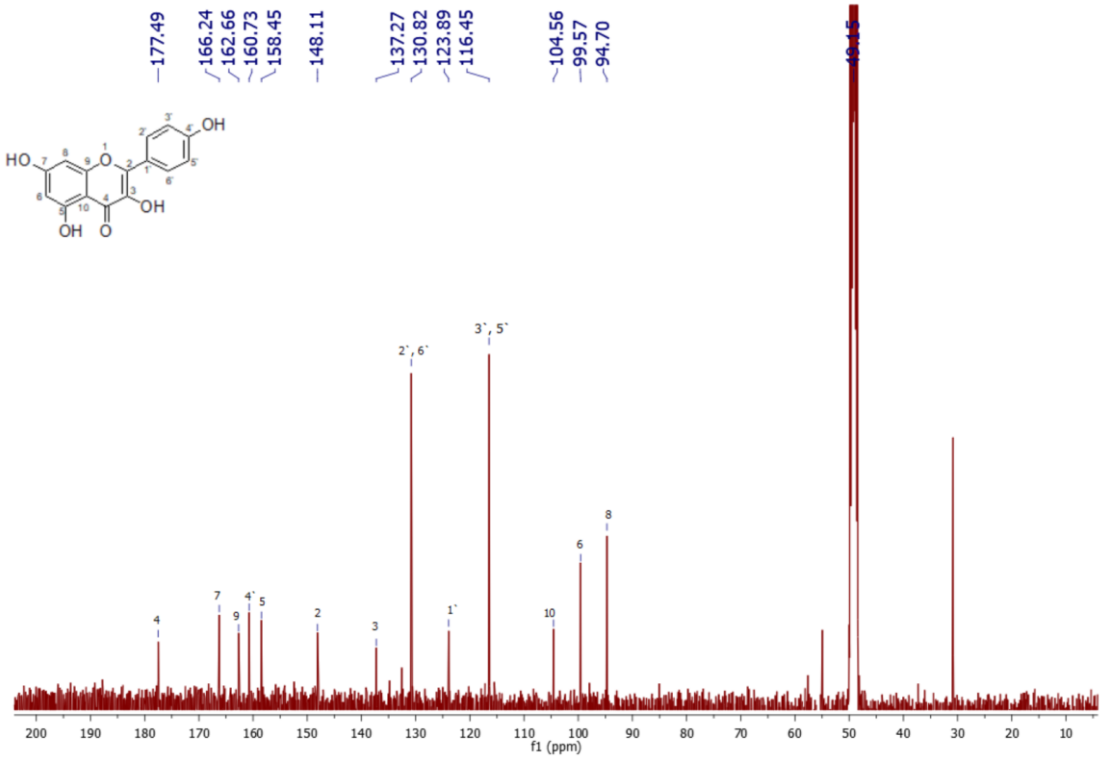
3.4 Conclusion

Four compounds (**1**–**4**) of the ethyl acetate extract of the leaves of *S. argel* were successfully purified, isolated and identified. The yields of compounds (**1**–**4**) in the ethyl acetate extract and dried of the leaves were determined. The compounds were identified as kaempferol and its glucosides **(1-3)** and sucrose **(4)**. The kaempferol and its glucosides have been reported to possess pharmacological properties that can be of therapeutic benefit in treating diseases such as compound **1** has cholinesterase inhibitory activity [52], and also compound **3** can used for the treatment of diabetes mellitus [98]. The extraction methods reported here must be useful for the isolation of these compounds.

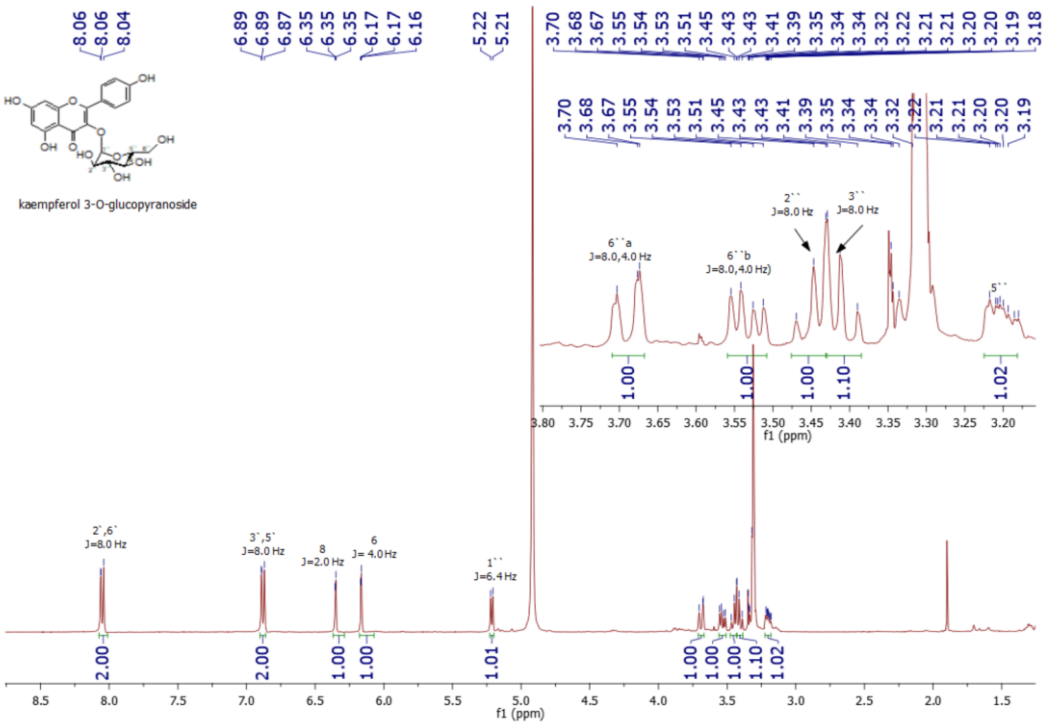
3.5 NMR spectra of the isolated compounds



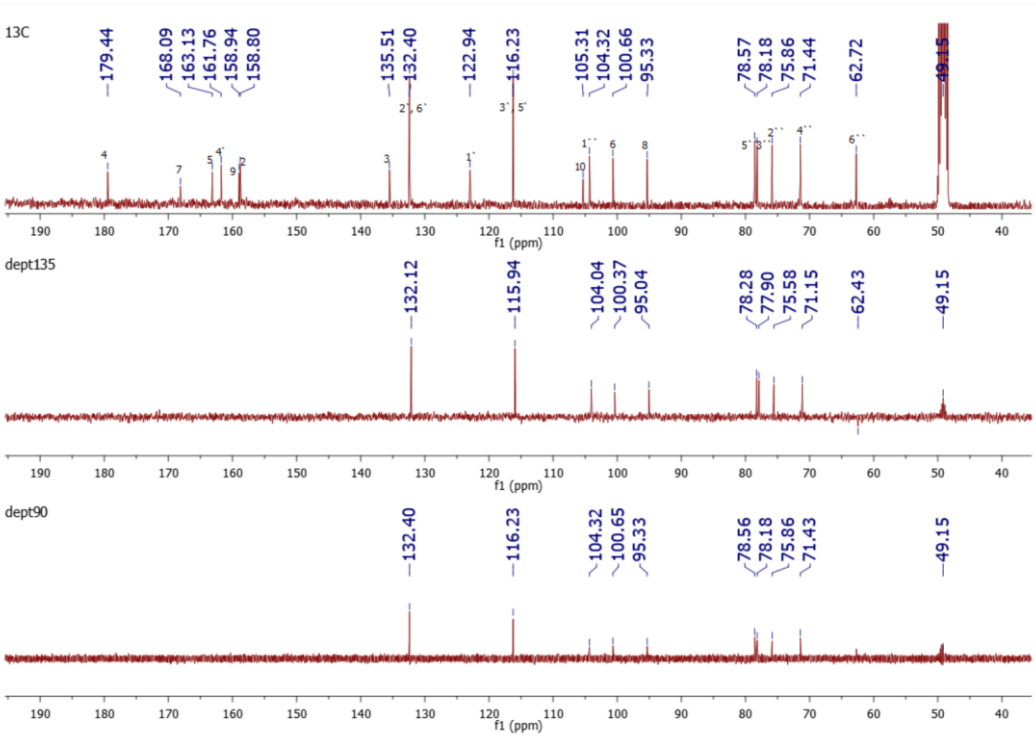
Spectrum 18 ^{1}H NMR spectrum of compound 1



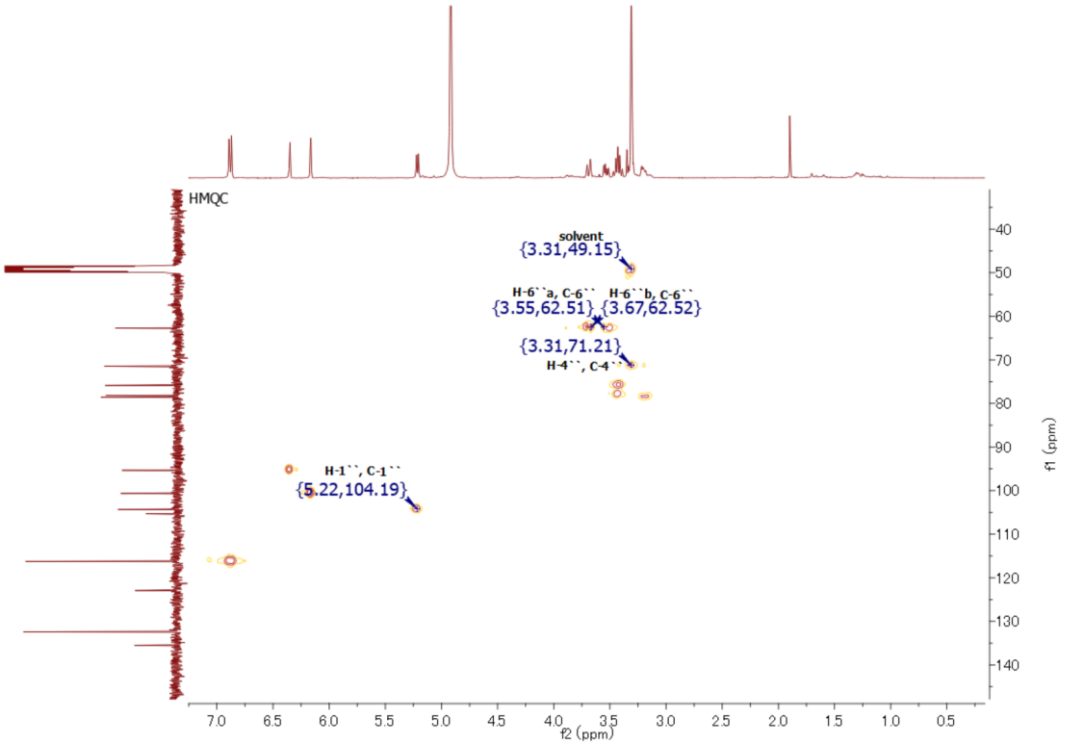
Spectrum 19 ^{13}C NMR spectrum of compound 1



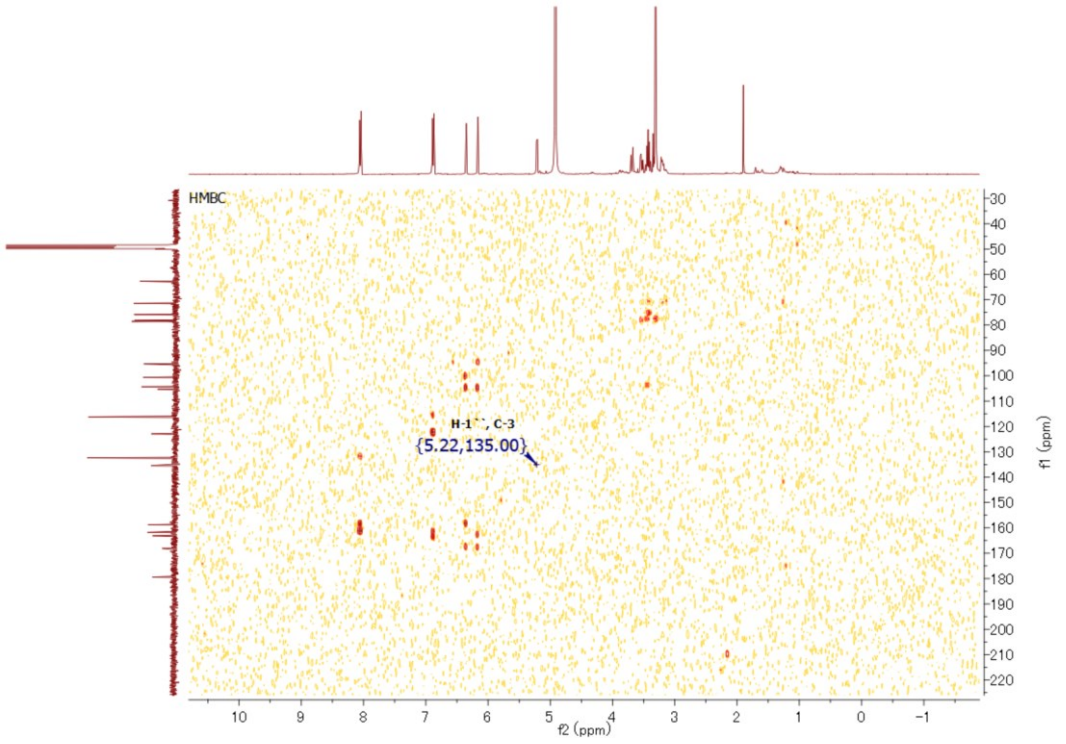
Spectrum 20 ^{1}H NMR spectrum of compound 2



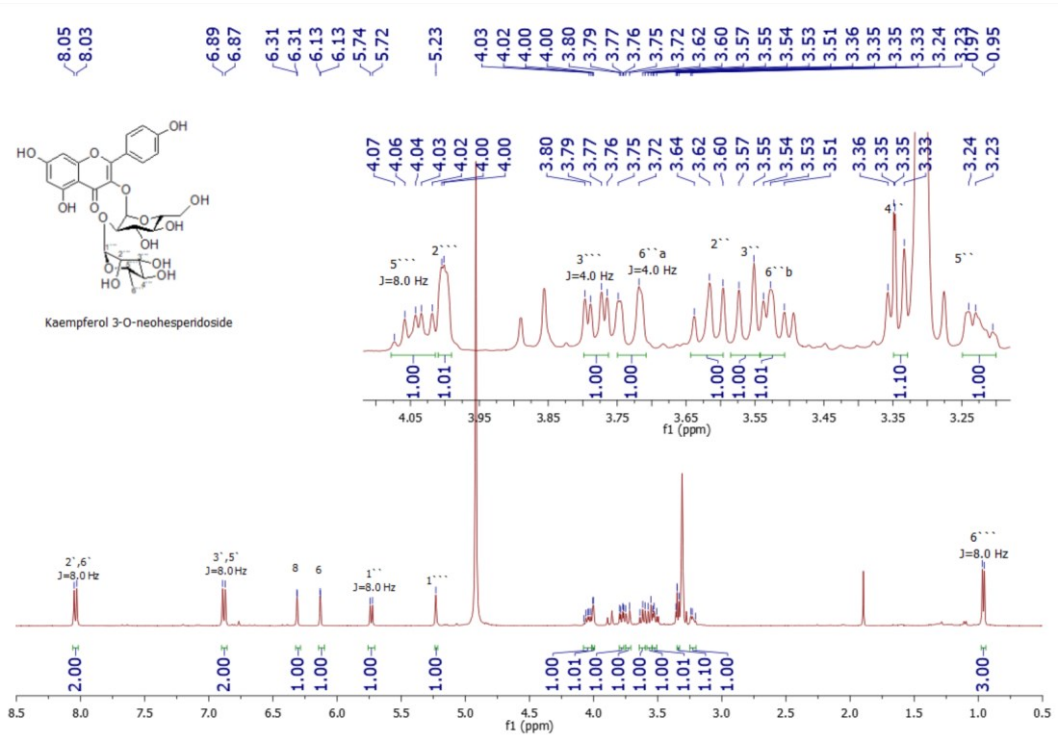
Spectrum 21 ^{13}C, DEPT 135, DEPT 90 NMR spectra of compound 2



