Comparative pharmacognostic and chromatographic assessment of COVID-ORGANICS (CVO) herbal product and Artemisia annua L.

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Many claims from herbal medicines have been developed to manage COVID-19. Tests for identity and purity are important parameters in the quality assurance of herbal medicines. The aim of this study is to confirm the presence of Artemisia annua as a component of COVID-ORGANICS, an acclaimed herbal product for the management of COVID-19 in comparism with A. annua cultivated at NIPRD, Abuja, Nigeria. Pharmacognostic and physicochemical investigations, thin layer and high-performance Liquid chromatography of Covid-Organics along with A. annua were carried out using standard procedures. Similar microscopic features viz T-shaped, glandular, unicellular and uniseriate trichomes, wavy epidermal cells with anomocytic stomata and prismatic calcium oxalate crystals characteristic of A. annua were observed. Moisture, total ash and sulphated ash contents ranged from 8.5 ± 0.0 to 10.4 ± 0.2%w/w; 7.8 ± 0.5 to 12.1 ± 0.1%w/w and 9.7 ± 0.3 to 15.9 ± 0.4%w/w, respectively while water and alcohol soluble extractive values ranged from 20.6 ± 0.5 to 33.7 ± 0.7%w/w; and 23.4 ± 0.6 to 38.2 ± 0.6%w/w respectively. TLC and HPLC profiles showed artemisinin in evaluated samples. The findings confirm that Covid-Organics contain A. annua. The domestication of A. annua in Nigeria along with studies carried out has shown the capacity and technical know-how to develop a phytomedicine from A. annua for management of COVID-19 associated symptoms. This is possible with the provision of the enabling environment in order to provide medicines’ security. Relevant policies are therefore needed to prioritise the development of the sector.

Keywords: Covid-Organics, Artemisia annua, pharmacognostic studies, chromatographic fingerprint

INTRODUCTION

Documentation on the use of traditional medicine to manage viral infections is common in literature. In China, traditional herbal medicine is often used with conventional medicine to treat SARS with most Chinese opting to receive traditional Chinese medicine for the treatment of Covid-19 (Yang et al., 2020). The use of traditional Chinese medicine in the treatment of SARS-CoV-2 is largely inspired by its earlier use in the
treatment of SARS caused by SARS coronavirus (SARS-CoV) (Zhong, 2004). In a controlled clinical study, supplementary treatment with traditional herbal medicine resulted in marked improvement of symptoms of SARS and shortened the disease course (Hsu et al., 2006). Glycyrrhizin, a major active constituent of liquorice root, and baicalin another Chinese herbal compound both inhibited the replication of clinical isolates of SARS virus (Cinat et al., 2003; Chen et al., 2004). Reports have shown that the use of Artemisia annua L. along with conventional orthodox medicines can be beneficial in the management of viral infections (Karamoddini et al., 2011). A. annua L. known as sweet wormwood is one of the most commonly used species in the genus Artemisia, family Asteraceae (Compositae). It is native to many countries in Asia and Europe but has since been domesticated in the medicinal plant garden of the National Institute for Pharmaceutical Research and Development (NIPRD), Nigeria towards the production of Artemisinin-Based Combination Therapies (ACTs) against Malaria (Figure 1) (Jegede and Brisibe, 2007; Jegede et al., 2012). The aequous, alcohol and non-polar solvent extracts of the aerial part of A. annua have been severally reported for their antimalarial, antifungal, antibacterial and antioxidant properties (Zheng, 1994; Allen et al., 1998; Tan et al., 1998). The antiviral properties of the methanol extracts of A. annua aerial parts against Herpes Simplex Virus type 1 (HSV1) have been reported (Karamoddini et al., 2011). A. annua has a well-established role in the management of tumors (Zhu et al., 2013). The ethanol extract of A. annua was reported by Li et al. (2005) to be active against SARS COV 1. The main compounds present in its essential oil appear to be camphene, β-camphene, isoartemisia ketone, 1-camphor, β-caryophyllene and β-pinene. In addition, other minor ingredients, such as artemisia ketone, 1, 8-cineole, camphane hydrate, and cuminal are also found in the volatile parts of A. annua (Verdian, 2009). Most common non-volatile compounds present in the aerial part are sesquiesterpenene lactone artemisinin and some of its biogenetic precursors arteannuin B and artemisinic acid, artemisin, dehydroartemisin, artemisinol, scopoletin, chrysosplenletin, chrysosplenol D, castcin, dihydroxy-6-methoxyacetophenone, eupatin, sistorsterol and coumarin 4-methylesculetin (Verdian, 2009; Zhu et al., 2013; Chougouo et al., 2016).

