様式 Form 7
（外国人招へい研究者）

2024 年　10 月　7 日

YYYY/MM/DD

独立行政法人日本学術振興会理事長　殿
To: President, Japan Society for the Promotion of Science

研究活動報告書
Research Report

１．受入研究者/ Host researcher

受入研究機関・部局・職 Name of Host Institution, Department and Title	千葉大学・大学院薬学研究院・教授
受入研究者氏名 Host Researcher's Name	石川　勇人

２．外国人招へい研究者/ Fellow

所属研究機関・部局・職 Name of Institution, Department and Title	University of Yaounde 1, Department of Organic Chemistry, Faculty of Science, Associate Professor
外国人招へい研究者氏名 Fellow's Name	KAMTO Eutrophe Le Doux

３．採用期間/ Fellowship Period

2023 年　10 月　1 日　～　2024 年　7 月　31 日

４．研究課題/ Research Theme

Chemical studies on flavonoids and alkaloids with antiviral activity from Cameroonian folk medicine

５．研究活動報告/ Research Report

（１）研究活動の概要・成果/ Summary of Research Results

Main Objective: Investigation of antiviral activity of flavonoids and alkaloids from selected Cameroonian medicinal plants.

Hence the present study aims to explore the phenolic and alkaloids constituents present in the hydroalcoholic extracts of selected plant organs: *Ormocarpum sennoides* (aerial part), *Tabernaemontana contorta* (fruits), and *Alstonia boonei* (leaves) by diverse chromatographic techniques and the evaluation of their antiviral properties.

I. Collection of plants and extraction

Selected plant organs were harvested in February 2022, at Mbalmayo, (*O. sennoides* subsp. *Zanzibaricum,* aerial part), as well as in January 2023 at Nkolbisson (*T. contorta*, fruits), and at Yaounde (*A. boonei*, leaves) in the Centre region of Cameroon. Air-dried and powdered part of each plant (*O. sennoides,* 631.63 g), (*T. contorta*, 400 g), and (*A. boonei*, 400 g) was extracted exhaustively by sonication with MeOH-H_2O (8:2) mixture for 1 h x 3

consecutively. The protocol was repeated 6 times. The resulting extracts were obtained after filtration and evaporation under reduce pressure as: (*O. sennoides,* a dark brownish extract of 83.03 g), (*T. contorta*, a brownish extract 65.37 g), and (*A. boonei* A dark greenish extract of 91.93 g), respectively.

II. Investigation of the phenolic constituents from *Ormocarpum sennoides*

II.1. Botany and ethnomedicinal uses

Ormocarpum sennoides subsp. *zanzibaricum* Brenan & J.B. Gillett belongs to Papilionoideae. It is a shrub of 1–5 m tall with a rounded crown. The pinnate leaves are larges and alternates with entire petiolates, leaflets 9-17, and raised, and secondary veins 3 or 4 on each side. *This plant is mainly distributed in* tropical and subtropical regions in dry and open woodlands (Gillet et al., 1971). It finds its application as a traditional medicine for the treatment of convulsion, burns, bone fracture, sexually transmitted diseases, children's diseases and for prenatal care (Pakia et al., 2003; Thamacin et al., 2014; Srividya et al., 2018).

II.2. Previous pharmacological and chemical studies on the plant

Early Pharmacological research on the leaf of *O. sennoides* showed its antioxidant properties. The ethanol extract showed high antioxidant activity in the range 1.2431 µL to 3.4939 µL (Arulappan et al., 2014; Srividya et al., 2014). Similar investigations revealed the anti-inflammatory, anti-arthritic and osteogenic activities of this plant (Srividya et al., 2015; Srividya and Sridevi, 2016).

On the other hand, only tree biflavanoids (trime-chamaejasmin, (+)- chamaejasmin, and (+)-liquiritigeninyl-(I-3,II-3)-naringenin), an isoflavonoid (glabridin), and one bisdihydrocoumarin (diphisin) among other classes of metabolites have been isolated from *O. sennoides* (Willd.) DC subsp. *zanzibaricum* roots to date (Chalo et al., 2020).

According to the literature, there was not much chemical and biological studies reported for this plant, nor the real potential of its phenolic content and therapeutic properties in regard of other species within the genus.