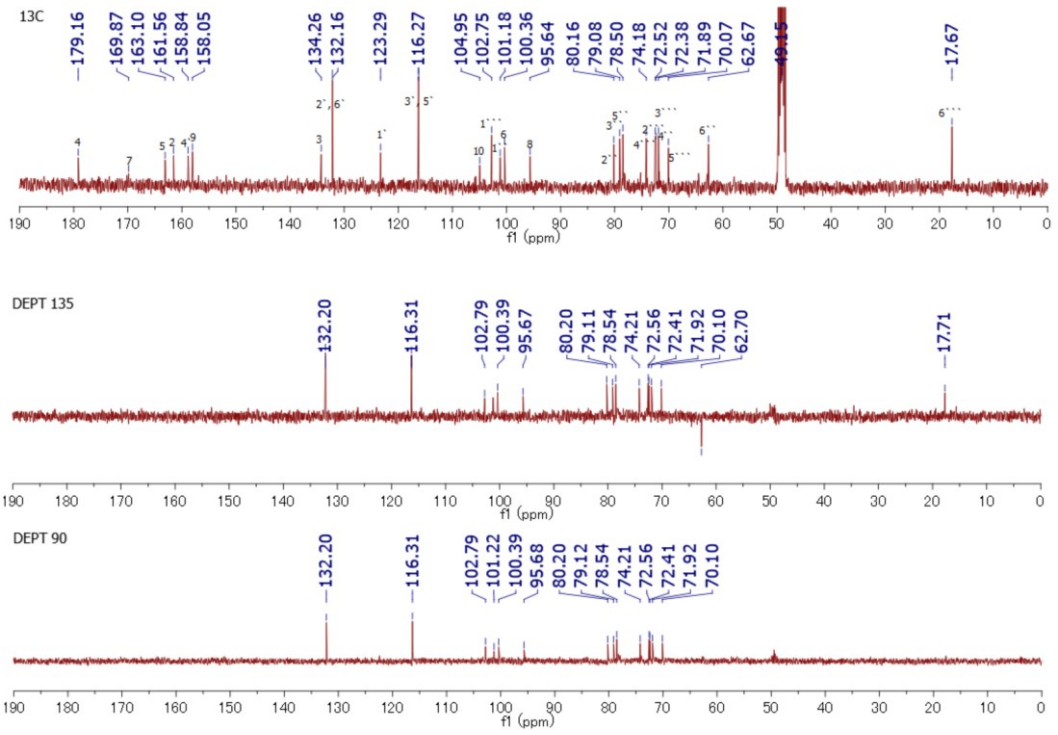
Spectrum 22 HMQC NMR spectrum of compound 2



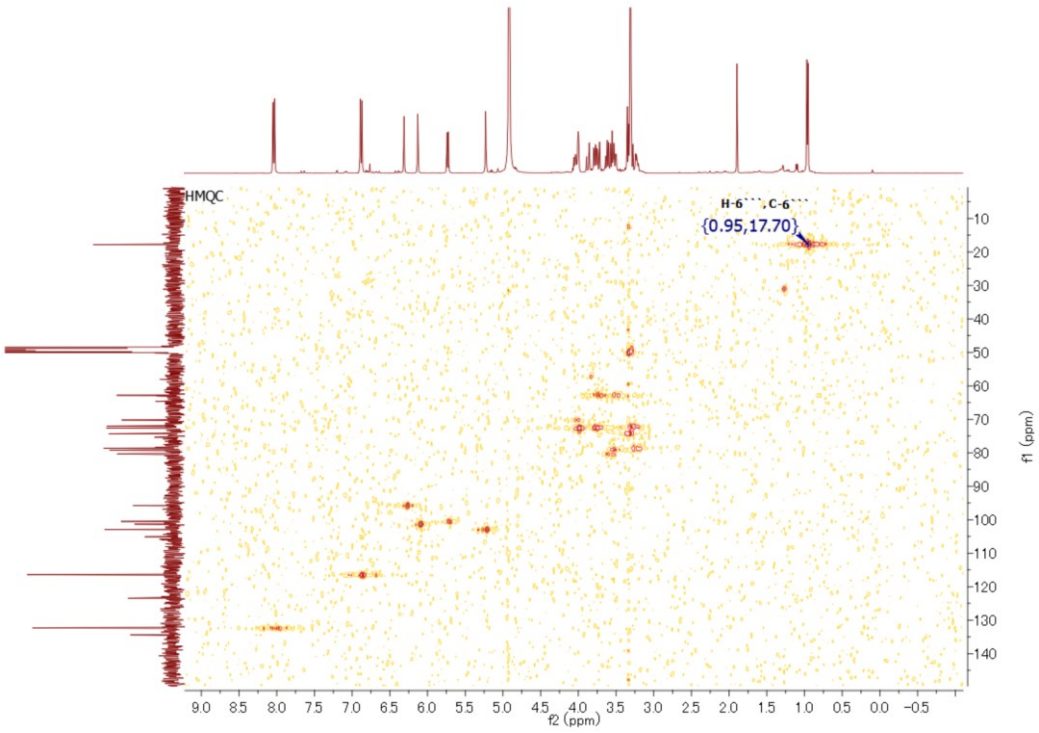
Spectrum 23 HMBC NMR spectrum of compound 2



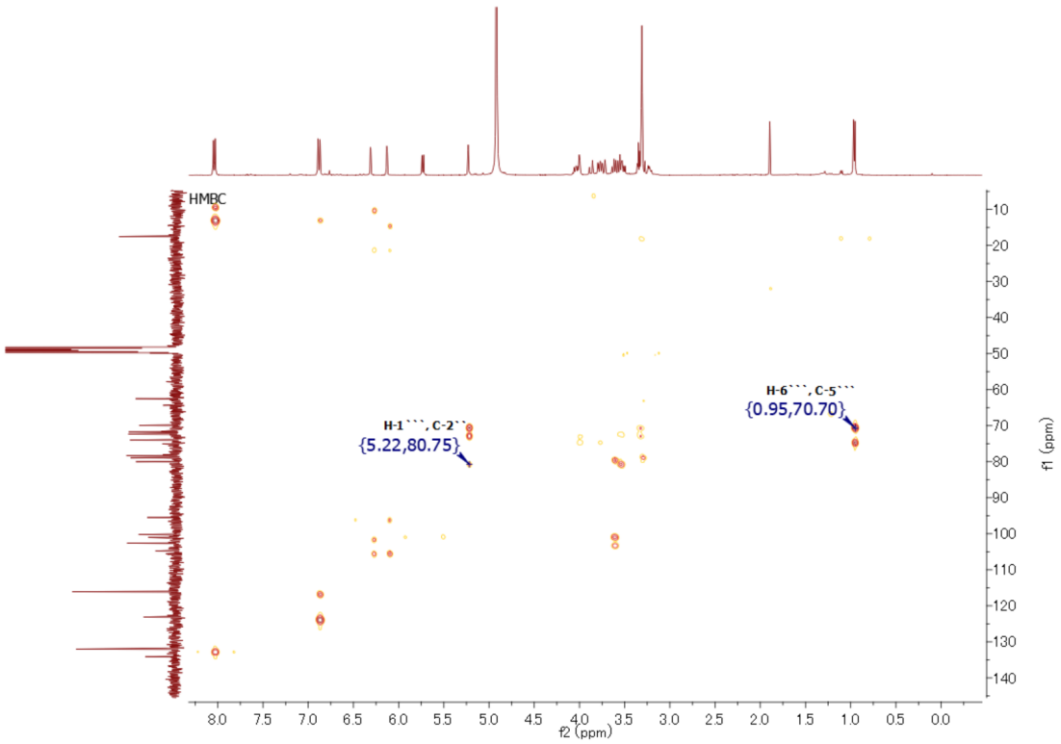
Spectrum 24 ^{1}H NMR spectrum of compound 3



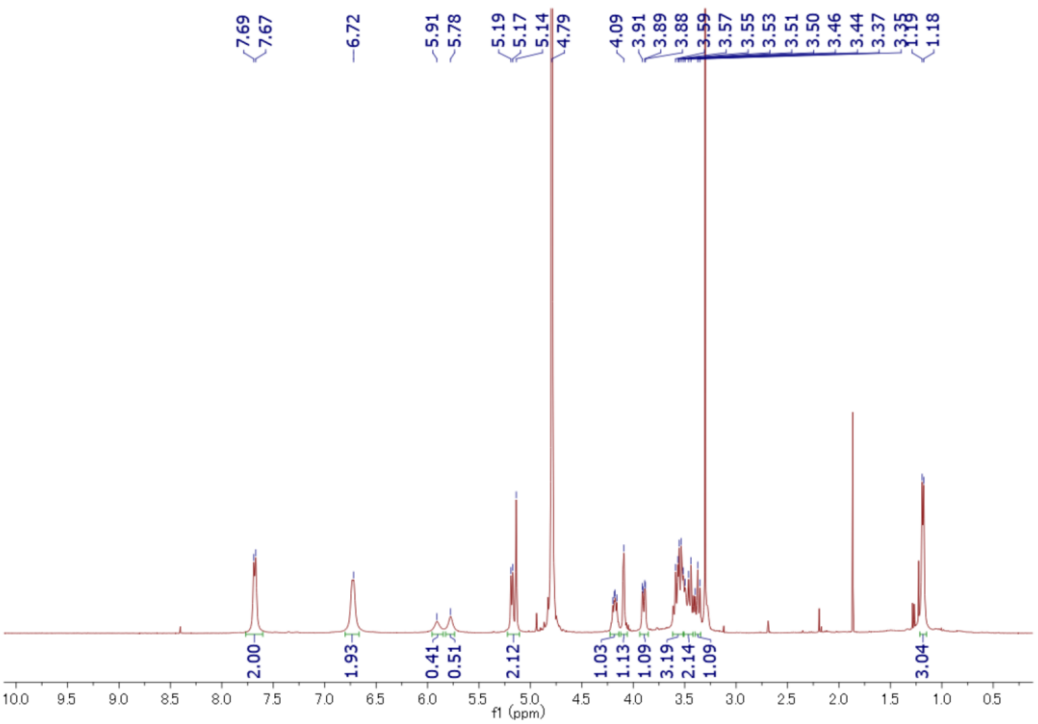
Spectrum 25 ^{13}C, DEPT 135, DEPT 90 NMR spectra of compound 3



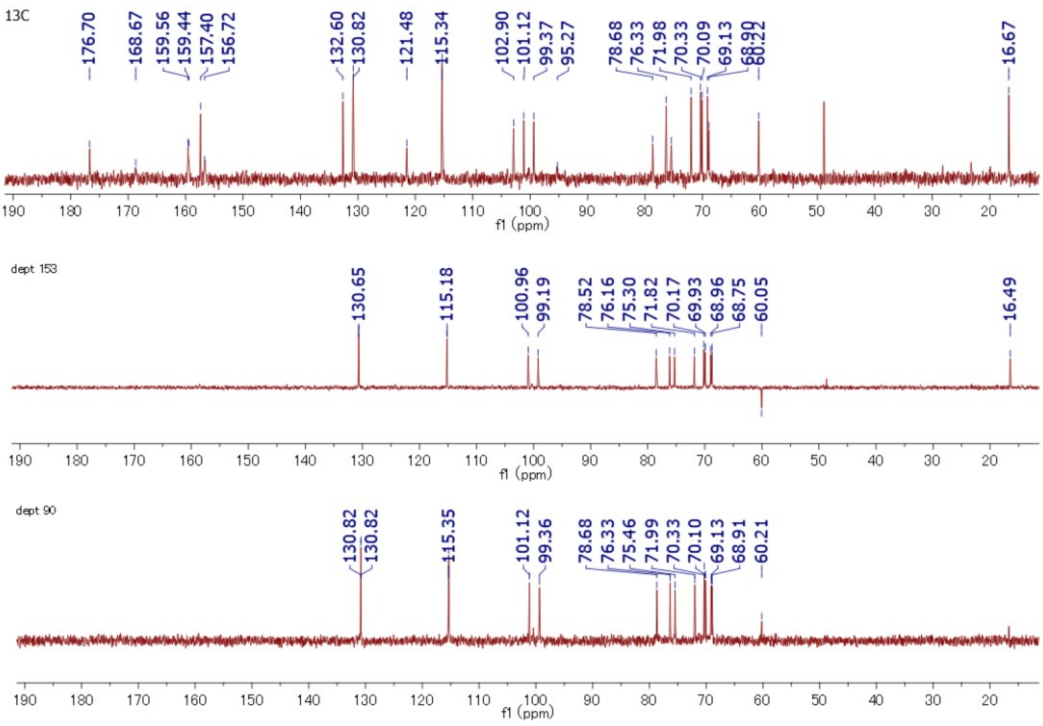
Spectrum 26 HMQC NMR spectrum of compound 3



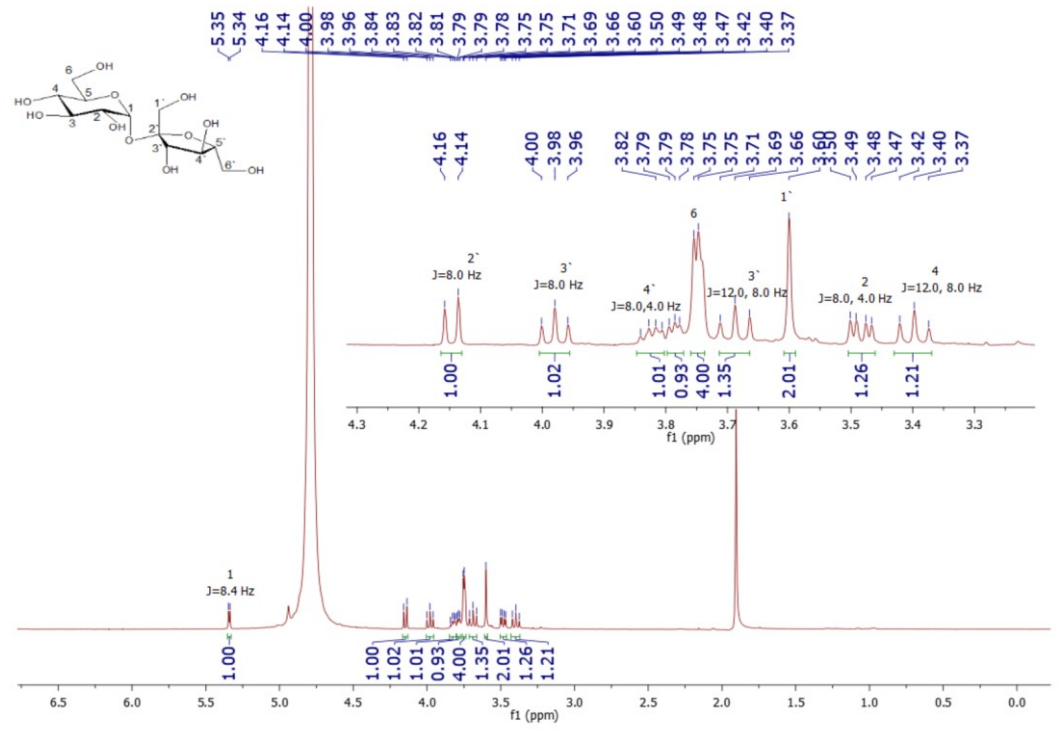
Spectrum 27 HMBC NMR spectrum of compound 3



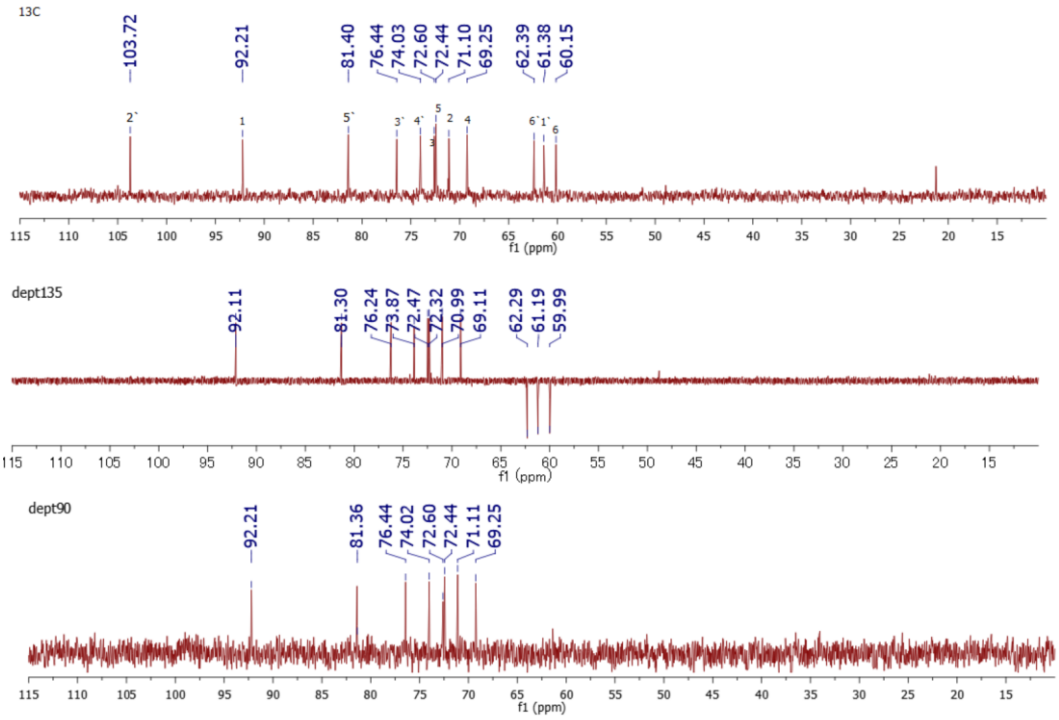
Spectrum 28 ^{1}H NMR spectrum of compound 3+D_2O