The herbal product named ‘COVID-ORGANICS’ (CVO) Tambavy Tisane Herbal Tea’ was produced by the Institute of Malagasy Recherches Appliquees, Ratsimamanga in Madagascar. The tea which is composed of A. annua (62%) and other medicinal plants (38%) was acclaimed by the Government of Madagascar to be effective in treatment and management of COVID-19 infection. The herbal tea is said to strengthen the immune system, treat viral infections, reduce fever and cure breathing difficulties.

The process of verification of claims is primarily aimed at substantiating claims of identity, purity, content, efficacy, and safety of herbal medicines and products. Hence the aim of this study is to identify and confirm the presence of A. annua as a major component of CVO as well as compare CVO Herbal product with samples of A. annua cultivated in NIPRD Garden.

MATERIALS AND METHODS

Sample collection and preparation

Two samples of the COVID-ORGANICS (CVO), Green and Orange pack designated as GPA and OPB respectively were used for the study. Two samples of A. annua cultivated in NIPRD Garden were collected at pre-flowering and flowering stages and designated as NFAa and FAa respectively.

Tests for identity and purity

All analyses were carried out according to guidelines specified in the WHO quality control guidelines for the assessment and evaluation of herbal medicines (WHO, 1992, 2011).

Organoleptic, macroscopic and microscopic evaluation

Macroscopic and organoleptic properties, viz; appearance, colour, taste and odour of all samples were documented. A minimum of 2 g of each powdered sample was soaked and cleared in sodium hypochlorite (3.5% w/v) overnight. The cleared particles were mounted in a mixture of glycerol in water (1:1) and viewed under the microscope at different magnifications. Tests for the presence of different metabolites using appropriate stains and indicators were carried out. Photomicrographs were taken using the TUCSEN camera and ARE Capture software.

Physicochemical evaluation

Test for physicochemical properties of the samples as crude drugs, viz; moisture content, ash content, sulphated ash, water and alcohol extractives, were carried out as described in the WHO guidelines for methodologies for research and evaluation of herbal medicines.

Extraction

Extracts were prepared as stated in the instructions for use of the CVO (as stated on the packs) and as indicated in USP method for identification of artemisinin, a major bioactive component of A. annua (International Pharmacopoeia, 2019). Three different extraction methods were thus employed to obtain extracts from the four dried powdered samples.

Method 1: The method prescribed on the leaflet for preparation of Madagascar COVIDORGANICS dried powdered sample was employed for the extraction of all samples. 1000 mg of each sample was extracted by infusion with 60 mL of boiling water and allowed to stand for 15 min, filtered and dried. Within 24 h, dried extracts were reconstituted in 20 mL of ethanol, filtered through a 0.45 μm syringe filter. Samples were transferred to 1.8 mL vials and used for TLC and HPLC analyses.

Method 2: Artemisinin was extracted from 1000 mg of CVO dried
powder and NIPRD A. annua dry leaf powder by refluxing with 50 mL of hexane (45°C) for 2 h, transferred to beakers and left to dry overnight in a fume hood. Within 24 h, samples were reconstituted in 20 mL of acetonitrile (two washes of 10 mL each) to obtain 50 mg per mL solution, filtered through a 0.45 µm nylon syringe filter. Samples were transferred to 1.8 mL vials and used for TLC and HPLC analyses (WHO, 2019).

**Method 3:** Artemisinin was extracted from 1000 mg of CVO dried powder and NIPRD A. annua dry leaf powder by maceration with 20 mL of ethanol (95% v/v) for 2 h with ultra-sonification to obtain 50 mg per mL solution filtered through a 0.45 µm nylon syringe-top filter. Samples were transferred to 1.8 mL vials and used for TLC and HPLC analyses. (https://www.mmv.org/docs).

**Thin layer chromatography (TLC) analysis**

Thin-layer chromatography analysis was performed using silica gel precoated aluminum sheet as stationary phase and a mixture of hexane and ethyl acetate (4:1) as the mobile phase. Spotting was done by the application of 10 µL of each of the test samples and artemisinin reference standard (0.1 mg/mL) separately. The spotted sheet was placed in the chromatographic chamber and the solvent-front was allowed to run up to 60 mm from the origin. The sheet was air-dried and sprayed with freshly prepared anisaldehyde/sulfuric acid solution and heated at 105°C for about 7 min. The chromatogram was examined in daylight. The artemisinin spot obtained with sample solution corresponds in position and appearance (pink) with that obtained with artemisinin reference standard solution (WHO, 2019).