Hence the present study aims to explore the phenolic constituents present in the hydroalcoholic extract of *Ormocarpum sennoides* (aerial part) using diverse chromatographic techniques and evaluate of their antiviral properties.

II.3. Materials and methods

II.3.1. Partition of the crude extract

In continuation, *O. sennoides* extract (50.01 g) was suspended in distilled water (250 mL), and successively extracted with hexane (6 x 250 mL), DCM (6 x 250 mL), EtOAc (6 x 250 mL) and water sat. n- BuOH (6 x 250 mL) to yield hexane (2.37 g), DCM (1.69 g), EtOAc (8.62 g), n-BuOH (16.41 g), and aqueous (20.51 g) fractions.

Prior to our targets, the crude extract and resulting fractions were submitted to Analytical HPLC methods on a SHIMADZU Corporation HPLC instrument equipped with a Capcell Pak C18UA120S-5, column (250 mm × 4-6 mm). The UV detection was set at 280 nm and the mobile phase was 0.1% Formic acid in water (solvent A) and acetonitrile (solvent B) at the following gradient: 0 min 90% A; 20 min 0% A; 40.33 min 0% A. The flow rate was 0.25 mL/min, while the column temperature was 40 °C to determine their phenolic potential.

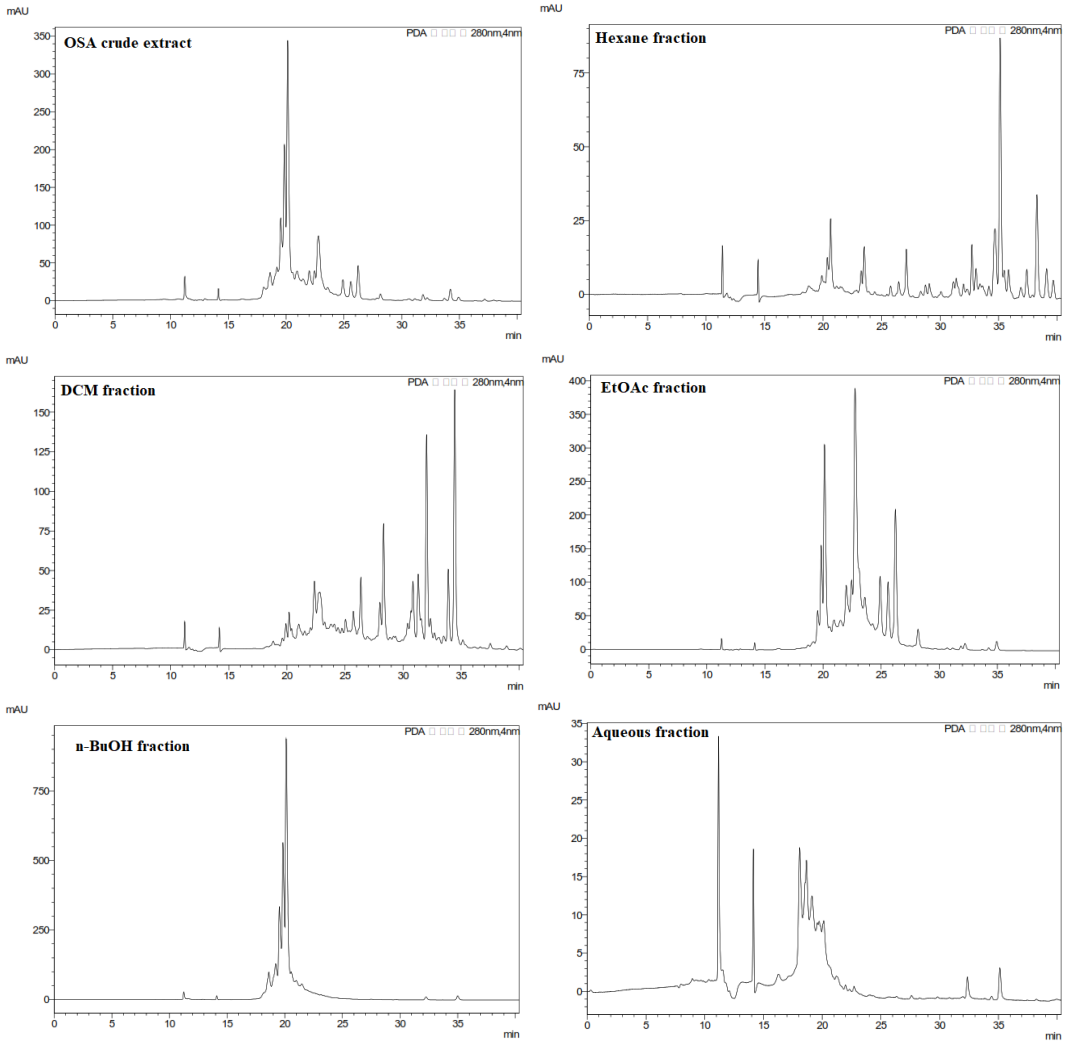


Figure 1. HPLC Chromatogram of *O. sennoides* aerial extract and its resulting fractions, Capcell Pak C18UA120S-5, 5 µm column (250 mm × 4-6 mm), UV detection at 280 nm. The mobile phase: 0.1% FA in water (solvent A) and acetonitrile (solvent B) at the following gradient: 0 min 90% A; 20 min 0% A; 40.33 min 0% A. The flow rate was 0.25 mL/min.

II.3.2. Fractionation of the EtOAc fraction