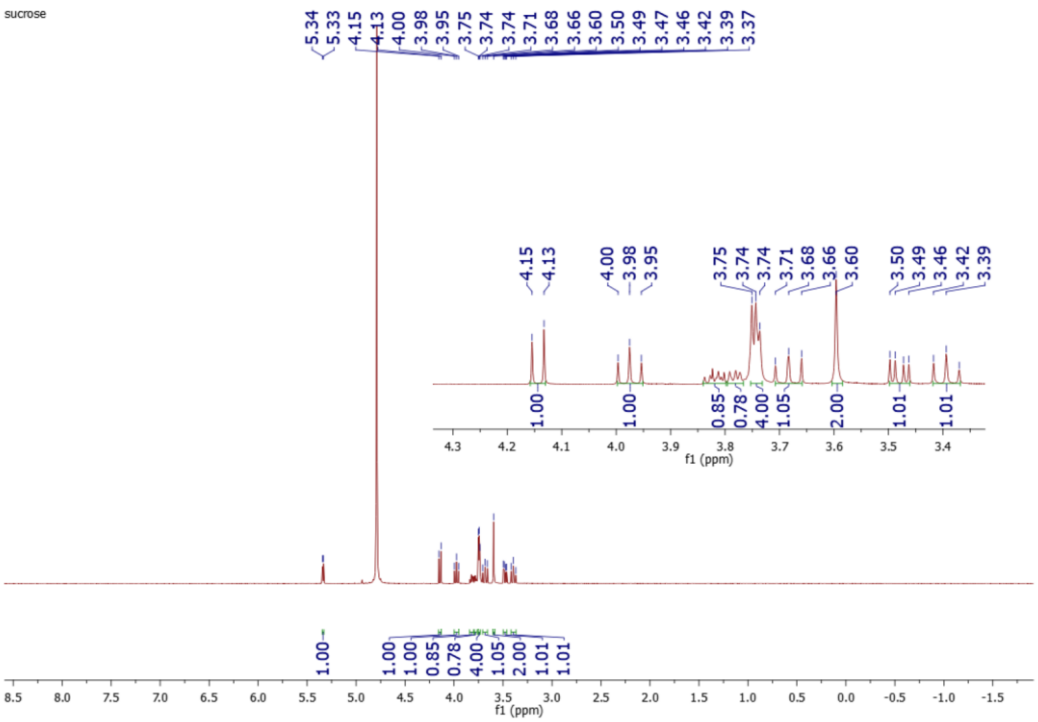
Spectrum 29 ^{13}C, DEPT 135, DEPT 90 NMR spectra of compound 3+ D_2O



Spectrum 30 ^{1}H NMR spectrum of compound 4



Spectrum 31 ^{13}C, DEPT 135, DEPT 90 NMR spectra of compound 4



Spectrum 32 ^{1}H NMR spectrum of sucrose

Chapter 4

Synthetic study of kaempferol 3−*O*−neohesperidoside

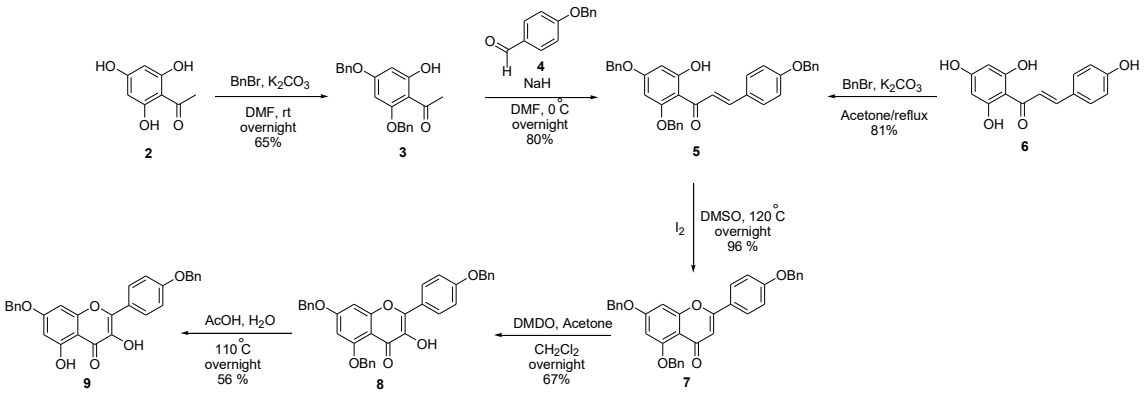
4.1 Introduction:

The first synthesis of kaempferol 3-*O*-neohesperidoside have been reported by Yamasaki *et al.* in 2011 [98], involving the *O*-glycosylation reaction of a flavonol with neohesperidosyl bromide by using the modified phase transfer catalyzed protocol (PTC) [16]. The flavonol was synthesized from the inexpensive commercially available 2',4',6' -Trihydroxy acetophenone **2**, which was selectively benzyl protected and the resulting **3** was subjected to aldol condensation with *p*-benzyloxybenzaldehyde **4** to furnish the corresponding chalcone **5**. Compound **5** was also obtained from the benzylation of the naringenin (4',5,7-Trihydroxy flavanonoe) **6** using excess amount of K_2CO_3. Subsequent cyclization of **5** using a catalytic I_2 in DMSO and high temperature led to the formation of the desired flavone **7** [100]. The flavone **7** was oxidized at C-3 position using DMDO (Maloney & Hecht, 2005) followed by opening of the formed epoxide with catalytic *p*-TsOH [101] to afford flavonol **8**, which is selectively debenzylated with acetic acid (aq.) at high temperature (110 °C) to give 56 % of **9**. This operation was selected to avoid interference in the glycosylation reaction resulting from H−bonding between the hydroxyl group at C−3 and C−4 carbonyl group (scheme 10). The flavonol would be synthesized from the commercially available 4-hydroxy cinnamic acid, which is treated with the thionyl chloride furnishing the expected acyl chloride. Then the hydroxyl groups of phloroglucinol and the 4-hydroxycinnamic acid chloride were protected with a methoxy group to provide trimethoxy benzene **10** and 4-methoxycinnamic

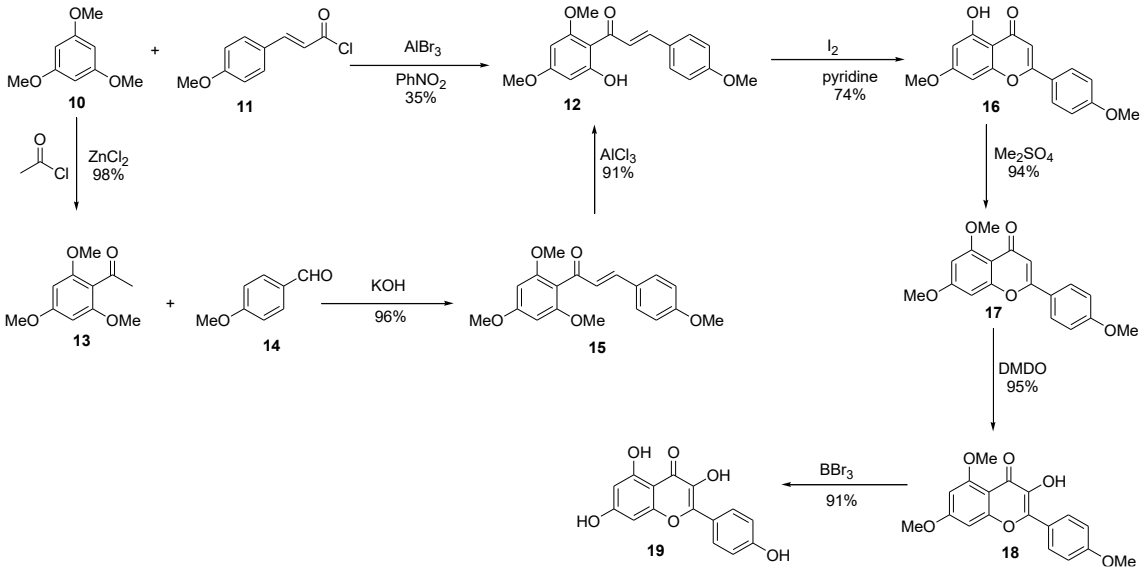
acid chloride **11** then the Friedel−Crafts reaction with $AlBr_3$ as a catalyst gave *o*-hydroxy chalcone **12** in 35% yield. To optimize the reaction yield, cross-aldol reaction of **13** and **14** was performed to provide **15**, followed by ortho-demethylation of acetophenone catalyzed with $AlCl_3$ to afford **12** in high yield (91%). Then **12** was cyclized with I_2 and pyridine furnishing the flavone **16**. The reaction was failed to obtain the C-3 hydroxy flavone directly from **16** with DMDO, since the free hydroxy group of flavone also affected the oxidative hydroxylation with DMDO. The methylation of **16** at 5-OH position followed by the epoxidation with DMDO generated the expected flavone **18** in 93% yield, then the demethylation of **18** with BBr_3 was uneventful providing aglycone kaempferol **19** in excellent yield (91%) (Scheme 11). Performing the reaction using acylation of phloroglucinol catalyze by Lewis's acid did not provide the tetrahydroxy-chalcone **19**, and also the cyclization of **12** with H_2O_2 or PhSSPh conditions was failed. Trace amount of flavanone was afforded after treated with K_2CO_3. The advantage of iodine oxidative cyclization is that the reaction can be accomplished in a relatively high yield and afford only the desired product **16** in one-pot. On the other hand, the oxidation of the commercially available naringenin **6** with I_2/pyridine led to the formation of **20**, which was fully protected with methyl group by using Me_2SO_4 to provide the methylated flavone **17**. Compound **17** could be converted to **18** and **19** [102, 103] (Scheme 12).

Hasan and co-workers reported the synthesis flavonol **19** using Algar−Flynn−Oyamada reaction [19, 104], starting from the acetylation of 3,5-dimethoxy phenol **21** to *O*-Acetyl 3,5-methoxy phenol **22,** which was converted to the corresponding 2-hydroxy-4,6-dimethoxy acetophenone **23** by utilizing Fries rearrangement. Then **23** reacted with 3,4-dimethoxybenzaldehyde **24** using 50% NaOH and hot ethanol gave methoxychalcone **12**. The oxidation of **12** at α/β unsaturated ketone position using H_2O_2 as oxidizing agent with base and MeOH furnished methoxyflavonl **18**, which was transformed into the flavonol **19** by the demethylation with TMSI/MeOH [105] (Scheme 13). In 2011, Yamasaki et.al reported the synthesis of protected neohesperidosyl bromide **30** [98]. As shown in Scheme 14, Zemplen deacetylation and subsequent benzylation of the commercially available 3,4,6-Tri-*O*-acetyl-D-glucal **25** yielded 3,4,6-Tri-*O*-benzyl-D-glucal **26**. Glucose **27** was then successfully synthesized by the epoxidation of the **26** using DMDO's reagent, through opening the epoxide ring by selective addition of acetyl group at the anomeric carbon. The glycosylation of **27** with rhamnose **28** was achieved using TMSOTf as a promotor. The diastereoselective bromination of the resulting acetyl disaccharide **29** in HBr/AcOH solution led to the formation of the fully protected neohesperidosyl bromide **30** in good yield (90%). On the other hand, Peng et al. synthesized the protected neohesperidosyl bromide **37** by applying a new protocol in 2005 (Scheme 15) [107]. A selective protection of the hydroxyl group at C−2 with AZMB group followed by the benzylation of the free 3−OH led to afforded 88% of **32**. Then **32**

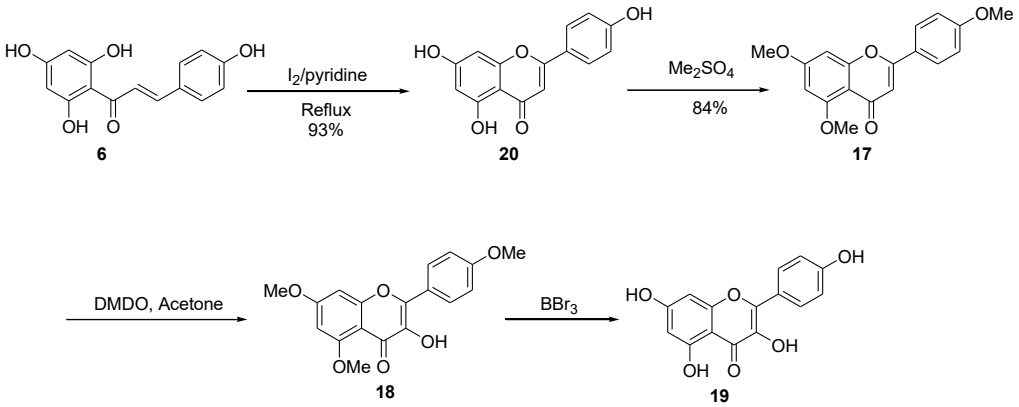
was treated with TsOH and acetic anhydride to give a fully protected **33**, which was selectively deprotected at C−2 by using Bu_3P to afford the glucoside **34**. The resulting **34** was then reacted with 2,3,4-Tri-*O*-benzoyl-L-rhamnpyranosyl trichloroacetimdate donor **35** under the promotion of TMSOTf to furnish the fully protected disaccharide **36** in 92% [109]. The allyl group at the anomeric carbon was treated with $PdCl_2$ then acetylated and brominated the corresponding acetate with HBr/AcOH provided the desired protected neohesperidosyl bromide **37** in 59%. The *O*-glycosylation of the protected neohesperidosyl bromide **30** with the flavonol **9** in using a mixture of tetrabutylammonium bromide (TBAB) and K_2CO_3 provided the protected flavonol disaccharide **38** in 47%. Finally, the deacetylation followed by debenzylation of **39** with H_2/Pd afforded 7% of **1**. [98] (Scheme 16).



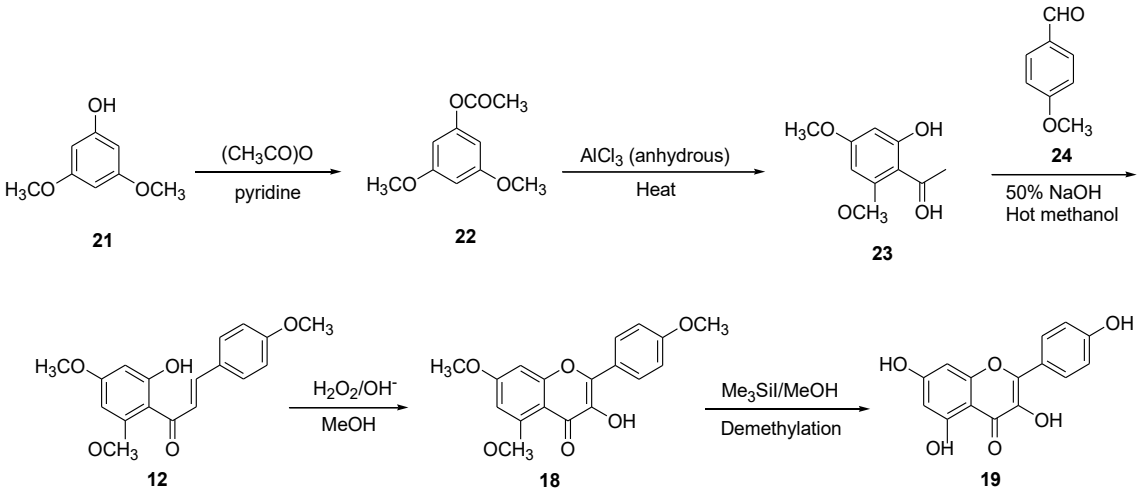
Scheme 10 Synthesis of 4',7-di-*O*−benzylkaempferol **9**



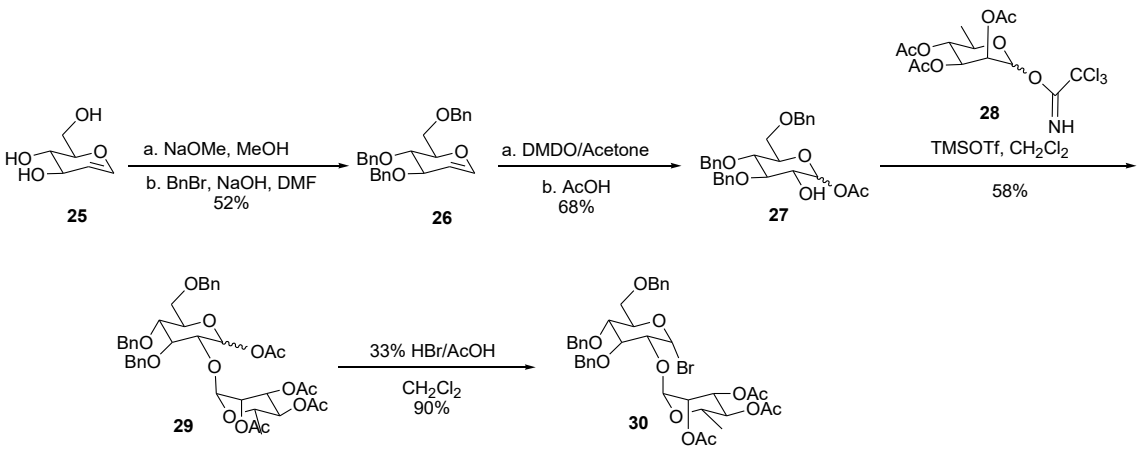
Scheme 11 Synthesis of kaempferol **19**