**High performance liquid chromatography (HPLC) analysis**

High-performance liquid chromatography was performed using a stainless-steel ODS column (15 cm × 4.6 mm, particle size: 5 µm). The mobile phase consisted of a 50:50 mixture of acetonitrile and water. Artemisinin reference standard solution (0.1 to 20 mg per mL) was used. The HPLC system was operated at a flow rate of 1.0 mL per minute. The ultraviolet detector was set at a wavelength of 210 nm. 20 µL of sample and reference solutions were injected separately. The run time was set to 15 min. The areas of the peak responses obtained in the chromatograms were documented and the percentage composition of each peak was calculated based on the total peak areas (WHO, 2019).

**RESULTS AND DISCUSSION**

**Organoleptic and macroscopic properties**

The macroscopical and organoleptic features of the samples of CVO and A. annua are presented in Table 1. Light to dark green colour was observed for all the samples while sweet-bitter taste was noted for CVO samples and bitter astringent taste for A. annua samples (Table 1). CVO samples (OPB and GPA) had similar appearance and odour. Their appearance differed slightly from those of A. annua (NFAa (Pre-flowering) and FAa (Flowering) collected from NIPRD garden. NFAa and FAa had a bitter astringent taste which is a characteristic of A. annua (Verdian, 2009) while the two CVO samples had a sweet–bitter taste indicating the presence of a sweetener in the product. GPA was sweeter and lighter in colour than OPB. Other parameters observed from the macroscopy include bark or root fragments, red and green leaf fragments bigger in size than the green leaf fragments of A. annua (Table 1).

**Qualitative microscopy**

Chemomicroscopic properties are shown in Table 2. All parameters assessed were present except mucilage which test negative for all the samples. Microscopic analyses of the samples showed the presence of A. annua with characteristic features such as T-shaped, glandular, unicellular and uniseriate trichomes observed; wavy epidermal cells with anomocytic stomata and
Table 1. Macroscopic/organoleptic properties of COVID-ORGANICS (CVO) and Artemisia annua.

| Organoleptic features | OPB | GPA | NFAa | FAa |
|-----------------------|-----|-----|------|-----|
| Appearance            | Powdered green and red fragments of leaf, fragments of root or stem bark parts | Powdered green leaf fragments |
| Colour                | Dark green | Light green | Light green | Dark-green |
| Particle size         | Moderately coarse | Moderately coarse | Fine-medium coarse | Fine-medium coarse |
| Odour                 | Distinct, strong and aromatic | Distinct, strong and aromatic | Bitter | Bitter-Astringent |
| Taste                 | Sweet-Bitter | Sweet-Bitter | Bitter-Astringent | Bitter-Astringent |

GPA- CVO Green pack; OPB- CVO Orange pack; NFAa- Artemisia annua at pre-flowering stage; FAa- Artemisia annua at flowering stage.

Table 2. Chemomicroscopic properties of COVID-ORGANICS (CVO) and Artemisia annua.

| Property                  | OPB | GPA | NFAa | FAa |
|---------------------------|-----|-----|------|-----|
| Lignin                    | +   | +   | +    | +   |
| Protein                   | +   | +   | +    | +   |
| Oil                       | +   | +   | +    | +   |
| Calcium oxalate crystals  | +   | +   | +    | +   |
| Mucilage                 | -   | -   | -    | -   |
| Cellulose                 | +   | +   | +    | +   |
| Starch                    | +   | +   | +    | +   |
| Tannins                   | +   | +   | +    | +   |

GPA- CVO Green pack; OPB- CVO Orange pack; NFAa- Artemisia annua at pre-flowering stage; FAa- Artemisia annua at flowering stage.