According to HPLC chromatograms, the EtOAc fraction was firstly selected for further investigation. Thus, EtOAc fraction (7 g) was taken in a minimum amount of water (10 mL) and then submitted to column chromatography (CC) using Diaion HP 20 resin, eluting with 600 mL/gradient of H_2O-MeOH solvent mixture at 20% MeOH, 40% MeOH, 60% MeOH, 80% MeOH, and 100% MeOH, successively to yield six fractions: OSEA1 (70 mg), OSEA2 (90 mg), OSEA3 (2.48 g), OSEA4 (2.51 g), OSEA5 (494 mg), and OSEA6 (1.22 g) after TLC monitoring.

II.3.3. Purification of selected fractions

Fraction OSEA3 (2.48 g) was repeatedly subjected to silica and Sephadex LH-20 gel columns chromatographic purifications steps to afford six compounds **1–6** (5 mg, 8 mg, 20.7 mg, 12,6 mg, 24.7 mg, 33.3 mg)

Fraction OSEA4 (2.13 g) was subjected over Silica gel column eluting with a gradient solvent system of

EtOAc-MeOH (10:0, 7:3, 4:6, 1:9 and 0:10) to collect 10 mL/tube. Nine fractions were obtained (OSEA4a–OSEA4i). Compound **8** (3.2 mg) was obtained directly as fraction OSEA4d (3.2 mg). Further purifications of both fractions OSEA4b (220.7 mg) and OSEA4f (317.9 mg) using Sephadex LH-20 gel column (MeOH–H_2O, 9:1 v/v) and yielded compounds **7** (11.3 mg), and **9** (4.7 mg), respectively.

Using the same method alternating silica and Sephadex LH-20 gel column chromatography, two compounds (**10**, **39.9 mg** and **11**, **31.8 mg**) were obtained from fraction OSEA5 (494 mg), while five ones (**12**, **4.3 mg; 13**, **9 mg**; **14**, **21.6 mg; 15**, **16.2 mg** and **11**, **6 mg**) were also purified from fraction OSEA6 (1.22 g).