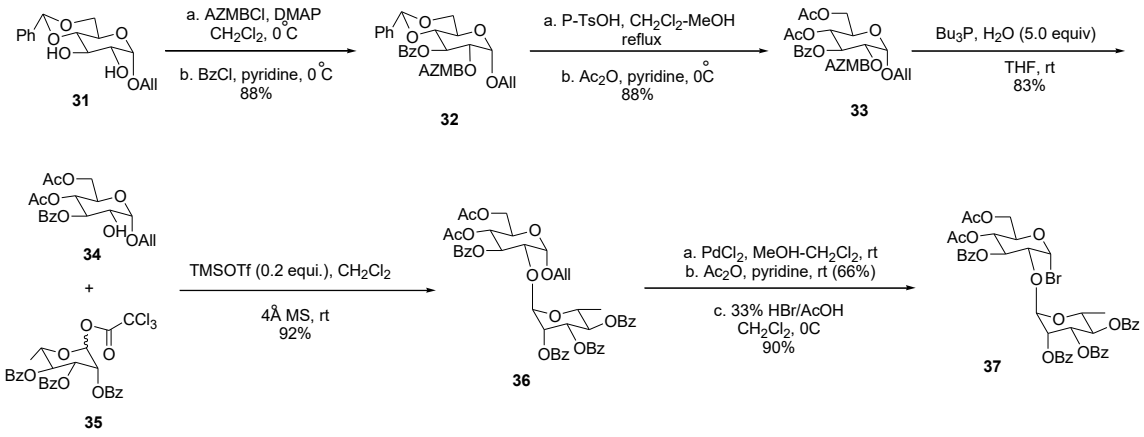
Scheme 12 Synthesis of kaempferol **19** using naringin **6**



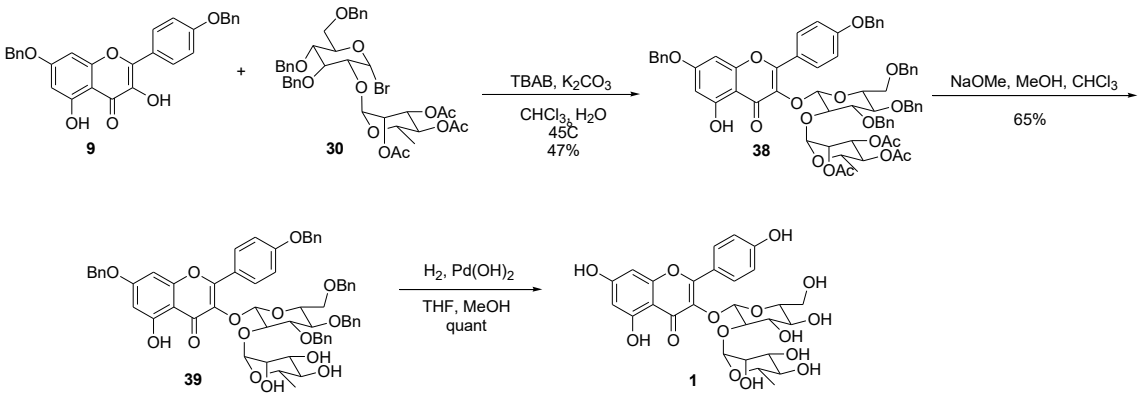
Scheme 13 Synthesis of kaempferol **19** using Algar −Flynn−Oyamada reaction



Scheme 14 neohesperidosyl bromide **30**



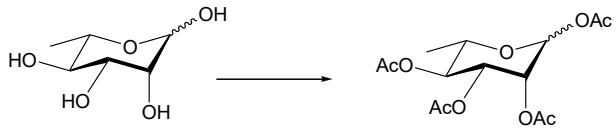
Scheme 15 neohesperidosyl bromide **37**



Scheme 16 Synthesis of Kaempferol 3−*O*−neohesperidoside **1**

4.2 Materials and Method

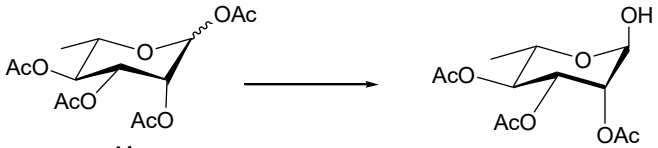
4.2.1 Synthesis of 1,2,3,4-Tetra-*O*-acetyl-L-rhamnopyranose 41 [110]



40 41

Commercially available **40** (3.0 g, 16 mmol) was added in four portions over 30 min to a mixture of sodium acetate (864 mg, 10.5 mmol) in acetic acid (20 mL, 22.0 g, 210 mmol) at rt, then the reaction was heated at 140 °C under reflux for 4 h. Cooled the reaction mixture to rt, then added to iced water and fractionated with EtOAc. The EtOAc layer was separated, washed with $NaHCO_3$ (aq.) and dried over $MgSO_4$. Then concentrated under vacuum pressure and purified with silica gel column (EtOAc: Hex = 1:1) to afforded anomeric mixture of **41** as a pale-yellow liquid (4.2 mg, 76%, α/β = 2/1). R_f = 0.57 (EtOAc: Hex = 1:3; Hanessian's stain and heat). The anomeric ratio was measured by comparison of integration of ^{1}H NMR signals of the anomeric protons of the mixture, α anomer. ^{1}H NMR ($CDCl_3$) δ 6.00 (s, 2H, 2.0 Hz), 5.31 (dd, 1H, *J* = 4.0 and 12.0 Hz), 5.24 (m, 1H), 5.08 (m, 1H), 3.96 (m, 1H), 2.21 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.05 (s, 3H), 1.23 (d, 3H, *J* = 4.0 Hz) ppm.; β anomer: 5.83 (d, 1H, *J* = 8.0 Hz), 5.46 (brs, 1H), 5.13 (d, 2H, *J* = 8.0 Hz), 3.69 (m, 1H), 2.20 (s, 3H), 2.09 (s, 3H), 1.99 (s, 6H), 1.29 (d, 3H, 8 Hz) ppm.

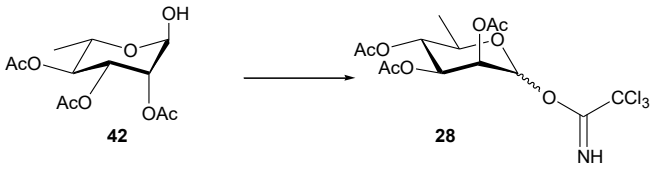
4.2.2 Synthesis of 2,3,4-Tri-*O*-acetyl-6-deoxy-α-L-mannopyranose 42 [110]



41 42

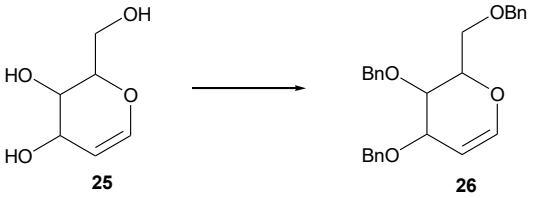
Acetic acid (1.0 mL, 16 mmol) was added to a solution containing ethylene diamine (965 µl, 869 mg, 14.5 mmol) in anhydrous THF (20.0 mL). The solution was stirred for 30 min at rt under N_2-gas, then a solution of **41** (4.0 g, 12 mmol) in THF (5 mL) was added, and the resulting solution was stirred overnight at rt. The solution was concentrated under vacuo and dissolved in ethyl acetate and washed with water, brine, 1M HCl (aq) solution, dried over anhydrous $MgSO_4$, and concentrated under vacuo. The residue was purified by silica gel column chromatography (EtOAc: Hex = 1:3) to give **42** as white crystal (2.0 g, 69 %). R_f = 0.33 (EtOAc: Hex = 1:1, TLC stained with Hanessian's stain). ^{1}H NMR ($CDCl_3$) δ 5.36 (dd, 1H, *J* = 3.6, 10 Hz), 5.25 (m, 1H), 5.14 (brs, 1H), 5.08 (t, 1H, *J* = 6.0, 14 Hz), 4.15 (m, 1H), 2.14 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.21 (d, 3H, *J* = 6.4 Hz) ppm; ^{13}C NMR ($CDCl_3$) δ 170.59, 170.45, 170.37, 92.24, 71.31, 70.52, 69.06, 66.51, 21.12, 21.01, 20.93, 17.63 ppm.

4.2.3 Synthesis of 2,3,4-Tri-*O*-acetyl-L-rhamnopyranosyl trichloroacetoimidate **28** [111]



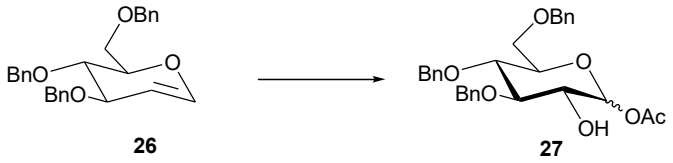
To a solution of compound **42** (2.0 g, 6.9 mmol) in CH_2Cl_2 (26 mL) were added trichloroacetonitrile (5.7 mL, 8.2 g, 56 mmol) and DBU (517 µL, 527 mg, 3.46 mmol). The mixture was stirred at rt for 30 min, then the mixture was concentrated and purified with silica gel column chromatography using 1:4 EtOAc: Hex as eluting solvent to afforded **28** as yellow liquid (1.9 g, 95 %). R_f = 0.38 (EtOAc: Hex = 1:4; Hanessian's-stain and heat). ^{1}H NMR ($CDCl_3$) δ 8.72 (s, 1H), 6.19 (d, 1H, *J* = 4.0 Hz), 5.45-5.43 (m, 1H), 5.37 (m, 1H), 5.18 (t, 1H, *J* = 12.0, 8.0 Hz), 4.11-4.04 (m, 1H), 2.17 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H), 1.26 (d, 3H, *J* = 4.0 Hz); ^{13}C NMR ($CDCl_3$) δ 170.50, 170.37, 170.32, 170.17, 170.14, 170.08, 163.84, 160.61, 94.83, 92.80, 92.28, 90.82, 71.30, 70.46, 69.48, 69.04, 68.31, 66.53, 60.65, 21.24, 21.12, 21.01, 20.98, 20.93, 20.84, 17.65, 14.36 ppm.

4.2.4 Synthesis of 3,4,6-Tri-*O*-benzyl-D-glucal **26** [98] [112]



A commercially available Tri-*O*-acetyl-D-glucal **25** (380 mg, 1.4 mmol) was dissolved in MeOH (6 mL). NaOMe (348 mg, 6.69 mmol) was then added to the solution. The mixture was stirred for 30 min at rt and neutralized with cation-exchange resin, filtered and concentrated under vacuo. The residue was dissolved in DMF (16 ml), then NaH (124 mg, 5.2 mmol) was added slowly at 0 °C. BnBr (0.8 mL, 7 mmol) was added and the reaction mixture was stirred under Ar for 12 h at 0 °C to rt. Water was added to quench the reaction, and products were extracted by EtOAc: Hex =1:1. The organic layer was washed with water, brine, and dried over Anh. Na_2SO_4 and concentrated under vacuo. Residue was purified by silica gel column chromatography (EtOAc: Hex = 1:10) to give compound **26** as pale-yellow solid (204 mg, 53%). R_f = 0.28 (EtOAc: Hex = 1/10; UV 254 nm). ^{1}H NMR ($CDCl_3$) δ 7.32-7.21 (m, 15H, H arom), 6.42 (dd, 1H, *J* = 1.2, 6.4 Hz, H-1), 4.87 (dd, 1H, *J* = 2.8, 6.4 Hz, H-2), 4.83 (d, 1H, *J* = 11.6 Hz, CH_2 ph), 4.64−4.52 (m, 5H, CH_2 ph), 4.21 (m, 1H, H-3), 4.08 (ddd, 1H, *J* = 2.8, 5.2 Hz, H-5), 3.87 (dd, 1H, *J* =6.0, 8.8 Hz, H-4), 3.81 (dd,, 1H, *J* =5.2, 10.4 Hz, H-6), 3.76 (dd, 1H, *J* =2.8, 10.8 Hz, H-6) ppm.

4.2.5 Synthesis of 1-*O*-Acetyl-3,4,6-tri-*O*-benzyl-D-glucopyranose 27



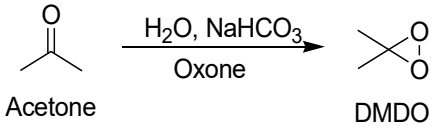
4.2.5.1 Synthesis of **27** *using DMDO/Acetone solution* [113]

Compound **26** (680 mg, 1.6 mmol) was dissolved in 10 mL of DMDO/Acetone, and the mixture was stirred under Ar atmosphere for 6 h at 0 °C. The mixture was concentrated under vacuo and dissolved in acetic acid (40 mL), then stirred under argon atmosphere at rt for 14 h. Water (20 mL) was added and extracted by EtOAc: Hex 1:1. The organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated under vacuo. Residue was purified by PLC; eluted by Tol: Acetone (4:1) to afforded **27** (291 mg, 43%). R_f = 0.5 (Tol: Acetone = 1:3; UV 254 nm and Hanessian's-stain + heat).

4.2.5.2 Synthesis of **27** *using in situ formed DMDO* [114]

To a vigorously stirred of cooled solution of **26** (90 mg, 0.21 mmol) in CH_2Cl_2 (1 mL), acetone (0.1 mL) and sat. $NaHCO_3$ (aq.) (2 mL), a solution of Oxone® (230 mg, 2.5 mmol) in H_2O (1.5 mL) was added dropwise over 15 min. Then the reaction mixture was vigorously stirred at 0 °C for 30 min and then at rt for an 2 h. Water was added and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_3 and concentrated under vacuo. Then 6 mL of acetic acid was added and stirred overnight under Ar at rt. To the solution was added H_2O, extracted with EtOAc and the organic layer washed with brine and dried over Na_2SO_3 and concentrated under vacuum pressure. The residue was purified by silica gel column chromatography using EtOAc: Hex 1:3 as eluted solvent to accomplished compound **27** (50.5 mg, 56%). R_f = 0.2 (EtOAc: Hex 1:3).

4.2.6 Preparation of DMDO/Acetone reagent [115]



Distilled water (20mL), acetone (30 mL) and $NaHCO_3$ (24 g, 0.285 mol) were stirred over 20 min at rt, Oxone® (25 gm, 0.041 mmol) was added to the reaction mixture and stirred over 15 min at rt, the DMDO/Acetone was evaporated using evaporator trap in dried ice/methanol (155 mmHg, 65 rpm) (Fig 8 and 9).

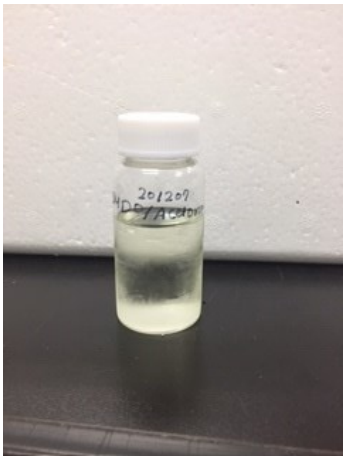
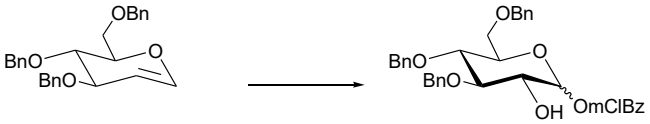


Fig. 8 DMDO/Acetone solution

Fig. 9 Evaporation of DMDO/Acetone solution

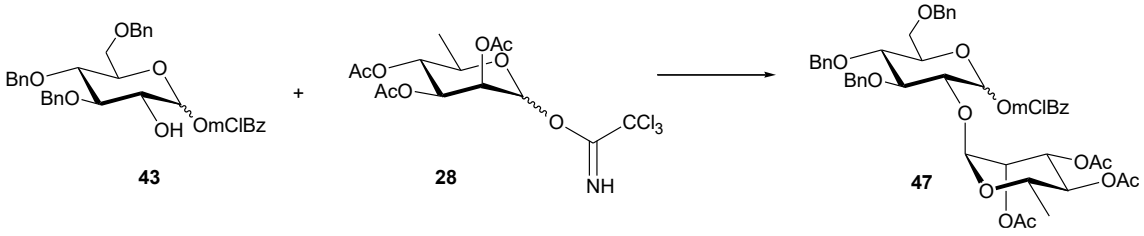
4.2.7 Synthesis of 1-*O*-*m*-chlorobenzoyl-3,4,6-tri-*O*-benzyl-D-glucopyranose 43[116]



26 **43**

Compound **26** (2.0 g, 4.8 mmol) was dissolved in 200 mL of CH_2Cl_2. 2.4 g of 70% *m*CPBA (9.6 mmol) was added, the reaction mixture was stirred overnight under nitrogen atmosphere at rt. The reaction mixture was quenched by saturated aqueous Na_2SO_3, and extracted by EtOAc. EtOAc layer was then separated, washed with water and brine, and dried by anhydrous $MgSO_4$, then the residue was subjected to purification using silica gel chromatography and 1:3 EtOAc: Hex, furnishing **43** as mixture of α/β anomer as white solid (2.6 g, 93%; α/β = 1/5). R_f = 0.37 (EtOAc: Hex 1:3; UV 254 nm and Hanessian's –stain and heat). The anomeric ratio was measured by comparison of integral intensities of the anomeric protons from ^{1}H NMR spectrum of the mixture; β anomer: ^{1}H NMR ($CDCl_3$) δ 8.08 (t, 1H, 2.0 Hz), 7.89 (m, 1H), 7.57 (m, 1H), 7.37-7.26 (m, 15H), 7.20 (m, 2H), 5.78 (d, 1H, *J* = 8.0 Hz), 4.95 (d, 1H, *J* = 11.2 Hz, CH_2 ph), 4.86 (dd, 2H, *J*= 6.0, 11.2 Hz, CH_2 ph), 4.64 (m, 2H), 4.51 (d, 1H, *J* = 12.0 Hz), 3.85 (m, 4H), 3.07 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$) δ 170.12, 164.16, 138.43, 138.02, 137.91, 134.84, 134.77, 133.95, 133.78, 131.21, 131.14, 130.41, 130.30, 130.01, 129.94, 128.85, 128.65, 128.58, 128.43, 128.22, 128.16, 128.14, 127.96, 94.97, 84.61, 75.96, 75.52, 75.09, 73.76, 73.10, 68.21 ppm.