Table 3. Microscopy of the powdered samples of COVID-ORGANICS (CVO) and Artemisia annua.

| Observed Microscopic features | OPB | GPA | NFAa | FAa |
|-------------------------------|-----|-----|------|-----|
| Epidermal cells               | Two (2) types of epidermal cells present: Wavy and polygonal wall cells | Two (2) types of epidermal cells present: Wavy and polygonal wall cells | One (1) type epidermal cells present: Wavy wall cells | One (1) type of epidermal cell present: Wavy wall cells |
| Stomata                       | Anomocytic types found on all samples | Wavy and polygonal wall cells | Wavy wall cells |
| Trichomes                     | T-shaped unicellular, Pin-tipped wavy walled, Uniseriate clothing hairs and Glandular hairs were found on all samples | | |
| Calcium oxalate crystal       | a) Druses | a) Druses | Prismatic | Prismatic |
|                               | b) Prismatic | b) Prismatic | | |
|                               | c) Rosette | c) Rosette | | |
| Oil globules                  | Present on all epidermal cells | | |
| Other cell inclusions         | Presence of Sclerids | Presence of Sclerids | - | - |

GPA- CVO Green pack; OPB- CVO Orange pack; NFAa- Artemisia annua at pre-flowering stage; FAa- Artemisia annua at flowering stage.

prismatic calcium oxalate crystals were also seen in all four samples. These results are in agreement with observations by Ferreira et al. (2010), who reported the presence of anomocytic stomata with numerous glandular and non-glandular trichomes on both surfaces of leaves. The presence of other plant fragments apart from A. annua in CVO was indicated by the presence of druses, rosette calcium oxalate crystals, hypostomatous polygonal epidermal cells and occasional sclerids depicting the presence of samples of other plant specimens (Table 3 and Figures 2 to 9). The relatively higher abundance of A. annua features in the CVO indicates that it is the major component of the product.

Physicochemical properties

Air-dried A. annua samples (NFA and FA) had a moisture content of 9.8 and 8.5% w/w respectively, while CVO (GPA and OPB) samples had a moisture content of 10.3 and 10.4% w/w respectively. The presence of a sweetener and other plant materials which may be root or
Figure 2. T-Shaped trichomes characteristic of *Artemisia annua*.

Figure 3. Pin-tipped wavy walled trichomes characteristic of *A. annua*.

Figure 4. Uniseriate clothing hairs characteristic of *A. annua*.
stem-bark in the product might be responsible for the higher moisture content in GPA and OPB. While the extractive values of CVO (GPA) were higher than those of all the other samples, those of OPB were similar to those of *A. annua* (NFAa and FAa). The ash content of GPA, OPB, NFAa and FAa were 9.4 ± 0.4%w/w, 12.1 ± 0.1%w/w, 9.1 ± 0.3%w/w and 7.8 ± 0.5%w/w respectively. Slight variations observed could be due to difference
in geographical location and collection sources, however very noticeable are the higher extractive values. The higher extractive value of GPA relative to OPB might be indicative of the presence of more sweetener in the former especially as GPA was sweeter than OPB. Verdian (2009) reported the average values of moisture content (9.2 w/w) and total ash (8.3 w/w) of A. annua leaves. All values observed for proximate analysis are within WHO allowable limits for herbal products (WHO, 2006) (Table 4).
Table 4. Physicochemical properties of the powdered samples of COVID-ORGANICS (CVO) and Artemisia annua.

| Parameters (%)              | GPA       | OPB       | NFAa      | FAa       | Monograph Limit (WHO, 2006) |
|-----------------------------|-----------|-----------|-----------|-----------|-----------------------------|
| Moisture content            | 10.3 ± 0.2| 10.4 ± 0.3| 9.8 ± 0.3 | 8.5 ± 0.0 | Not more than 14%           |
| Water soluble Extractive value | **33.7 ± 0.7** | 20.6 ± 0.5 | 23.9 ± 0.7 | 22.0 ± 0.8 | -                           |
| Alcohol soluble Extractive value | **38.2 ± 0.6** | 25.3 ± 0.8 | 23.4 ± 0.6 | 28.7 ± 0.1 | Not less than 1.9%          |
| Total Ash content           | 9.4 ± 0.4 | 12.1 ± 0.1| 9.1 ± 0.3 | 7.8 ± 0.5 | Not more than 8%            |
| Sulphated Ash               | 12.8 ± 0.1| 10.9 ± 0.4| 11.2 ± 0.1| 9.7 ± 0.3 | -                           |

GPA: COVID-ORGANICS Green pack; OPB: COVIDORGANICS Orange pack; NFAa: Artemisia annua at pre-flowering stage; FAa: Artemisia annua at flowering stage

Figure 10. TLC profile of hexane, ethanolic extract and hot water infusion of CVO and Artemisia annua (OPB, NFAa, FAa and GPA).

