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Evaluation of the elemental, nutritional and antioxidant properties of Cov-Pla herbal preparations

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The outbreak of the Covid-19 pandemic has had a dramatic effect on human existence and still shows no sign of abating. Scientists worldwide are therefore working assiduously to get new drug treatments to help mitigate the crisis. Some of those efforts involve research to obtain Covid-19 treatments from natural sources. The present study is aimed at evaluating the elemental, nutritional and antioxidant properties of Cov-Pla1, Cov-Pla2, Cov-Pla3 and PlaBoost herbal preparations. Elemental analysis was carried out using AAS after acid digestion of the samples. Proximate analysis of the formulations was done using the official AOAC methods while the antioxidant assay was carried out using the DPPH free radical scavenging method. The results of the study showed that the concentration of the heavy metals in all the samples were within acceptable regulatory limits. Proximate analysis revealed that the suspensions had protein content between 1.52-1.68 % and carbohydrate content of 0.79 – 1.08 % with low content of fat, crude fibre and ash. The formulations were found to be free of microbial contamination and stable for thirty days. Antioxidant evaluation revealed that Cov-Pla3 had the strongest free radical scavenging capacity with IC_{50} of 27.29 µg/mL while PlaBoost had the least (IC_{50}: 251 µg/mL). The result of the study indicates that the formulations are free of metallic and microbial contaminants. In addition, proximate analysis has established some diagnostic parameters which will aid future authentication and purity assessment of the formulations. The formulations were all found to possess considerable antioxidant activity which will provide collateral benefit in relieving oxidative stress associated with Covid-19 infection.

Key words: Covid-19, heavy metals, trace elements, proximate analysis, antioxidant.

INTRODUCTION

The COVID-19 pandemic caused by the SARS-CoV-2 coronavirus has had significant impact on human life globally causing extraordinary disruptions to international health and travel (Abu-Rayash and Dincer, 2020). There are presently no specific drugs for treatment of this novel disease and management is mainly symptomatic in nature. Several vaccine candidates are at various stages of clinical trials but none has so far been certified to be safe and effective for use in humans (Koirala et al., 2020). There is undoubtedly a pressing demand for new drugs active against this viral disease in order to mitigate the present epidemic. A number of strategies are being pursued for the discovery of new therapeutics for the disease. These include repurposing of existing drugs (Santos et al., 2020), computer aided drug discovery (Onawole et al., 2020) and *de novo* synthesis and screening of small molecules against the SARS-CoV-2 virus (Ahmed-Belkacem et al., 2020; Jockusch et al., 2020). Another approach which is fast gaining traction for the discovery of new drugs against this disease is the exploration of plant material for herbal medicines against the disease (Cao et al., 2020; Wang et al., 2020).

Herbal medicines have been defined as "medicinal products containing as active ingredients only plants, parts of plants or plant materials, or combinations thereof, whether in the crude or processed state" (Aronson, 2016). Herbal medicines have been used by mankind since ancient times for treatment of different ailments (Mitscher, 2007). It has been estimated that a large percentage of the world population, especially those in the low- and middle-income countries still rely on herbal medicines to meet their healthcare needs (Bodeker et al., 2017).

Of particular interest is the use of herbal medicines in the treatment of viral infections; for example the Alpha-zam herbal formulation was found to be a potent and selective inhibitor of the hepatitis C virus (Oyero et al., 2016) while KIOM-C, a compound mixture of several herbs was found to have anti-viral action against a panel of different viral organisms (Talactac et al., 2015). It is therefore expedient to search for potential therapies for the Covid-19 pandemic from the extensive collection of medicinal plants available locally.

The Plateau State Government in North central Nigeria set up the Plateau State research committee on Covid-19 and other infectious diseases with a mandate to carry out research directed at obtaining potential new treatments from both conventional and alternative sources for the treatment of Covid-19. After extensive literature reviews combined with *in silico, in vitro* and *in vivo* studies, the committee formulated three herbal suspensions coded Cov-Pla1, Cov-Pla2 and Cov-Pla3, each containing a combination of five (5) plants with proven anti-viral activity in addition to other biological activities. A herbal tea coded PlaBoost containing 6 different plants was similarly formulated.

Determination of the quality and purity of herbal preparations is an essential step in the standardization of herbal medicines. This entails establishing important parameters such as physicochemical properties, heavy and traces metal content, determination of ash values, moisture content, proximate analysis, stability studies and assessment of microbial sterility among a host of other evaluations (Mukherjee, 2019). This paper reports the findings of the elemental, nutritional and antioxidant evaluation of the Cov-Pla formulations.

MATERIALS AND METHODS

Chemicals/Solvents

All solvents (methanol, ethanol, acetonitrile, phosphoric acid, and ethyl acetate) were of analytical grade and purchased from Sigma Aldrich (Germany). DPPH and Vitamin C Standard were also obtained from the same source.

Organoleptic assessment

The organoleptic properties of the formulations were assessed to determine their appearance, taste and odour/smell.

Elemental analysis

Heavy metal content and trace element content in the formulations were determined using atomic absorption spectrophotometry (AOAC, 2019).

Nutritional analysis

This was performed using the official methods of analysis of the Association of Official Analytical Chemists (AOAC) International as specified: Moisture Content by Loss on drying, Crude Protein determined by Kjedahl titration method, Fat content by acid hydrolysis method and crude fibre by digestion method (AOAC, 2019).