4.2.8 Synthesis of 1-*O*-*m*-chlorobenzoyl-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-3,4,6-tri- *O*-benzyl-D-glucopyranose 47 [117]

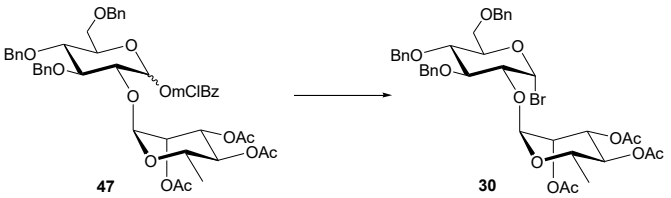


A mixture of **43** (170 mg, 0.29 mmol), **28** (200 mg, 0.44 mmol) and 113 mg of activated 4 Å MS in anh.CH_2Cl_2 (5 mL) were stirred 30 min at -20 °C. Then was added a cooled solution of TMSOTf in anh. CH_2Cl_2 (0.1 M, 1.3 mL, 0.123 mmol., The reaction mixture was stirred 1 h under N_2 gas at -20 °C, the mixture was neutralized with Et_3N, filtered through vacuum filtration and concentrated under vacuo. The residue was purified by silica gel column chromatography and eluted with EtOAc: Hex = 1:4 gave anomeric mixture of **47** (90 mg, 32 %, α/β = 1/4) (Table 5, Entry 7)). R_f = 0.14 (EtOAc: Hex 1:3; UV 254 nm and Hanessian's –stain and heat). ^{13}C NMR ($CDCl_3$) δ 170.14, 170.04, 169.92, 163.88, 137.96, 137.89, 137.86, 134.74, 133.77, 131.23, 130.28, 129.93, 128.65, 128.61, 128.56, 128.22, 128.07, 128.02, 127.84, 127.74, 98.33, 93.67, 90.84, 85.46, 75.89, 75.77, 75.07, 73.81, 70.80, 69.56, 69.14, 68.92, 68.84, 68.09, 66.95, 60.62, 53.96, 31.94, 29.46, 21.27, 20.94, 20.87, 20.79, 17.62, 14.10 ppm.

4.2.9 Activation of 4Å Molecular sieves (MS)

A powder of 4Å MS was heated in microwave (500 w) for 2 min and then the flask containing the powder was connected to vacuum pressure. This method was repeated until removing the moisture from the powder.

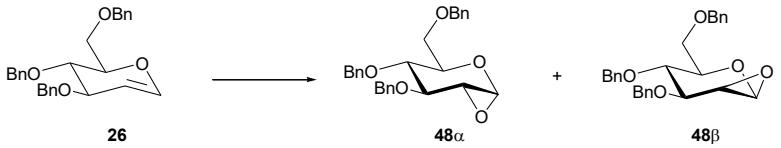
4.2.10 Synthesis of 2-*O*-(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-3,4,6-tri-O-benzyl-α-D-glucopyranosyl bromide 30 using 47



To a cooled solution of compound **47** (76 mg, 0.088 mmol) in anh.CH_2Cl_2 (1 mL), 25% HBr/AcOH (100 µL, 143 mg, 0.432 mmol), the reaction mixture was stirred under nitrogen gas at 0 °C for 30 min. Water was added and extracted with $CHCl_3$, the organic layer was washed with 5% $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated under vacuo. The residue was purified by plate coated silica gel chromatography (PLC) and eluted with EtOAc: Hex = 1:3 to afforded **30** (16%, table 6, entry 6)). R_f = 0.2 (EtOAc: Hex 1:3; UV 254 nm and Hanessian's –

stain and heat). The % value was determined by ^{1}H NMR using triphenyl methane as internal standard (δ = 5.5 ppm, relaxation delay = 20 s).

4.2.11 Synthesis of 1,2-Anhdro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose 48



To a mixture of **26** (1.0 g, 2.4 mmol) and freshly prepared *m*CPBA−KF complex (1.8 g, 12.1 mmol), was added CH_2Cl_2 (150 mL). The mixture was stirred overnight at rt under N_2 gas, then the insoluble complex was filtered off and the solvent was evaporated under vacuo. The compound was recrystallized from EtOAc: Hex (0.5:3), then the white solid was filtered, washed with hexane and dried under reduce pressure to give a mixture of pure sugar epoxide **48** (645 mg, 65%, α/β = 7/1). R_f = 0.35 (EtOAc: Hex = 1:1; PMA-stain and heat); α anomer ^{1}H NMR ($CDCl_3$) δ 7.38-7.17 (m, 15H, H Bn), 4.99 (dd, 1H, *J* = 4.0 Hz, H-1), 4.83-4.52 (m, 6H, 3CH_2 Bn), 3.99 (dd, 1H, 12 Hz, H-3), 3.79-3.63 (m, 4H, H-4,5,6,6`), 3.07 (d, 1H, H-2). ^{13}C NMR ($CDCl_3$) δ 138.37, 138.11, 137.70 (Cq Bn), 128.88-127.89 (C_{arom}), 79.14 (C1), 77.70 (C3), 74.77, 74.43, 73.77 (CH_2 Bn), 72.48 (C6), 69.62 (C5), 68.39 (C4), 52.78 (C2).

4.2.12 Synthesis of *m*CPBA−KF complex (Camp′s reagent) [118]

KF (2.1 g, 36 mmol) was activated by heating, then 400 mL of anh. CH_2Cl_2 and 2.2 g of 70% *m*CPBA (8.9 mmol) were added. The mixture was vigorously stirred at rt under N_2-gas for 30 min. Then the insoluble complex was filtered off by vacuum filtration, washed with CH_2Cl_2 and dried under reduced pressure afforded *m*CPBA−KF complex as white solid powder (3.4 g, 94%) (Fig. 10).

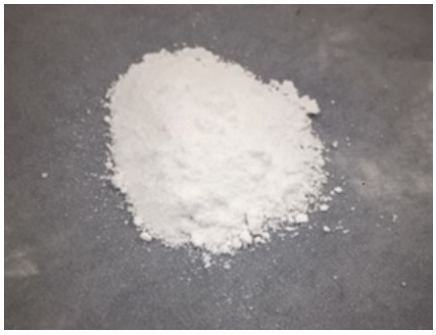
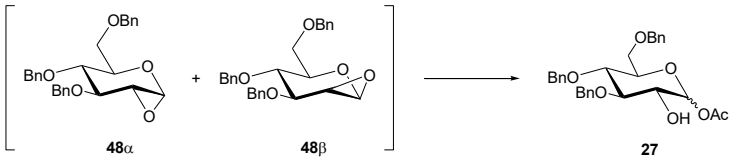


Fig. 10 *m*CBPA−KF complex

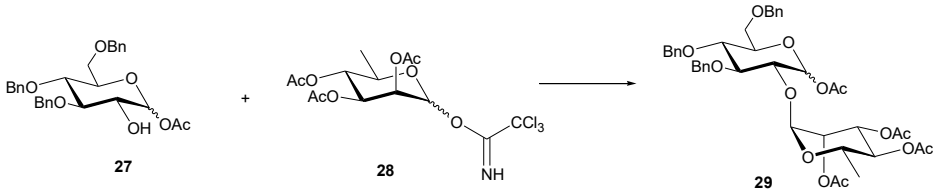
4.2.13 Synthesis of 1-*O*-Acetyl-3,4,6-tri-*O*-benzyl-D-glucopyranose 27 using 48 [113]



48α 48β 27

To a stirred and cooled solution of **48** (780 mg, 1.80 mmol) in anh.CH_2C1_2 (10 mL) was added 234 µL of acetic acid, then the reaction mixture was stirred over night at 0 °C under N_2-gas. The mixture was diluted with CH_2Cl_2 and washed with aq. $NaHCO_3$, then the organic layer was dried over anh. $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography and EtOAc: Hex 1:3 to give **27** as pale-yellow liquid (573 mg, 73% α/β = 1/12). R_f = 0.28 (EtOAc: Hex 1:3). The anomeric ratio was measured by comparison of integral intensities of the anomeric protons from ^{1}H NMR spectrum of the mixture; α anomer, ^{1}H NMR ($CDCl_3$) δ 7.36-7.15 (m, 15 H, H_{arom}), 5.24 (dd, 1H, *J* = 4 Hz, H-1), 4.95-4.49 (m, 6H, CH_2 Bn), 4.08-3.82 (m, 6H, sugar protons), 2.13 (s, 3H, H_{Ac}). ^{13}C NMR ($CDCl_3$) δ 169.85 (C=O Ac), 138.59, 138.26, 138,02 (Cq Bn), 128.85-127.95 (C_{arom}), 92.12 (C1), 82.53, 75.89, 75.65 (C2, C4, C5), 75.11,73.82, 73.32 (CH_2 Bn), 68.92 (C6), 21,19 (CH_3 Ac). C3 overlapped with the solvent peak; β anomer, ^{1}H NMR ($CDCl_3$): δ 7.36-7.15 (m, 15 H, H_{arom}), 5.52 (d, 1H, *J* = 8 Hz, H-1), 4.92-4.56 (m, 6H, CH_2 Bn), 4.56-4.47 (m, 2H, H-6 and 6`), 3.78 (m, 2H, H-3 and 4), 3.61-3.57 (m, 2H, H-2 and H-5), 2.14 (s, 3H, H_{Ac}); ^{13}C NMR ($CDCl_3$): δ 169.77 (C=O Ac), 138.54, 138.09, 138,02 (Cq Bn), 128.85-127.95 (C_{arom}), 94.22 (C1), 84.76, 75.89,75.52 (C2, C4, C5), 75.11,73.77, 73.17 (CH_2 Bn), 68.26 (C6), 21,29 (CH_3 Ac). C3 was overlapped with the solvent peak.

4.2.14 Synthesis of 1-*O*-Acetyl-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-3,4,6-tri- *O*-Benzyl-D-glucopyranose 29

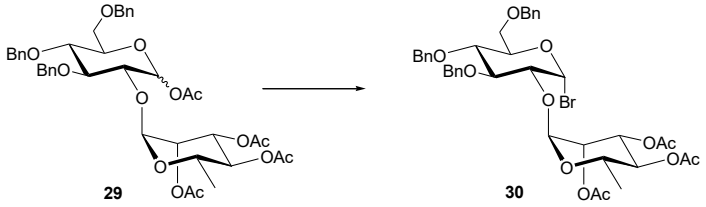


27 28 29

A mixture of **27** (340 mg, 0.76 mmol) and freshly prepared of **28** (241 mg, 0.49 mmol) and 109 mg of freshly activated 4 Å MS in anh.CH_2Cl_2 (5 mL) were stirred 30 min at -20°C under N_2. Then added a cooled solution of TMSOTf in anh. CH_2Cl_2 (0.1 M, 2.3 mL, 0.23 mmol) to the solution. The reaction mixture was stirred under N_2 at -20 °C for 1 h. The mixture was neutralized with Et_3N, filtered through vacuum filtration and concentrated under vacuo. The residue was purified by silica gel column chromatography and eluted with EtOAc: Hex = 1:4 to give a mixture of **29** (284 mg, 60 %, α/β = 1/2). R_f = 0.13 (EtOAc: Hex 1:3; UV 254 nm and Hanessian's –stain

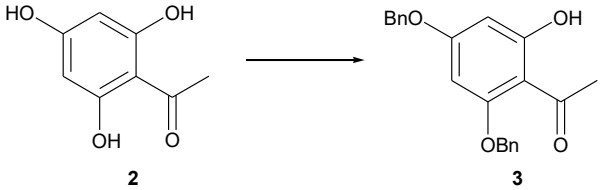
and heat), the anomeric ratio was measured by comparison of integral intensities of the anomeric protons from ^{1}H NMR spectrum of the mixture. β−anomer ^{1}H NMR ($CDCl_3$) δ 7.33−7.27 (m, 15H), 5.59 (d, 1H, *J* = 8.0 Hz), 5.28 (dd, 1H, J = 3.4, 1.8 Hz), 5.21. ^{13}C NMR ($CDCl_3$) δ 170.27, 170.13, 170.09, 169.91, 169.34,138.17, 138.01, 137.92, 128.62, 128.54, 128.21, 128.11, 128.02, 127.98, 127.81, 99.66, 98.56, 92.99, 91.28, 85.19, 81.76, 75.89, 75.66, 75.06, 73.77, 73.07, 70.99, 70.73, 69.70, 69.20, 68.04, 67.45, 66.95, 21.26, 20.98, 20.86, 17.44 ppm.

4.2.15 Synthesis of 2-*O*-(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide 30 using 29



To a cooled solution of compound **29** (40 mg, 0.050 mmol) in anh.CH_2Cl_2 (500µL), 25% HBr/AcOH (60 µL, 84 mg, 0.26 mmol), the reaction mixture was stirred under nitrogen at 0 °C for 30 min. Water was added and extracted with $CHCl_3$, the organic layer was washed with 5% $NaHCO_3$, brine, dried over $MgSO_4$ and concentrated under vacuo. The residue was purified by PLC (EtOAc: Hex = 1:3) to give **30** (5 mg, 12%). R_f = 0.2 (EtOAc: Hex 1:3; UV 254 nm and Hanessian's –stain and heat). ^{1}H NMR ($CDCl_3$) δ 7.35−7.15 (m, 15H), 6.48 (d, 1H, *J* = 3.7 Hz), 5.38 (dd, 1H, *J* = 4.0 Hz), 5.32 (dd, 1H, *J* = 4.0, 8.0 Hz), 5.11 (t, 1H, *J* = 8.0, 12.0 Hz), 4.96 (d, 1H, *J* = 1.7 Hz), 4.85 (dd, 2H, *J* = 8.0, 12.0 Hz), 4.59 (t, 2H, *J* = 14.1 Hz), 4.52 (m, 2H), 4.19 (dd, 1H, *J* = 9.8, 6.2 Hz), 4.08−4.03 (m,2H), 3.80−3.76 (m, 2H), 3.67−3.63 (m, 2H), 2.11 (s, 3H), 2.07 (s, 3H), 1.99 (s, 3H), 1.23 (d, 3H, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$) δ 170.20, 170.13, 169.95, 138.36, 138.20, 138.08, 137.97, 137,74,128.67, 128.49, 128.36, 128.21, 128.16, 128.10, 128.01, 127.92, 100.66, 99.91, 95.98, 92.61, 91.79, 85.08, 81.63, 81.55, 80.97, 80.66,75.49, 75.23, 75.17, 73.73, 71.11, 70.80, 70.53, 69.80, 69.42, 69.27, 68.77, 68.34, 67.65, 67.21, 29.92, 21.04, 17.53 ppm.

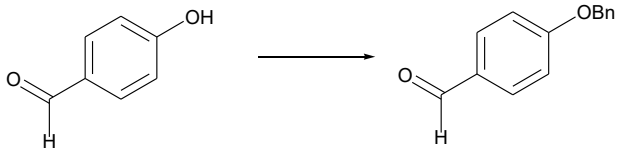
4.2.16 Synthesis of 2,4-dibenzyloxy-6-hydroxyacetophenone 3 [98]



2',4',6'-Trihydroxyacetophenone **2** (1.0 g, 6.0 mmol) was dissolved in DMF (35 mL), then BnBr (1.55 mL, 13.1 mmol) and K_2CO_3 (1.8 g, 13 mmol) were added. The mixture was stirred overnight at rt under Ar. Water (30 mL) was added and extracted by EtOAc (150 mL). Organic layer was

washed with brine solution, dried over Na_2SO_4 and concentrated under vacuum pressure. Residue was purified by silica gel column chromatography (EtOAc: Hex = 1:1) to give **3** as white solid (1.743 g, 87%). Silica gel TLC R_f = 0.73 (EtOAc: Hex = 1:1). ^{1}H NMR ($CDCl_3$) δ 7.41-7.38 (m, 10H, H-Bn), 6.17 (d, 1H, J = 4.0 Hz), 6.10 (d, 1H, H = 4.0 Hz), 5.06 (s, 4H, $2CH_2$ Bn), and 2.55 (s, 3H, CH_3) ppm. ^{13}C NMR ($CDCl_3$) δ 203.42 (C=O), 167.78 (C9), 165.30 (C7), 162.20 (C5), 136.05 and 135.81 ($2CH_2$ Bn), 129.01-127.40 (Carom Bn), 94.92 (C6), 92.58 (C8), 71.33 and 70.49 (Cq Bn) and 33.55 (CH_3) ppm.