Thin layer chromatography

TLC fingerprint of the hot water infusion, n-hexane and 95% ethanol extracts are shown in Figure 10. The fingerprint indicated the presence of artemisinin in all four samples (OPB, GPA, NFAa and FAa (Figure 10).

High performance liquid chromatography

Percentage composition of chemical constituents determined by HPLC of the four samples extracted with 95% ethanol, hot water and n-hexane are presented in Tables 5 to 7 respectively while the chromatograms are shown in Figures 11 to 22 with artemisinin appearing except the hot water extract of OPB (Figure 12).

The HPLC profiles of all the extracts had variable components peaks. For the ethanol extract, CVO samples (GPA and OPB) had 16 and 17 peaks respectively, while FAa and NFAa samples had 15 peaks each. Out of a possible 23 peaks, 8 peaks cut across the four samples. The number of peaks with the same retention time and comparable percentage composition in the CVO samples (GPA and OPB) were 14. The number of peaks with same retention time and comparable percentage composition in the NIPRD samples (FAa and NFAa) were 10. The number and correspondence of profile peaks in these samples is a pointer to the similarities or disparity in the chemical profiles of the extracts, which is an indication of the variation in the medicinal plants’ composition of the samples. The peak corresponding to artemisinin had a retention time of 5.29 to 5.59 and the percentage composition in the samples were 4.27, 6.32, 5.32, and 15.49 for GPA, OPB, FAa and NFAa respectively.

For the hexane extract, GPA and OPB had 16 and 17 peaks respectively, while FAa and NFAa had 21 and 19 peaks respectively. The number of peaks that cut across the four samples were 14 in number. The number of peaks with same retention time and comparable percentage composition in GPA and OPB were 18 in number while in FAa and NFAa, they are 19. Since hexane has been reported to be a suitable solvent for extraction of artemisinin from A. annua, the percentage
Table 5. Percentage composition of chemical constituents of CVO and Artemisia annua extracted with 95% ethanol (determined by HPLC).

| Peak and Retention Time (min) | FAa Ethanol extract (% composition) | NFAa Ethanol extract (% composition) | OPB Ethanol extract (% composition) | GPA Ethanol extract (% composition) |
|------------------------------|-------------------------------------|--------------------------------------|-------------------------------------|-------------------------------------|
| 1.784±0.2                   | 15.12                               | -                                    | -                                   | -                                   |
| 1.855±0.2                   | 14.52                               | 7.97                                 | 44.96                               | 41.91                               |
| 2.049±0.1                   | -                                   | -                                    | 12.15                               | 13.40                               |
| 2.241±0.2                   | 30.25                               | 20.04                                | 15.32                               | 20.14                               |
| 2.801±0.1                   | 12.76                               | 20.11                                | 6.85                                | 8.33                                 |
| 3.317±0.2                   | -                                   | 1.69                                 | 1.69                                | 1.43                                 |
| 3.711±0.2                   | 12.66                               | 21.38                                | 4.53                                | 4.36                                 |
| 4.295±0.2                   | 2.81                                | 4.67                                 | 0.85                                | 0.73                                 |
| 4.752±0.2                   | 5.32                                | 3.53                                 | 1.34                                | 1.01                                 |
| 5.292±0.2                   | 5.32                                | 3.53                                 | -                                   | -                                   |
| 5.589±0.2                   | -                                   | 15.49                                | 6.32                                | 4.27                                 |
| 7.059±0.2                   | 1.11                                | 0.43                                 | 0.93                                | 0.91                                 |
| 7.88±0.0                    | 0.38                                | -                                    | -                                   | 0.40                                 |
| 8.168±0.2                   | 0.45                                | 0.05                                 | 0.60                                | -                                    |
| 9.06±0.2                    | 0.44                                | 0.06                                 | 0.48                                | 0.49                                 |
| 9.634±0.2                   | 1.32                                | 2.15                                 | 0.91                                | 0.67                                 |
| 10.208±0.1                  | -                                   | 1.03                                 | 0.54                                | 0.40                                 |
| 10.848±0.0                  | 0.11                                | -                                    | -                                   | -                                    |
| 11.376±0.2                  | -                                   | 0.04                                 | 0.07                                | -                                    |
| 11.951±0.0                  | 0.87                                | -                                    | -                                   | -                                    |
| 12.15±0.2                   | -                                   | 1.21                                 | 0.93                                | 0.70                                 |
| 13.211±0.0                  | 1.25                                | -                                    | -                                   | -                                    |
| 13.575±0.2                  | -                                   | 1.76                                 | 0.75                                | 0.84                                 |

Composition of the peak corresponding to artemisinin can be taken as a good indication of the relative artemisinin contents of the samples.