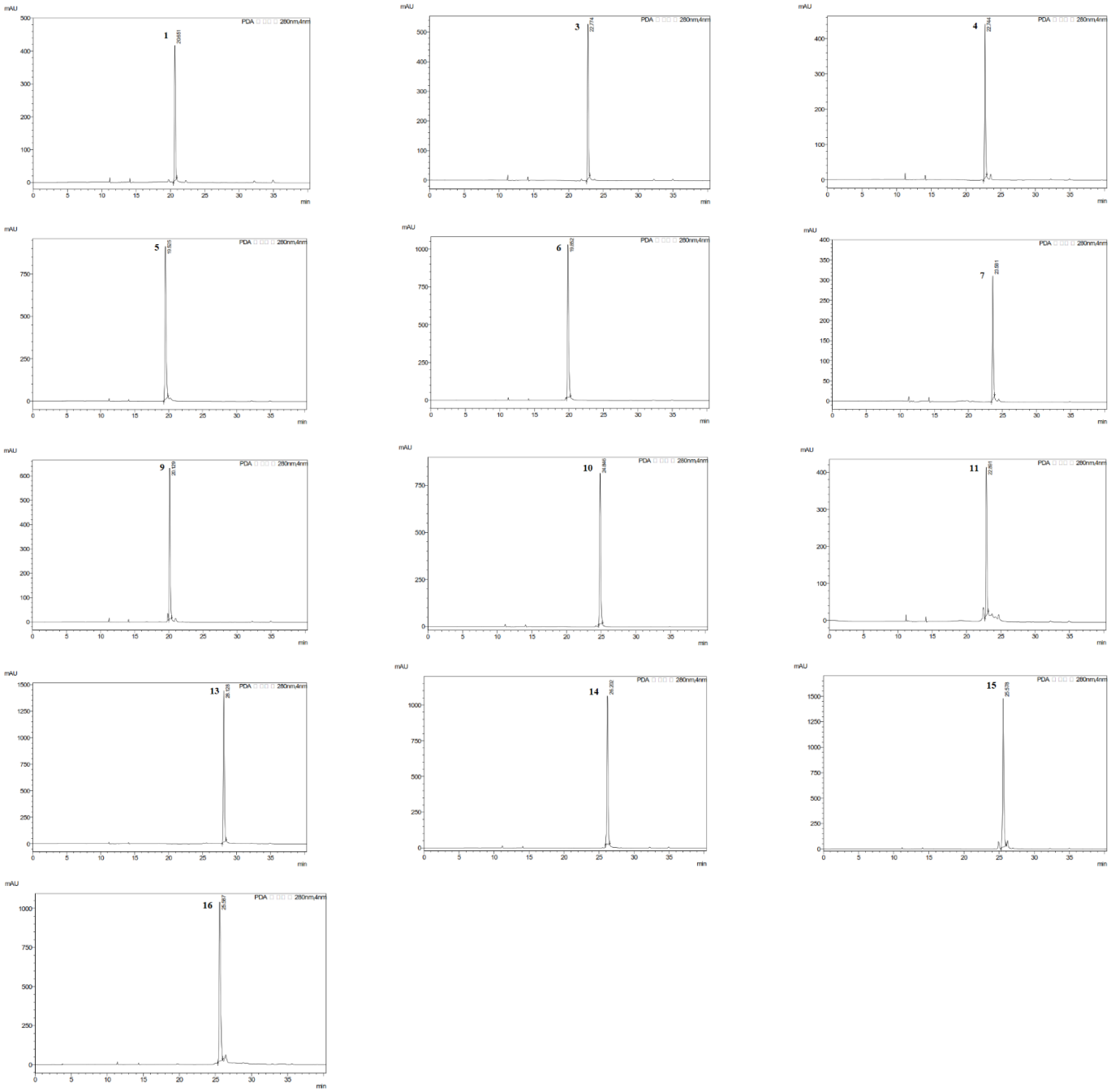
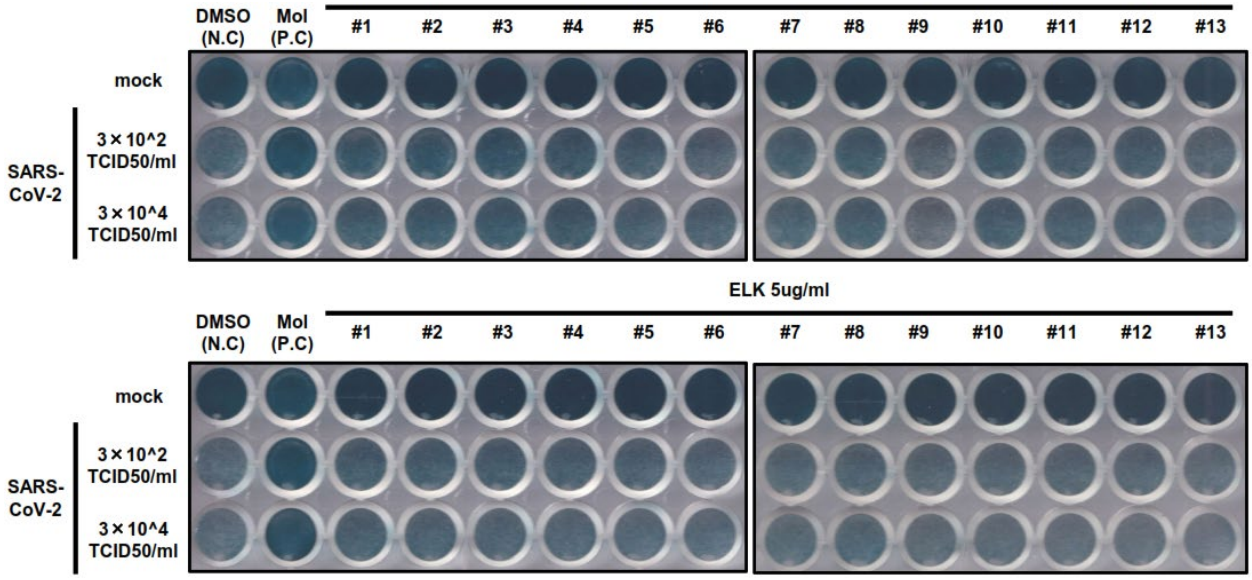