4.2.17 Synthesis of *p*-benzyloxy benzaldehyde 4 [98]

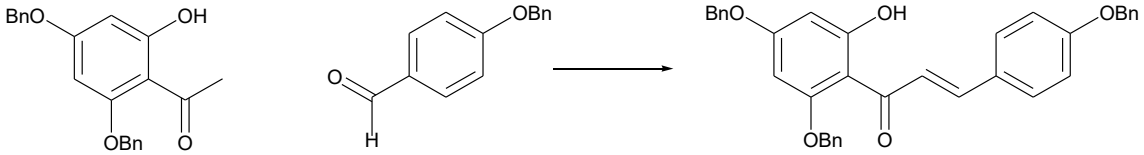


4

4-hydroxybenzaldehyde (2 g, 16 mmol) was dissolved in acetone (70 mL), then BnBr (2.1 mL, 18 mmol) and K_2CO_3 (2.5 g, 18 mmol) were added. The mixture was stirred overnight at rt under Ar. Water (30 mL) was added and extracted by EtOAc (90 mL). Organic layer was washed with brine solution, dried over Na_2SO_4 and concentrated under vacuum pressure to give **4**, which was recrystallized from 1:3 EtOAc: Hex to give white crystal (3.5 g, 100 %). R_f = 0.65 (EtOAc: Hex = 1:1). ^{1}H NMR ($CDCl_3$) δ 9.89 (s, 1H, CHO), 7.85 (d, 2H, *J* = 8 Hz), 7.45-7.35 (m, 5H, H-Bn), 7.09 (d, 2H, *J* = 8 Hz) and 5.16 (s, 2H, CH_2- Bn) ppm. ^{13}C NMR ($CDCl_3$): δ 191.02, 163.95, 136.03, 132.22, 130.34, 128.96, 128.56, 127.71, 115.36 and 70.49 ppm.

Synthesis of 3-(4-benzyloxyphenyl)-1-(2,4-benzyloxy-6-hydroxyphenyl) prop-2-en-1-one 5

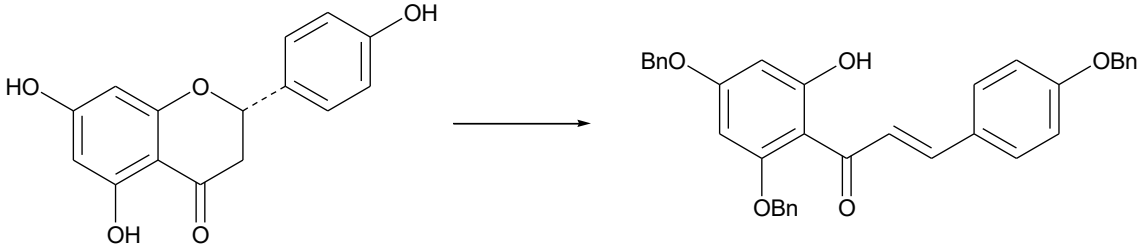
4.2.18.1 Synthesis of 5 *using 3 and 4*



3 **4** **5**

2',4'-dibenzyloxy-6-hydroxy acetophenone **3** (840 mg, 2.4 mmol) and *p*-benzyloxy benzaldehyde **4** (510 mg, 2.4 mmol) were dissolved in DMF (26 mL), then NaH (230 mg, 9.6 mmol) was added. The mixture was stirred overnight at rt under Ar, and then water (30 mL) was added and extracted by EtOAc (200 mL). Organic layer was washed with brine, dried over Na_2SO_4 and concentrated under vacuum pressure. Residue was purified by silica gel column chromatography (EtOAc: Hex = 1:3) to give **5** as bright yellow solid color [98] (1.0 g, 85%). R_f = 0.5 (EtOAc: Hex 1:3).

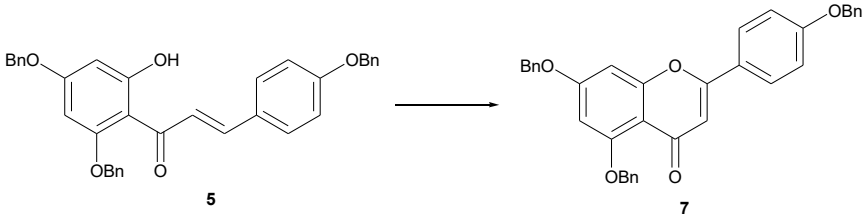
4.2.18.2 Synthesis of **5** *using 6*



6 **5**

Acetone (100 mL) was added to naringenin **6** (3.0 g, 11 mmol). BnBr (4.0 mL, 34 mmol) and K_2CO_3 (9.0 g, 65 mmol) were added. The reaction mixture was heated at reflux (80 °C) for 7 h under N_2. After the solution was cooled and evaporated under vacuo, the residue was dissolved in EtOAc, washed with water, dried over Na_2SO_4 and concentrated under vacuo. The residue was purified by silica gel column chromatography, and EtOAc: Hex (1:3) to give compound **5** as bright yellow solid [101] (3.3 g, 54 %). ^{1}H NMR ($CDCl_3$) δ 7.82 (dd, 2H, *J* = 15.6 Hz), 7.51-7.34 (m, 15 H), 7.01 (d, 2H, *J* = 8.8 Hz), 6.79 (d, 2H, *J* = 8.8 Hz), 6.23 (d, 1H, *J* = 2.4 Hz), 6.17 (d, 1H, *J* = 2.0 Hz), 5.10 (s, 2H), 5.08 (s, 2H), 5.05 (s, 2H) ppm. ^{13}C NMR ($CDCl_3$) δ 192.78, 169.02, 165.27, 161.88, 160.47, 142.96, 136.74, 136.09, 135.67, 130.35, 129.13, 129.03, 128.92, 128.88, 128.77, 128.55, 128.36, 128.20, 127.87, 127.66, 127.43, 125.41, 115.19, 106.50, 95.24, 92.67, 71.59, 70.48, 70.24 ppm.

4.2.19 Synthesis of 4',5,7-Tri-*O*-benzylapigenin 7 [101]

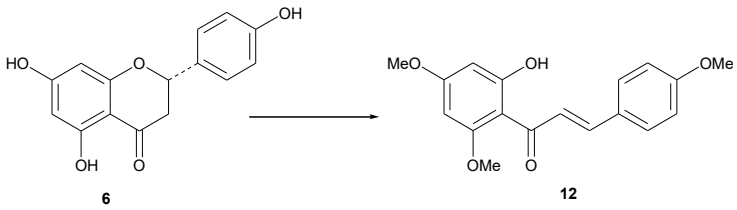


5 **7**

3-(4-benzyloxy phenyl)-1-(2,4-benzyloxy-6-hydroxy phenyl) prop-2-en-1-one **5** (3.0 g, 5.5 mmol) was dissolved in DMSO (50 mL). Iodine (78 mg, 0.6099 mmol) was added. The reaction mixture was stirred at reflux (110 °C) overnight under N_2. The mixture was cooled to rt, water (30 mL) was added and extracted by EtOAc (150 mL). Organic layer was washed with 1N HCl (aq.), brine, dried over $MgSO_4$ and concentrated under vacuum pressure. Residue was purified by silica gel column chromatography (EtOAc: Hex = 1:1) to give **7** as off white (2.3 g, 75 %). R_f = 0.23 (EtOAc: Hex = 1:2; violet spot with UV (254 nm)). ^{1}H NMR ($CDCl_3$) δ 7.83 (d, 2H, *J* = 9.2 Hz), 7.62 (d, 2H, *J* = 7.6 Hz), 7.46-7.37 (m, 15H), 7.09 (d, 2H, *J* = 9.2 Hz), 6.65 (d, 1H, *J* = 2.0 Hz), 6.59 (s, 1H), 6.50 (d, 1H, *J* = 2.4 Hz), 5.24 (s, 2H), 5.15 (s, 2H), 5.12 (s, 2H) ppm. ^{13}C NMR ($CDCl_3$) δ 177.64, 163.03, 161.37, 160.90, 159.86, 136.61, 136.46, 135.89, 128.96, 128.90, 128.77, 128.63, 128.42, 127.81, 127.66, 126.79, 124.24, 115.41, 109.97, 107.93, 98.52, 94.42,

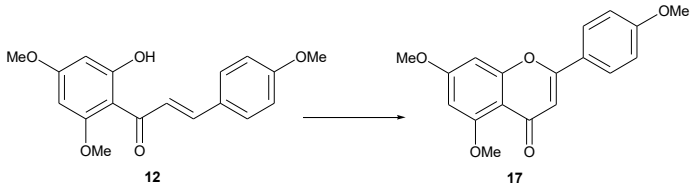
70.92, 70.67, 70.34 ppm.

4.2.20 Synthesis of 3-(4-Methylphenyl)-1-(2,4-methoxy-6-hydroxyphenyl) prop-2-en-1-one 12 [103]



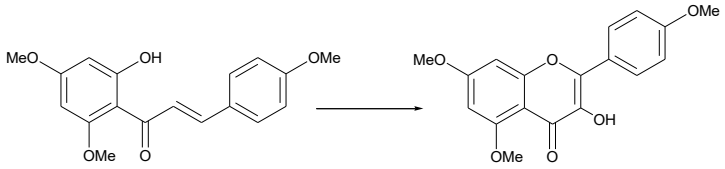
A mixture of naringenin **6** (500 mg, 1.846 mmol), K_2CO_3 (1500 mg, 11.00 mmol), 10 ml acetone and Me_2SO_4 535 µl (694 mg, 5.5 mmol) was refluxed (80 °C) for 4 h. the reaction mixture was cooled to room temperature, added water, extracted by dichloromethane, dried over $MgSO_4$ and concentrated under reduced pressure. Then residue was purified by silica gel column using EtOAc/ Hex (1/3) as eluting solvent to afford **12** as yellow solid (348.5 mg, 70%). R_f = 0.25 (EtOAc: Hex = 1:3). ^{1}H NMR ($CDCl_3$) δ 7.80 (d, 2H, *J* = 1.6 Hz), 7.57 (d, 2H, *J* = 8.7 Hz), 6.93 (d, 2H, *J* = 8.7 Hz), 6.11 (d, 1H, *J* = 2.3 Hz), 5.96 (d, 1H, *J* = 2.3 Hz), 3.92(s, 3H), 3.86(s, 3H), 3.84 (s, 3H) ppm; ^{13}C NMR ($CDCl_3$) δ 192.0, 168.49, 166.12, 162.55, 161.45, 142.63, 130.26, 128.37, 127.88, 125.18, 114.45, 106.41, 93.86, 91.33, 55.97, 55.72, 55.53 ppm.

4.2.19 Synthesis of 4′,5,7-Tri-*O*-Methylapigenin 17 [103]



(243 mg, 0.77 mml) of **12** was dissolved in Anh. DMSO and added (20.8 mg, 0.16 mmol) of Iodine, then the reaction mixture was heated overnight at 110 °C. the mixture was cooled to rt, diluted with ethyl acetate, washed with 1N HCl, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silicagel column chromatography using a mixture of EtOAc/ Hex (1/1), EtOAc (100%), CH_2Cl_2 (100%) and MeOH (100%) as eluted solvents. The elution with MeOH (100%) led to give **17** as (185 mg, 76%), R_f = 0.18 (EtOAc 100%, violet spot with UV (254 nm)). ^{1}H NMR ($CDCl_3$) δ 7.79 (d, 2H, J = 8.0 Hz), 6.97 (d, 2H, J = 8.0 Hz), 6.59 (s, 1H), 6.54 (d, 1H, J = 2.0 Hz), 6.35 (d, 1H, J = 1.6 Hz), 3.94 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H) ppm; ^{13}C NMR ($CDCl_3$) δ 177.59, 163.81, 161.90, 160.59, 160.52,159.55, 127.42, 123.32, 114.15, 108.71, 107.07, 95.61, 92.66, 56.22, 55.73, 55.36 ppm.

4.2.21 Synthesis of 4′,5,7-Tri-*O*-methylapigenin 18 [105]



A mixture of MeOH and 20% NaOH was added to **12** (118 mg, 0.376 mmol) and 1.5 ml of H_2O_2 (30%) was added. The reaction mixture was stirred overnight at 0 °C. then the mixture was acidified by 5% HCl (25 ml) and extracted with dichloromethane, dried over $MgSO_4$ and concentrated under vacuum pressure. The residue was purified by silica gel column chromatography and EtOAc/ Hex (1/1) to give **18** as yellow solid (18.4 mg, 15%), R_f = 0.25 (EtOAc: Hex = 1:1). ^{1}H NMR ($CDCl_3$) δ 8.18 (d, 2H, *J* = 8.9 Hz), 7.03 (d, 2H, *J* = 8.9 Hz), 6.55 (d, 1H, *J* = 1.7 Hz), 6.35 (d, 1H, *J* = 1.7 Hz), 3.98 (s, 3H), 3.91 (s, 3H), 3.89 (s, 3H) ppm; ^{13}C NMR ($CDCl_3$) δ 172.09, 164.43, 160.74, 159.00, 142.42, 137.57, 129.01, 123.68, 114.13, 106.35, 95.79, 92.50, 56.55, 55.96, 55.53 ppm.

4.3 Results and discussion

Kaempferol 3-*O*-neohesperidoside **1** was isolated from the *Solenostemma argel*. Flavonoid **1** was reported to have insulin-mimetic activity. The first synthesis of **1** has been reported in 2011 using PTC protocol [98]. In the present study, we will synthesize **1** using the same protocol by following different procedure for the synthesis of flavonol and disaccharide bromide **30**. For the synthesis of disaccharide bromide **30**; the hydroxyl groups of the known compound **40** was protected by acetyl groups by using NaOAc and acetic anhydride gave 76% of **41**, which is selectively deprotected the anomeric acetyl group to afforded **42** as pure α anomer. Hemiacetal **42** was then activated into the corresponding TCA derivative **28** in excellent yield (95%) as pure α anomer (Scheme 17).

The next challenge was made to accomplish the synthesis of 1−O−Acetyl−3,4,6 −tri−*O*−benzyl−D−glucopyranose **27.** 3,4,6-Tri−*O*−benzyl−D−glucal **26** was prepared from commercially available 3,4,6 Tri−*O*−acetyl−D−glucal **25** by deacetylation and subsequent benzylation (Scheme 18). Then **26** was subjected to epoxidation reaction by following the reported procedure in the previous studies. In scheme 18A; **26** was treated with DMDO/acetone followed by addition of excess amount of AcOH [113] obtained 43% of **27**. However, DMDO was unstable and possessed serious safety problems. Its low concentration made its scaling up to be difficult. On the other hand, the epoxidation of **26** using in situ formed DMDO was achieved, in which, a vigorous stirring of **26** in CH_2Cl_2 with Oxone, acetone and saturated aqueous $NaHCO_3$ at 0 °C followed by

opening of the formed epoxide with acetic acid [114] furnished **27** in 56 % yield (Scheme 18B), Although the methods partially overcame the DMDO's problem, the reaction was carried out in acidic and hydrous conditions to afford the formation of diol instead of **27**. Furthermore, as shown in Scheme 18C, the double bond of **26** was oxidized with *m*CPBA in CH_2Cl_2 at rt followed by ring opening by the *m*-chlorobenzoate anion generated in situ afforded **41** in good yield (93%) with high stereoselectivity (α/β = 1/5). Comparing with the reported finding [116], (α/β = 5/1), α anomer was the major; whereas our obtained product was β anomer as a major isomer. Then, **41** was treated with excess of acetic acid to furnish **27** (Table 8, entry 6). The results showed the reaction was not proceeded. Similarly, we failed to obtain **44** and **46** when using different conditions (Table 8, entries 1 and 5). Therefore, KF was added to *m*CPBA in CH_2Cl_2 under anhydrous conditions to reduces the solubility of *m*CPBA and to avoid the undesired products that form from the opening of the epoxide ring by *m*-chlorobenzoate anion (scheme 18D). Obtaining pure sugar epoxide was failed when oxidized **26** with in situ formed *m*CPBA−KF complex by procedure described by Bellucci and co-worker [119] (table 9). Compound **43** was formed as the major product. On the other hand, good results were obtained when *m*CPBA−KF was filtered off from the reaction mixture and used the solid complex for the epoxidation reaction of **26** (Table 10). The mixture of pure sugar epoxides **48** were obtained in good yield 80% and high stereoselectivity (α/β = 6/1) when used 1:5 molar ratio **26**: *m*CPBA−KF complex (entry 4)

in anh. CH_2Cl_2 at rt; with low yield of **43** (0.5%). Furthermore, the reaction conditions of the preparation of *m*CPBA−KF complex were optimized and good results were achieved when a 1:4 molar in anh. CH_2Cl_2 was used (Table 11, entry 3). The purification of sugar epoxide mixture **48** with silica gel chromatography led to the formation of diol by opening the oxirane ring with the acidic silica, therefore, **48** was recrystallized and directly selectively opened by an addition of acetic acid in anh.CH_2Cl_2 at rt under anhydrous conditions furnishing a mixture of **27** in 73% yield (α/β = 1/12).