For the hot water infusion extract, GPA and OPB had 13 and 10 peaks respectively, while FAa and NFAa had 13 peaks each. 4 peaks had the same retention time across the four samples while for GPA and OPB had 5 peaks with the same retention time. However, a single peak was responsible for 99 and 60% composition in GPA and OPB, respectively indicating that they were the major components in the hot water infusion of the two CVO samples. The number of peaks with the same retention time and comparable percentage composition in FAa and NFAa were 7 in number. The major components in FAa and NFAa were 37.01 and 55.48% respectively. The number and correspondence of profile peaks in these samples is a pointer to the similarities or disparity in the chemical profiles of the extracts, which is an indication of the variation in the medicinal plants’ composition of the samples. The peak corresponding to artemisinin was present in all the samples except the water extract of OPB (Figures 12, 14 to 23). This could be because the artemisinin concentration in the samples was below machine sensitivity since artemisinin is known to be poorly extracted in water. While the HPLC analysis confirmed the presence of artemisinin in the CVO samples (GPA and OPB), the water infusion prescribed in the use of the product does not extract sufficient artemisinin. Rather, the major components in the water extracts were at peaks retention time of 1.73 to 1.85. More work needs to be done to isolate and characterize these major compounds in the infusion which may be a source of major bioactivity.

This study has shown that CVO contains A. annua and other plants. A. annua has been reported to contain bioactive compounds with potential antiviral properties (Karamoddini et al., 2011). It has a history of being safe and easily available for therapies and has shown significant activity against SARS coronavirus. The ethanol extract of the whole plant of A. annua has been reported to have a significant inhibition against two strains of SARS-CoV (BJ001, BJ006) in Vero cell-based CPE/MTS screening (Shi et al., 2005). A. annua derivative, artesunate, is also a promising novel drug to treat pulmonary fibrosis by inhibiting pro-fibrotic molecules associated with pulmonary fibrosis. Pulmonary fibrosis is observed in SARS coronavirus-2 (SARSCoV-2) infection with increased severity, mediated by Interleukin-1 (Conti et al., 2020).
Table 6. Percentage composition of chemical constituents of CVO and *Artemisia annua* extracted by hot water infusion (determined by HPLC).

| Peak and Retention Time (min) | FAa infusion (% composition) | NFAa infusion (% composition) | OPB infusion (% composition) | GPA infusion (% composition) |
|-----------------------------|-------------------------------|-------------------------------|-------------------------------|-----------------------------|
| 0.192±0.0                  | 0.02                          | -                            | -                            | -                           |
| 0.328±0.0                  | 0.14                          | -                            | -                            | -                           |
| 0.757±0.0                  | -                             | -                            | -                            | 0.086                       |
| 1.056±0.0                  | -                             | 0.01                         | -                            | -                           |
| 1.739±0.2                  | 37.01                         | 55.48                        | 38.58                        | 99.48                       |
| 1.852±0.2                  | 28.88                         | 23.59                        | 59.97                        | -                           |
| 2.167±0.2                  | 33.48                         | 11.91                        | -                            | -                           |
| 2.574±0.0                  | -                             | -                            | -                            | 0.14                        |
| 2.808±0.0                  | -                             | 3.78                         | -                            | -                           |
| 3.537±0.2                  | -                             | 0.67                         | 0.04                         | -                           |
| 3.739±0.2                  | 0.11                          | 2.11                         | 0.16                         | 0.15                        |
| 4.349±0.2                  | 0.11                          | 1.41                         | 0.98                         | 0.01                        |
| 5.064±0.0                  | 0.03                          | -                            | -                            | -                           |
| 5.515±0.1                  | 0.02                          | 0.77                         | -                            | 0.02                        |
| 6.033±0.0                  | 0.03                          | -                            | -                            | -                           |
| 6.799±0.1                  | 0.03                          | -                            | -                            | 0.01                        |
| 7.161±0.1                  | 0.13                          | -                            | -                            | 0.01                        |
| 7.907±0.1                  | -                             | 0.01                         | -                            | 0.01                        |
| 8.205±0.0                  | -                             | -                            | 0.01                         | -                           |
| 9.687±0.2                  | 0.02                          | 0.02                         | 0.02                         | 0.01                        |
| 10.443±0.0                 | -                             | 0.04                         | -                            | -                           |
| 11.196±0.2                 | -                             | 0.10                         | -                            | 0.04                        |
| 11.677±0.1                 | -                             | 0.12                         | 0.13                         | 0.01                        |
| 11.847±0.0                 | -                             | -                            | -                            | 0.02                        |
| 12.369±0.0                 | -                             | -                            | 0.11                         | -                           |