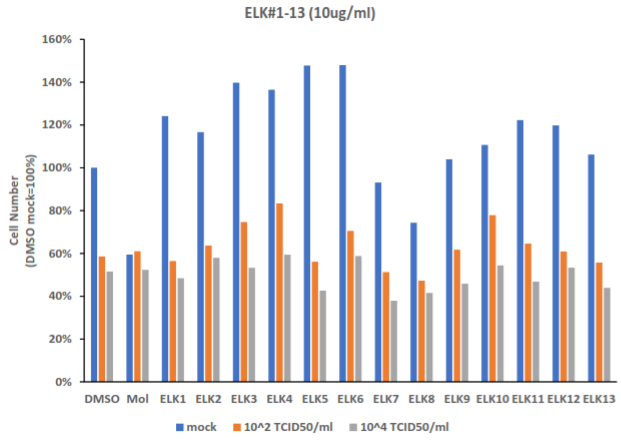
Figure 17. HPLC Chromatograms of some isolates

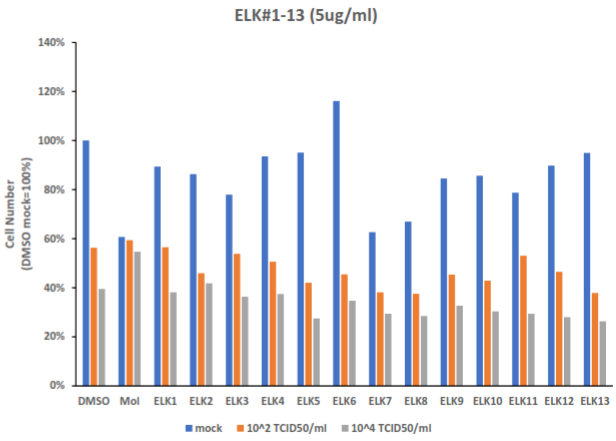
II.4. Evaluation of the antiviral properties of the isolates

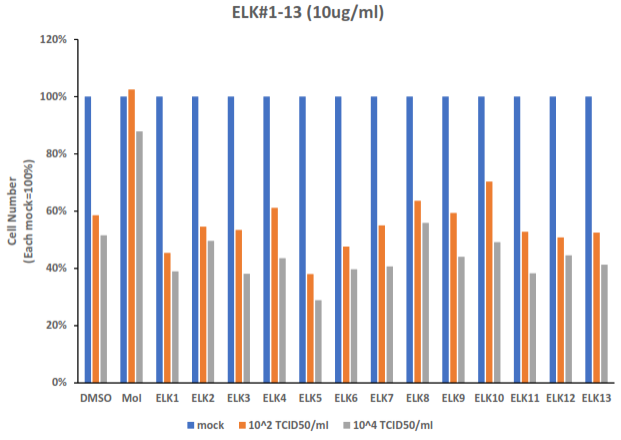
Some of the aforementioned isolates (**3–11**, **13–16**) were submitted for the antiviral assay against SARS-CoV-2 virus at different concentrations. But none of the aforementioned compounds showed interesting antiviral activity.