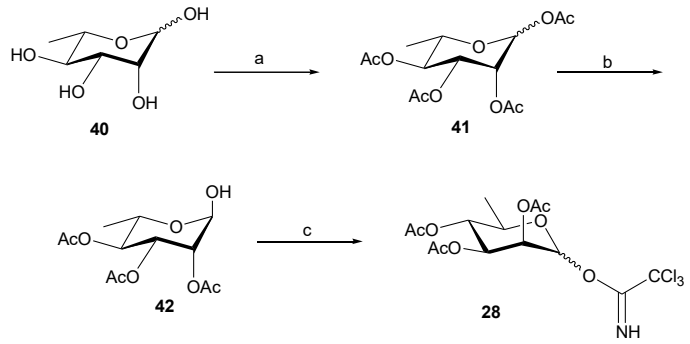
Glycosylation reaction was achieved using two different glycosyl acceptor. In the first reaction (Scheme 19A), the glycosyl acceptor **43** was glycosylated with freshly prepared rhamnosyl donor TCA derivative **28**. TMSOTf was employed as a promotor at rt without workup (Table 12, entry 1). Workup with the reagent stated in entries 2 to 5 afforded the disaccharide **47** mixed with the excess of the acceptor and trichloroacetamide as byproducts. These byproducts were difficult to remove from the product mixture due to their physical properties [120]. Heukendroff and Jensen suggested that the trichloroacetamide could be removed from the organic solvents by basic extraction since the amide protons are slightly acidic due to the electron withdrawing trichloromethyl group. Therefore, NaOMe and Et_3N were used as workup reagents (entries 6 and 7) to afforded **47** mixed with excess of the acceptor and TCA was completely removed from the reaction mixture. Furthermore, in the mechanism of the activation of TCA donor, the Lewis acid

(TMSOTf) reacts with the nitrogen atom in the trichloroacetamidate to furnishing the highly reactive oxocarbenium ion intermediate (Scheme 20). The activated TCA will compete the glycosylation reaction with glycosyl acceptor to afford the corresponding trichloroacetamide that may lead to a low recovery of the disaccharide and high recovery of the acceptor [121, 122]. Based on this mechanism, the donor **28** and acceptor **43** were premixed with freshly activated 4 Å MS in the Anh. CH_2Cl_2 in -20 °C for 30 min under anhydrous conditions, where a cooled solution of TMSOTf in anh. CH_2Cl_2 was added [123]. The reaction was again proceeded in anhydrous conditions for 30 min and quenched with Et_3N to afford 27% of the mixture of the disaccharides **47** and the acceptor in ratio (7:1) entry 7. And the ratio of the anomeric mixture of **47** was found to be α/β = 1/4. Thereafter, the **47** was treated with acetic acid to replace the *m*ClBz group with acetyl group (Table 13, entries 1 and 2). The results showed the reactions were not proceeded. The same results were obtained when treated **47** with prepared 33% HBr (47−49%) in AcOH (entries 3, 4 and 5), whereas the reaction proceeded and the disaccharide bromide **30** was obtained in 16% yield when reacted **47** with 25% HBr/AcOH (entry 6). In the second glycosylation reaction (Scheme 20B), the glycosyl donor **27** with freshly prepared rhamnosyl donor **28** in the presence of the TMSOTf in anh. CH_2Cl_2 at -20 °C for 30 min afforded a mixture disaccharide **29** in 60 % (α/β = 1/2). Similarly, **30** was not obtained when treated **29** with prepared 33% HBr (47−49%) in AcOH (Table 14, entry 1). It was successfully reacted with the

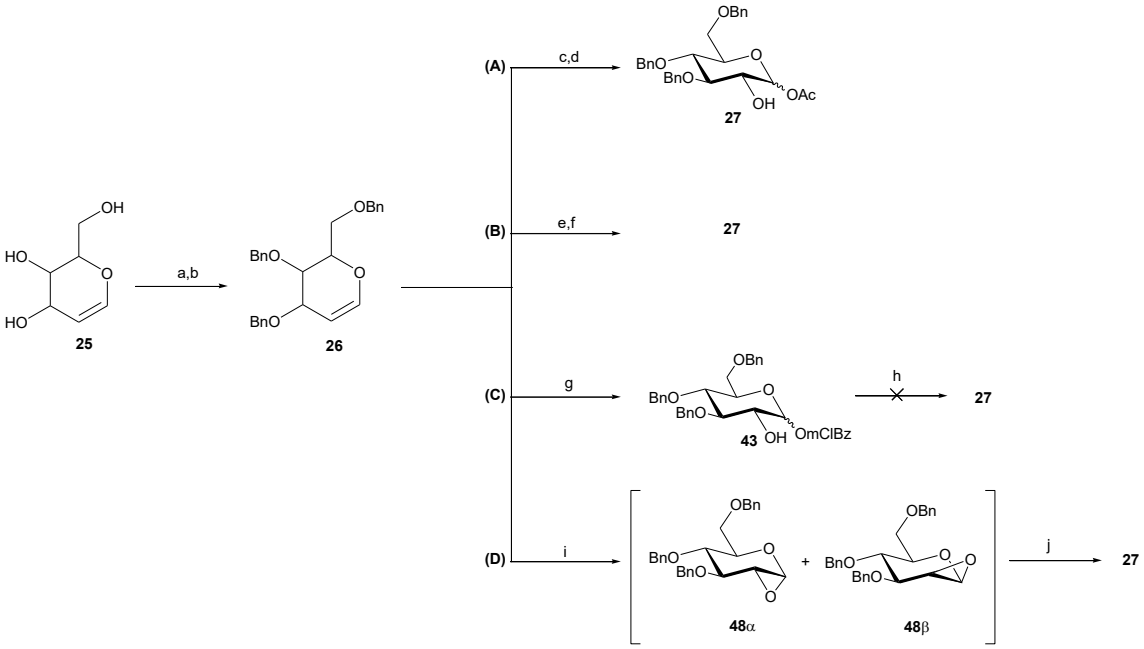
commercially available 25% HBr/AcOH (entry 2) to furnish **30**. On the other hand, **30** was found unstable upon silica gel chromatography and the crude was therefore directly used for the glycosylation with flavonol**.**

The investigation was made to synthesize the flavonol **8**. The hydroxyl groups of the inexpensive commercially available compounds **2** and 4−hydroxybenzaldehyde were protected with benzyl group to afforded **3** and **4**, respectively (Scheme 21A and B). Then **3** and **4** were subjected to aldol condensation using NaH in DMSO at 0 °C to accomplished **5** in 85 %. Whereas the benzylation of naringenin **6** afforded only 54 % of **5**, the flavone **7** was obtained in good yield (73 %) by subsequent cyclization in the presence of catalytic I_2 in DMSO at 140 °C (Scheme 21C). Furthermore, **7** was reacted with DMDO/Acetone (5 eq.) led to poor yields of the desired flavonol **8** (0.4 %) (Table 15, entry 1), Conditions of the epoxidation of flavone **7** were investigated (entries 2 to 5). The results showed that **8** was not obtained (scheme 21E). Moreover, as shown in Scheme 12D, the desired flavonol **8** was not obtained when treated **5** with hydrogen peroxide (Table 16, entry 1 and 2). On the other hand, the reaction of **5** with *m*CPBA led to the formation a new compound **47** instead of **8** (entries 3 to 8). ^{1}H NMR analysis of **47** showed one of the protons on A–ring was disappeared and one hydroxy group was connected to the ring.

On the other hand, naringenin **6** has been protected with methyl group by refluxing with Me_2SO_4 in the presence of K_2CO_3 to afforded the methylated chalcone **12** in good yield 70% (scheme 22). By following the Algar−Flynn−Oyamada reaction, Chalcone **12** was treated with alkaline 30% H_2O_2 in MeOH followed by opening the epoxide ring by acidifying the reaction mixture with 5% $HCl_{(aq.)}$ to accomplished the desired flavonol **18** in a moderate yield 15% (Table 17, entry 1). Furthermore, the epoxidation of **12** with other oxidizing agent were studied (entries 2, 3 and 4), the results showed that **18** was not obtained and **50** was formed when using triazox and *m*CPBA−KF as oxidant (scheme 22), the position of the hydroxy group on the aromatic ring A was confirmed by the absence of one the two protons signal in the region 5.5 and 5.6 ppm (region of protons of carbon 6 and 8) in the proton NMR spectrum

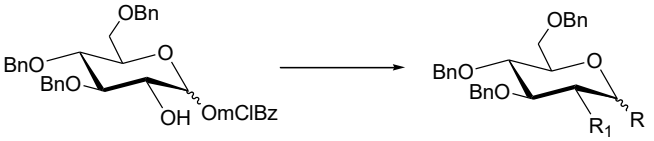


Scheme 17 Synthesis of 2,3,4-Tri-*O*-acetyl-L-rhamnopyranosyl trichloroacetoimidate (**28**). Reagents and conditions: (a) NaOAc, Ac_2O, 140°C (reflux), 4 h, 76%. (b) Ethylene diamine, Anh. THF, AcOH, rt, overnight, 69%. (C) CH_2Cl_2, CCl_3CN, DBU, rt, 30 min, 95%.



Scheme 18 Oxidation reaction of (**26**). Reagents and conditions: (a) NaOMe, MeOH, rt (b) DMF, NaH, BnBr, 0°C to rt, 12 h, 53%. (c) DMDO/Acetone, 0 °C, 6 h. (d) AcOH, rt, 14 h, 43%. (e) CH_2Cl_2, Acetone, $NaHCO_3$, Oxone, 0°C (30 min), rt (2 h), (f) AcOH, rt, 50.5%. (g) CH_2Cl_2, *m*CPBA, rt, 89% (α/β). (h) Table 8 (i) Table 9 and10 (j) Anh. CH_2Cl_2, AcOH, 12h, rt, 73%.

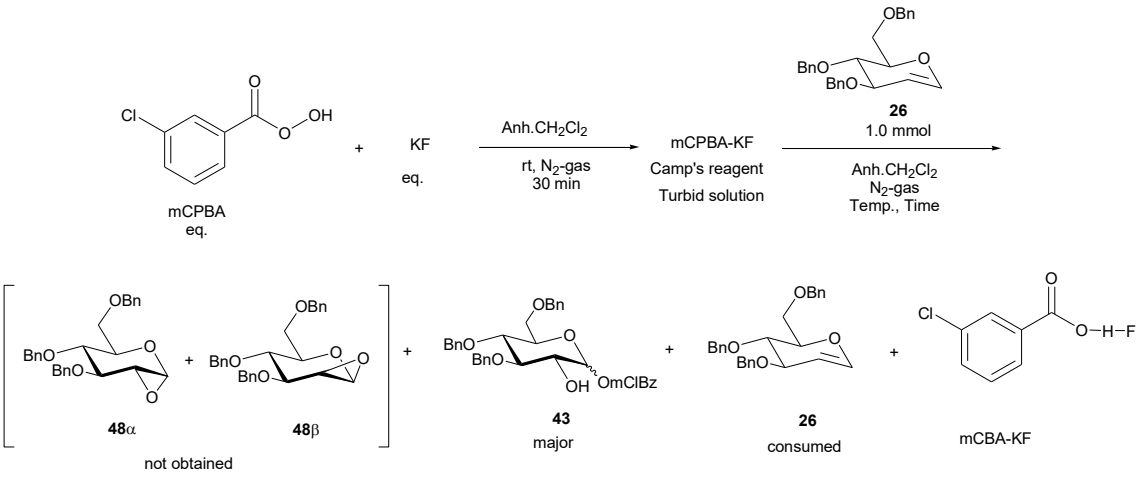
Table 8 Substitution reaction conditions of *m*ClBz group



43 **27, 44-46**

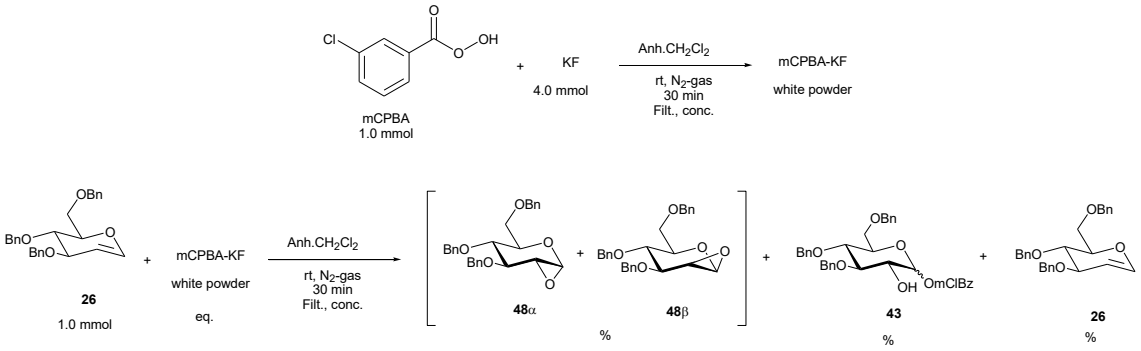
Entry	Conditions	compound	R	R_1	%
1[110]	NaOAc (1.0 eq.), Ac_2O (17.0 eq.), 140 °C (reflux), 3 h	44	OAc	OAc	0
2[101]	Br_2 (1.5 eq.), 0 °C, 1 h	45	Br	OH	0
3	CH_2Cl_2, Br_2 (2.0 eq.), rt, 3 h	45	Br	OH	0
4	CH_2Cl_2, Br_2 (2.0 eq.), 40 °C, (1.5 h), rt (3 h)	45	Br	OH	0
5[114]	MeOH, Ac_2O, Pyridine, rt, overnight	46	OCH_3	OAc	0
6	AcOH (excess), rt, overnight	27	OAc	OH	0

Table 9 Oxidation of **26** using in situ formed *m*CPBA–KF complex [119].



Entry	mCPBA eq.	KF eq.	Temp.	Time
1	4	8.5	40 °C	5 h
2	4	8.5	rt	4 h
3	2.4	5.0	rt	30 min
4	2.5	5.0	rt	overnight
5	0.68	5.0	rt	1 h 15 min

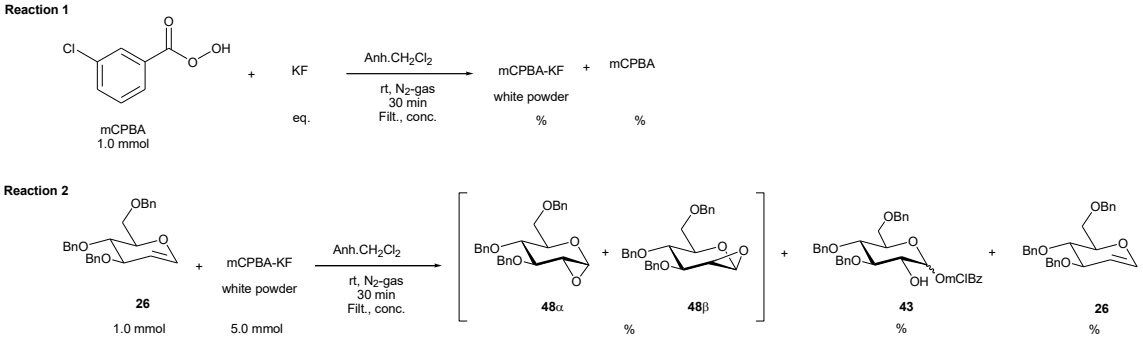
Table 10 Oxidation of **26** using *m*CPBA−KF complex powder[a]



Entry	mCPBA−KF eq.	Temp.	48 % (α/β)	43 %	26 %
1	2	Rt	8.05 (β is major)	10	23
2	3	Rt	73 (6/1)	14	9
3	4	Rt	78 (6/1)	6	7
4	5	Rt	80 (6/1)	6	5
5	6	Rt	76 (6/1)	7	5
6	7	Rt	47 (3/1)	16	8
7	8	Rt	50 (3/1)	17	13
8	9	Rt	9	23	12
9	5	0 °C	62 (3/1)	6	11

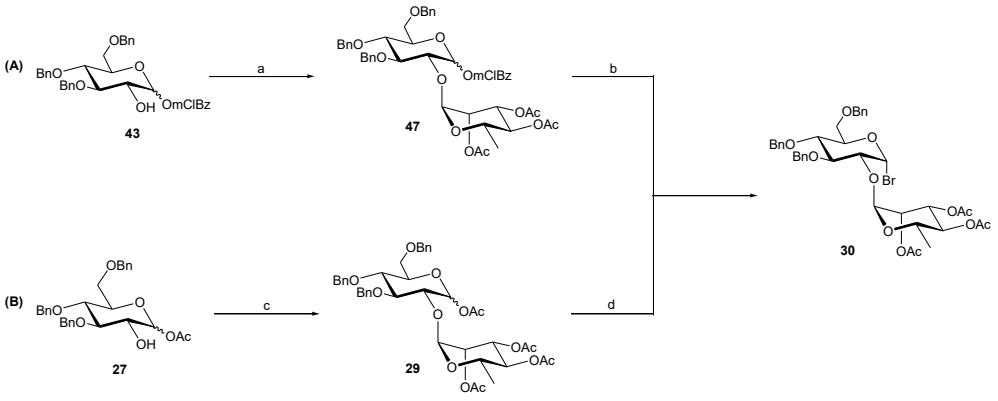
[a] All the reactions were carried out using activated KF (1 h at 120 °C) in Anh.CH_2Cl_2 under N_2-gas. The crude reaction mixture was analyzed by ^{1}H NMR using triphenyl methane as an internal standard (δ 5.5 ppm, relaxation delay = 20 s). The anomeric ratio was measured by comparison of integral intensities of the anomeric protons from ^{1}H NMR spectrum of the mixture.