Table 7. Percentage composition of chemical constituents of CVO and *Artemisia annua* extracted by hexane (determined by HPLC).

| Peak and Retention Time (min) | FAa hexane extract (% composition) | NFAa hexane extract (% composition) | OPB hexane extract (% composition) | GPA hexane extract (% composition) |
|-----------------------------|-----------------------------------|------------------------------------|------------------------------------|-----------------------------------|
| 1.024±0.0                  | 1.79                              | -                                  | -                                  | -                                 |
| 1.269±0.2                  | 0.32                              | 1.47                                | -                                  | -                                 |
| 1.483±0.1                  | 0.27                              | 0.52                                | -                                  | -                                 |
| 1.711±0.1                  | 4.87                              | 3.39                                | 9.55                               | 8.45                              |
| 1.831±0.2                  | 4.01                              | 2.50                                | 7.31                               | 7.17                              |
| 2.259±0.2                  | 4.74                              | 3.86                                | 8.28                               | 11.96                             |
| 2.432±0.1                  | 1.54                              | 1.90                                | 2.76                               | 2.58                              |
| 2.79±0.2                   | 7.47                              | 7.76                                | 5.62                               | 7.63                              |
| 3.144±0.2                  | 2.86                              | 2.62                                | 2.05                               | 1.64                              |
| 3.699±0.1                  | 16.58                             | 26.83                               | 14.47                              | 14.84                             |
| 4.303±0.1                  | 8.95                              | 8.70                                | 3.55                               | 3.54                              |
| 4.749±0.1                  | -                                 | -                                  | 3.54                               | 3.40                              |
| 5.313±0.2                  | 14.31                             | 15.17                               | 9.24                               | 7.83                              |
| 6.599±0.2                  | 1.55                              | 1.00                                | 0.63                               | -                                 |
| 7.084±0.2                  | 4.95                              | 4.42                                | 3.89                               | 4.80                              |
| 8.101±0.1                  | 1.15                              | -                                  | 0.47                               | 0.45                              |
| 8.495±0.1                  | 1.68                              | 0.87                                | -                                  | -                                 |
Table 7. Contd

| Value       | 2.01 | 1.38 | 2.43 | 3.63 |
|-------------|------|------|------|------|
| 9.06±0.1    |      |      |      |      |
| 9.603±0.1   | 8.20 | 5.82 | 8.63 | 6.35 |
| 11.476±0.1  | 0.86 | 0.01 |      |      |
| 12.242±0.2  | 4.64 | 4.84 | 9.20 | 6.70 |
| 13.631±0.2  | 7.23 | 6.95 | 8.38 | 9.04 |

**Figure 11.** HPLC chromatogram of FAa hot water infusion.

**Figure 12.** HPLC chromatogram for OPB hot water infusion.
Conclusion

This study shows that the COVID-ORGANICS samples contain *A. annua* as the major component. They also contain other plant materials possibly including a sweetener. The two products had similar pharmacognostic and phytochemical characteristics as *A. annua* grown by NIPRD in Nigeria. There was low artemisinin content in the hot water infusion of the products prepared following the instructions by the manufacturer with one of the samples having undetectable levels. Despite other limitations, the inherent potentials of *A. annua* as a phytomedicinal remedy for several conditions have been identified. This
is indicative of the considerable potential that exists in the sector. It is therefore critical that government and other stakeholders prioritise the development of the phytomedicinal sector.
Figure 17. HPLC chromatogram for GPA extracted with 95% ethanol.

Figure 18. HPLC chromatogram for OPB extracted with 95% ethanol.

CONFLICT OF INTERESTS
The authors have not declared any conflict of interests.

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Figure 21. HPLC chromatogram for GPA extracted with hexane.

Figure 22. HPLC chromatogram for OPB extracted with hexane.

of the NIPRD Artemisia annua samples.

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