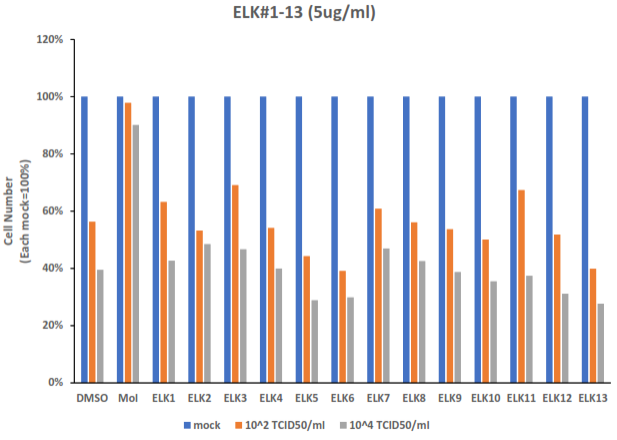


ELK 5ug/ml









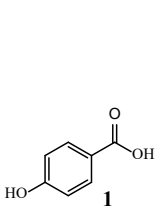
III. Summary

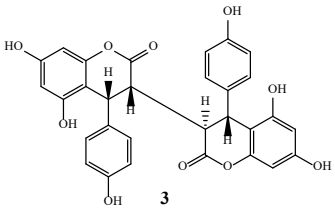
Air-dried and powdered part of *O. sennoides,* (631.63 g), *T. contorta*, (400 g), and *A. boonei*, (400 g) was extracted exhaustively by sonication with MeOH-H_2O (8:2) mixture to afford hydroalcoholic crude extracts after

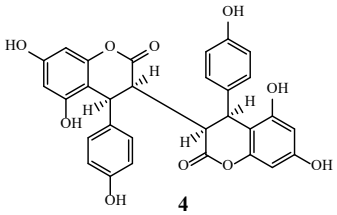
filtration and evaporation under reduce pressure: (*O. sennoides,* 83.03 g), (*T. contorta*, 65.37 g), and (*A. boonei* 91.93 g), respectively.

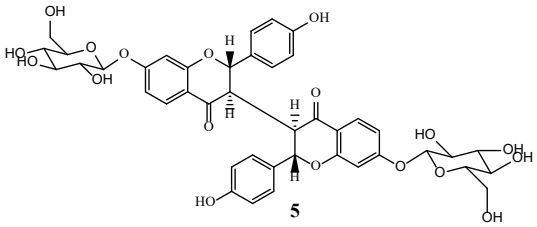
The study carried out on the aerial part of *O. sennoides subsp zanzibaruim* yielded to the isolation of sixteen phenolic compounds. Fourteen of them were totally characterized, while the structural elucidation of the remaining ones is ongoing. They consist of:

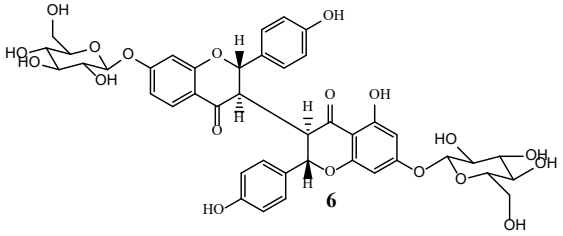
- **Compound 1**: 4-hydroxybenzoic acid, ESI-MS: m/z 139 $[M+H]^+$)
- **Compound 2**: Structural elucidation is ongoing
- **Compound 3**: 3″-Epidiphysin, (ESI-MS: m/z 543 $[M+H]^+$)
- **Compound 4**: Diphysin, ESI-MS: m/z 543 $[M+H]^+$)
- **Compound 5**: 7,7″-Di-O-glucosyl-(I-3,II-3) biliquiritigenin, (New glycosidic biflavonoid,-ESI-MS: m/z 835 $[M+H]^+$)
- **Compound 6**: 7,7″-Di-O-glucosyl liquiritigeninyl-(I-3,II-3)-naringenin, ESI-MS: m/z 851 $[M+H]^+$)
- **Compound 7**: 5-O-Methyldiphysin, ESI-MS: m/z 557 $[M+H]^+$)
- **Compound 8**: Isovitexin, (ESI-MS: m/z 433 $[M+H]^+$)
- **Compound 9**: 7,7-Di-O-glucosylchamaejasmin (Ormocarpin), ESI-MS: m/z 565 $[M+Na]^+$)
- **Compound 10**: Campylospermone A, ESI-MS: m/z 511 $[M+H]^+$)
- **Compound 11**: 7-O-Glucosylchamaejasmin, ESI-MS: m/z 705 $[M+H]^+$)
- **Compound 12**: Structural elucidation is ongoing
- **Compound 13**: Sikokianin C, ESI-MS: m/z 557 $[M+H]^+$)
- **Compound 14**: (+)-Chamaejasmin, ESI-MS: m/z 543 $[M+H]^+$)
- **Compound 15**: Liquiritigeninyl-(I-3,II-3)-naringenin, ESI-MS: m/z 527 $[M+H]^+$)
- **Compound 16**: (I-3,II-3)-Biliquiritigeninyl, ESI-MS: m/z 511 $[M+H]^+$)