Table 11 Synthesis of *m*CPBA−KF complex powder[a]



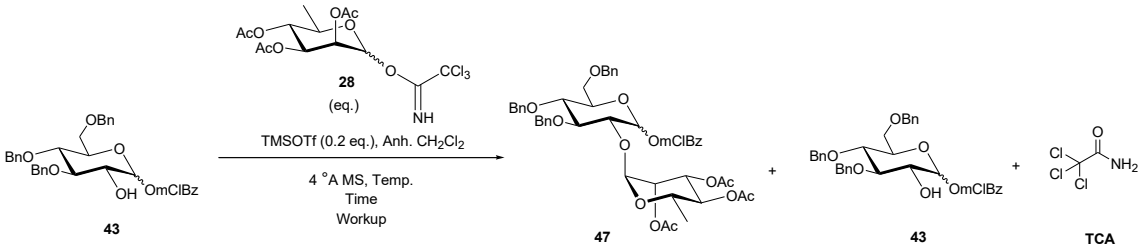
Entry	Reaction 1			Reaction 2		
	KF eq.	*m*CPBA−KF %	Excess *m*CPBA %	**48 % (α/β)**	**43 %**	**26 %**
1	2.0	87	11	74	9.0	16.5
2	2.89	60	42	70	2.0	16.0
3	4.0	101	12	88	0.5	5.0
4	5.0	96	17	63	0.3	21.0
5	6.0	102	14	70	1.0	35.5

[a] All the reactions were carried out using activated KF (1 h at 120 °C) in Anh.CH_2Cl_2 under N_2-gas. The crude reaction mixture was analyzed by ^{1}H NMR using triphenyl methane as an internal standard (δ 5.5 ppm, relaxation delay = 20 s).



Scheme 19 Synthesis of disaccharide bromide **30**. [a] Reagents and conditions: (a) compound **28**, Table 12 (b) Table 13 (c) compound **28**, TMSOTf (0.3 eq.), Anh.CH_2Cl_2, 4Å MS, -20 °C, 30 min, 60% (d) Table14.

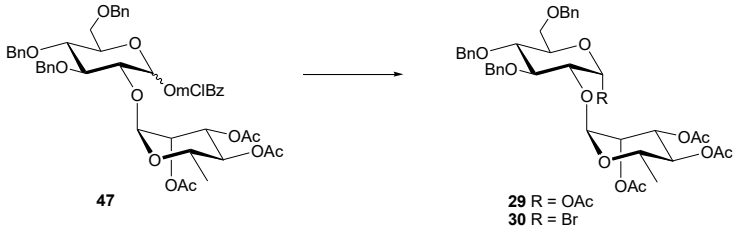
Table 12 Reaction conditions of the synthesis of **47**



Entry	43+28 mg	28 eq.	Time	Work up	mg (47: 43: TCA) [a]
1	41	1.4	13 h	–	14 (2:1:1)
2	41	1.4	2 h	HCl, NaOH, brine	5.4 (1:1:3)
3	41	1.4	o.n	NaOMe	9 (1:1:3)
4[107]	26	4.6	13 h	Et_3N	7 (1:2:3)
5	42	1.4	o.n	Et_3N (8 eq.), cat. DMAP, HCl, NaOH, brine	7 (1:1:1)
6[98]	142	4.7	13 h	NaOMe	57 (3:1:0)
7[c]	337	1.5	1 h	Et_3N	90 (7:1:0)

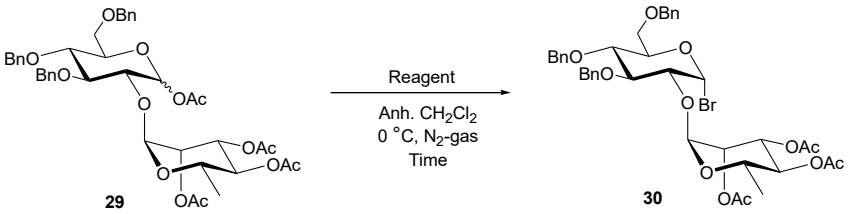
[a] products ratios were measured by comparison of the integral intensities of the protons from ^1H NMR spectrum of the mixture, [b]TMSOTf = 0.3 eq., -20 °C.

Table 13 Reaction conditions of the synthesis of **29** and **30** using **47**

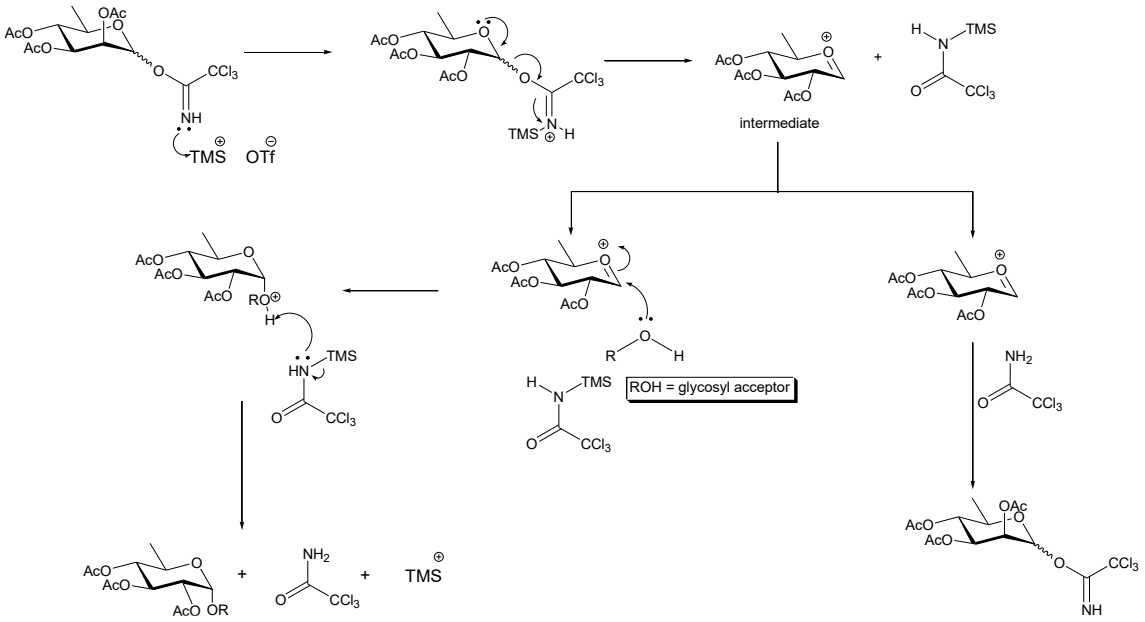


Entry	Reagent	Solvent	Temp.	time	R	%
1[113]	AcOH (excess)	–	rt	o.n	Ac	0
2	$pdCl_2$ (0.6 eq.), (Anh. Py, Ac_2O)	$MeOH/CH_2Cl_2$	rt	o.n, (3 h)	Ac	0
3[98]	33% HBr (47−49%) in AcOH (0.4 eq.)	CH_2Cl_2	rt	30 min	Br	0
4	33% HBr (47−49%) in AcOH (excess)	CH_2Cl_2	rt	o.n	Br	0
5[107]	HBr (47−49%),	CH_2Cl_2	0 °C	2 h	Br	0
6[a]	25% HBr/AcOH	CH_2Cl_2	0 °C	30 min	Br	16

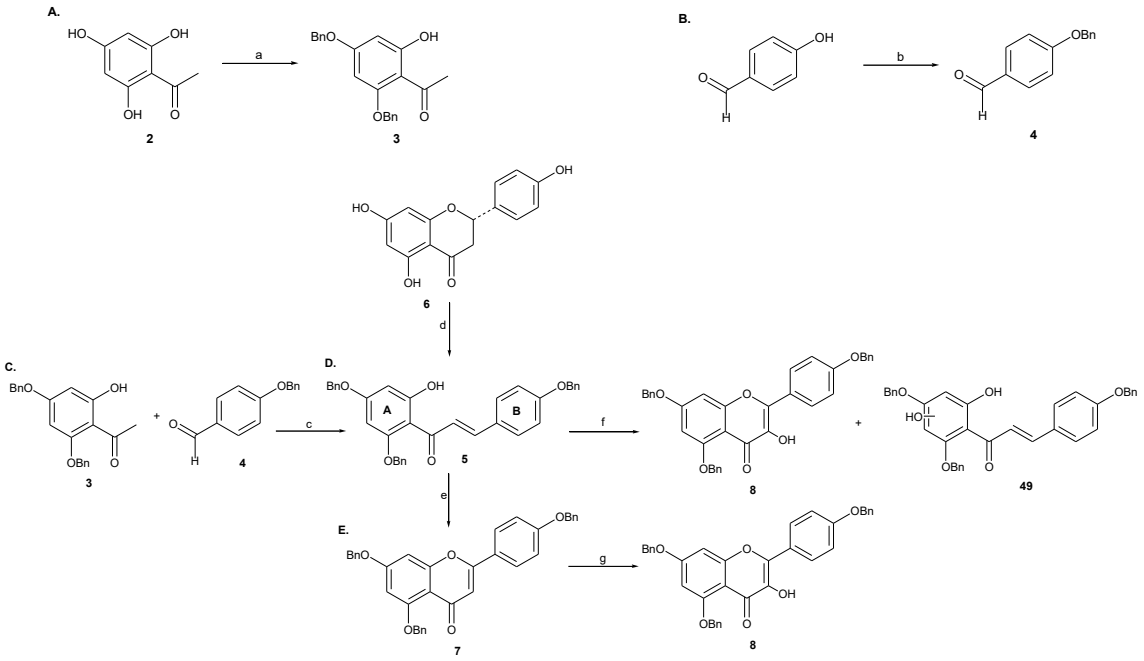
Table 14 Reaction conditions of the synthesis of **30** using **29**



Entry	Reagent	time	%
1[a]	33% HBr/AcOH (14 eq.) (is 47-49% HBr in AcOH)	o.n	0
2	25% HBr/AcOH (5 eq.) (Commercial HBr/AcOH)	30 min	16

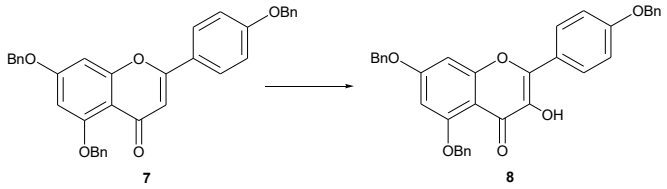


Scheme 20 Glycosylation reaction mechanism of the disaccharide



Scheme 21 Synthesis of flavonol **8**. Reagents and conditions: (a) BnBr, K_2CO_3, DMF, rt, 87%. (b) BnBr, K_2CO_3, DMF, rt, 100%. (c) NaH, DMF, rt, 85%. (d) BnBr, K_2CO_3, acetone, reflux (8°C), 54%. (e) DMSO, I_2, reflux (110 °C), 75%. (f) Table16 (g) Table 15.

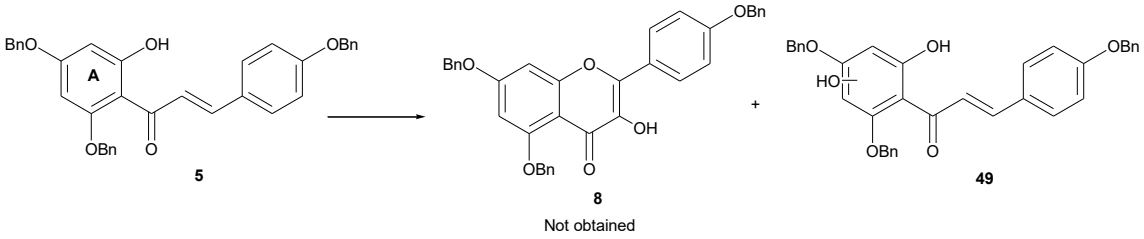
Table 15 Oxidation reaction conditions of **7**



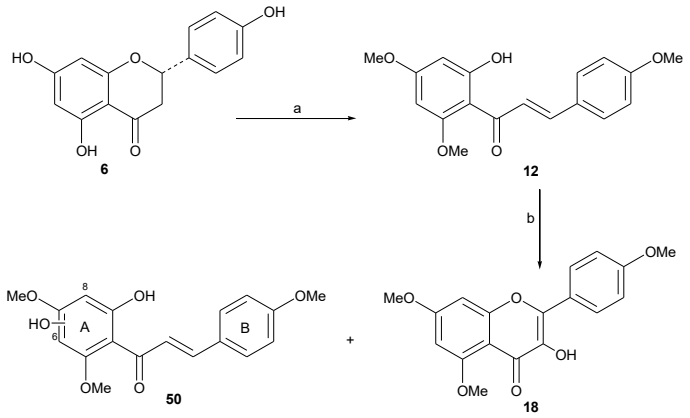
Entry	**Oxidizing agent (eq.)**	**solvent**	**Temp.**	**Time**	**8%**
1[101,98]	DMDO/Acetone (5)	CH_2Cl_2	0 °C	o.n	0.4
2	*m*CPBA (2)	CH_2Cl_2	rt	o.n	0
3	*m*CPBA (2)	1,4-dioxane	rt	o.n	0
4 [126], a	Acetone, carbonate buffer, 9% oxone, PH = 9	CH_2Cl_2	18 °C (rt)	16 h (1h)	0
5	DMDO/Acetone (1.8)	CH_2Cl_2	0 °C	o.n	0

[a] CH_2Cl_2: acetone (4:3 v/v)

Table 16 Oxidation reaction conditions of **5**

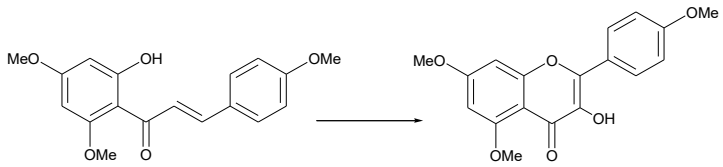


Entry	Oxidant (eq.)	Solvent	Additives	Temp.	49%
1[103]	30% H_2O_2 (7)	MeOH, 1,4-dioxane	5% NaOH	rt	0
2[105]	18% H_2O_2 (12)	MeOH	20% NaOH, 5% cold HCl	0 °C	0
3	*m*CPBA (2)	CH_2Cl_2	-	rt	23.5
4	*m*CPBA (2)	1,4-dioxane	-	rt	27.0
5	*m*CPBA (2)	1,4-dioxane	-	0 °C	7.0
6	*m*CPBA (2)	1,4-dioxane	-	101 °C	7.5
7	*m*CPBA (0.5)	1,4-dioxane	-	rt	9.0
8	*m*CPBA (4)	1,4-dioxane	-	rt	mixture



Scheme 22. Synthesis of flavonol **18**. Reagents and conditions: (a) Me_2SO_4, K_2SO_4, acetone, reflux (80 °C), 4h, 70%. (b) Table 17.

Table 17 Oxidation reaction conditions of **12**



Entry	Oxidant	Solvent	Additives	Temp.	Time	18%
1[105]	30% H_2O_2	MeOH	20% NaOH, 5% HCl	0 °C→ rt	o.n	15
2[124]	$PhI(OAc)_2$	MeOH	20% NaOH, 5% HCl	0 °C→ rt	o.n	0
3[127]	Triazox	CH_2Cl_2	2-methyl-2-butene	rt	1h, 15 min	0
4	*m*CPBA−KF	CH_2Cl_2	-	rt	o.n	0

4.4 Conclusion

*m*CPBA−KF was synthesized as white powder and non−hygroscopic. The complex was successfully used in the synthesis of a pure sugar epoxide in excellent yield (80%), and high stereoselectivity (α/β = 6/1) was observed. *m*CPBA−KF did not induce the ring opening arising by the *m*-chlorobenzoate anion generated in situ. The epoxide ring was opened with acetic acid followed by glycosylation with rhamnose donor **28** to afford anomeric mixture of the disaccharide **29,** which was easily aromatized with 25% HBr/AcOH to obtain pure α anomer of disaccharide bromide **30**. The disaccharide **47** was first synthesized in this study by the glycosylation of 1−*O*−*m*-chlorobenzoyl−3,4,6−Tri−*O*−benzyl−D−glucopyranose **43** and rhamnosyl TCA donor **28**. Compound **47** was also successfully used in the synthesis of the disaccharide bromide **30,** accordingly, *m*CPBA or *m*CPBA−KF was useful in the epoxidation of the glucal and synthesis of the disaccharide bromide donor in the last stage.

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