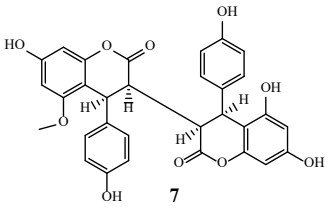


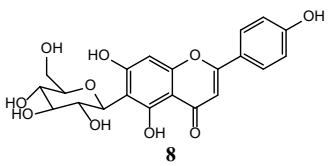


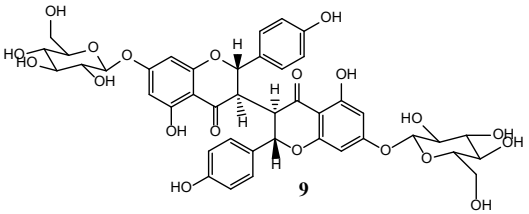












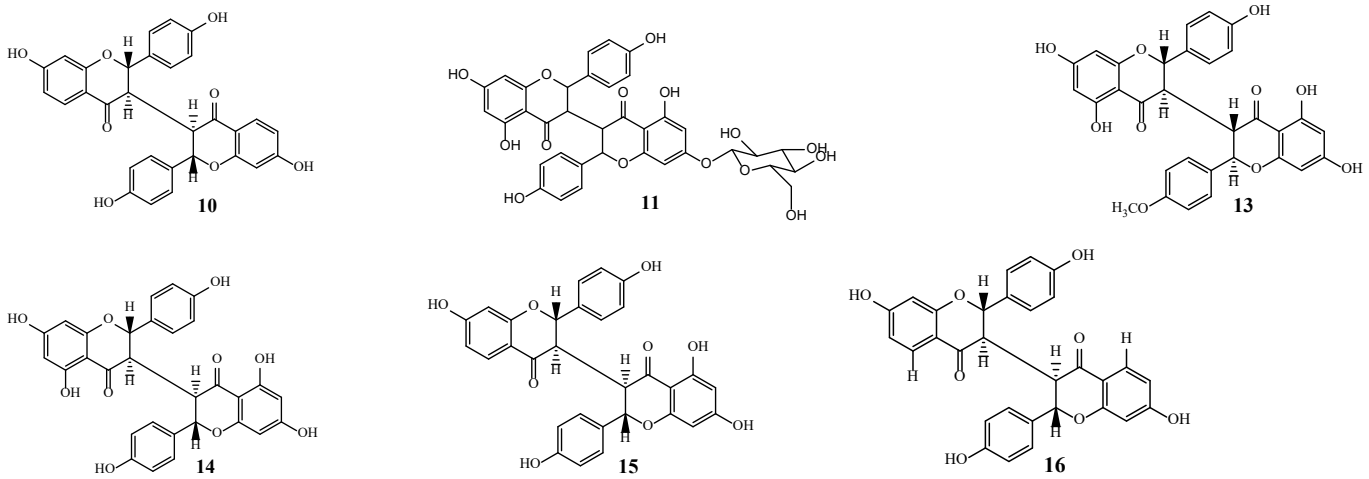


Figure 18. Structures of the isolated compounds from *Ormocarpum sennoides*

The antiviral evaluation of some of the isolates (**3–11**, **13–16**) were performed against SARS-CoV-2 virus at different concentrations without potent antiviral effect.

References

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（2）主な研究発表（雑誌論文、学会、集会、知的財産権等）/ Main Research Publications

（３）その他/ Remarks

Further chemical investigations will be carried out on remaining fractions and other plant extracts, while pharmacological studies will be performed against other viruses and/or some microbial agents.