Microbiological evaluation

The percentage potency of the formulations against Gram positive,

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Gram negative and fungal organisms in comparison to standard antibiotics was evaluated using official methods (AOAC, 2019). The formulations were similarly assessed for sterility and the presence of bacterial contamination.

Phytochemical analysis

Preliminary phytochemical screening was carried out using standard methods in order to identify the secondary metabolites present in the formulations (Harborne, 1998).

Stability studies

Qualitative stability studies which encompassed monitoring the colour and other organoleptic properties of the formulations in addition to checking for fungal growth were carried out over a thirty (30) day period.

Evaluation of antioxidant activity (DPPH Free Radical Scavenging Activity)

The antioxidant activity (free radical scavenging activity) of extract on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined according to the method earlier reported (Odumosu et al., 2015). About 12.5 mg of the extract (Cov-Pla1, Cov-Pla2, Cov-Pla3 and PlaBoost herbal tea) was dissolved in methanol using 25 mL volumetric flask. The following concentrations of the extract were prepared 500, 250, 125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95, and 0.98 µg/mL. All the solutions were prepared with methanol as solvent. A quantity (2 mL) of each prepared concentration was mixed with 4 mL of 50 µM DPPH solution in methanol. Experiment was done in triplicate. The mixture was vortexed for 10 s to homogenize the mixture and test tubes were incubated for 30 min at room temperature in the dark. Thereafter, the absorbance was measured at 515 nm on a UV-Vis spectrophotometer (Shimadzu, UV-1620PC, Japan). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Vitamin C was used as standard with the following concentrations 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.7812, 0.391 and 0.195 µg/mL. Blank solution was prepared by mixing 2 mL of methanol with 4 mL of 50 µM DPPH solution. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Finally, the IC_{50} value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the separate linear regression of the mean of the antioxidant activity against concentration of the test extract (µg/mL).

RESULTS

The results of the organoleptic evaluation of the formulations are presented in Table 1. From the table, it can be seen that the suspensions had a golden-brown colour and possessed a bitter taste with a sweet after taste. Also, they all had a characteristic odour with an acidic pH of about 5.

The concentrations of the heavy metal contaminants found in the formulations are presented in Table 2. It can be seen that they all contain minute quantities of lead with PlaBoost herbal tea having the greatest concentration of 1.252 mg/kg of lead while arsenic and mercury were absent in the formulations.

The concentrations of the micronutrient elements found in the formulations are presented in Table 3. It can be observed that the formulations are rich in iron and zinc but Cov-Pla suspensions contain small quantities of selenium and calcium with manganese absent while selenium, calcium and manganese were not detected in PlaBoost herbal tea.

The results of the proximate analysis of the suspensions are shown in Table 4 while those of PlaBoost herbal tea can be found in Table 5. It can be seen that the Cov-Pla suspensions have high moisture content ranging between 96.88 and 97.12% while the herbal tea has a low moisture content of 9.12%. The ash and extractive values of the formulations are similarly displayed in the tables.

The results of the antimicrobial potency evaluation of the formulations are displayed in Table 6 while the results of the sterility testing of the formulations are shown in Table 7. It can be seen that all the formulations are sterile and completely free of any bacterial contaminants.

Phytochemical screening of the formulations revealed the presence of flavonoids, saponins and steroids as their major bioactive components (Table 8). Qualitative stability studies of the formulations also revealed stability was maintained for thirty (30) days with no change in the organoleptic properties of the formulations over this period (Table 9).

The results of the antioxidant evaluation are shown in Table 10. It revealed that Cov-Pla3 had the strongest free radical scavenging capacity with IC_{50} of 27.29 µg/mL while PlaBoost had the least (IC_{50} of 251 µg/mL). All the formulations however had lower free radical scavenging capacity compared to the standard Ascorbic acid.

DISCUSSION

Although herbal medicines are usually touted as safe due to their natural source, studies have revealed that this is not always true as some herbal preparations have been shown to produce serious adverse effects (Ekor, 2014). They have also been shown to be prone to adulterationby unscrupulous individuals especially those with high demand. It is therefore important to ensure that all herb based medicines are non-toxic and of high quality to ensure the safety of their users. This study was therefore carried out to establish some properties of the Cov-Pla herbal preparations such as their elemental and nutritional

Table 1. Organoleptic properties of the formulations.

S/N	Parameter	Cov-Pla1	Cov-Pla2	Cov-Pla3
1	Appearance	Golden Brown	Golden Brown	Golden Brown
2	Taste	Bitter with sweet after taste	Bitter with sweet after taste	Bitter with sweet after taste
3	Odour	Characteristic of Plant Extract	Characteristic of Plant Extract	Characteristic of Plant Extract
4	pH	5.53	5.37	5.52

Table 2. Evaluation of heavy metal contaminants in the formulations.

S/N	Metal	Cov-Pla1	Cov-Pla2	Cov-Pla3	PlaBoost Herbal Tea
1	Lead (mg/kg)	0.229 ±0.04	0.125±0.03	0.112±0.02	1.252±0.07
2	Arsenic (mg/kg)	0.00	0.00	0.00	0.00
3	Mercury (mg/kg)	0.00	0.00	0.00	0.00

Table 3. Evaluation of the micronutrient content of the formulations.

S/N	Micronutrient	Cov-Pla1	Cov-Pla2	Cov-Pla3	PlaBoost Herbal Tea
1	Iron (mg/kg)	0.823±0.032	1.124±0.051	0.572±0.014	393.38±7.56
2	Zinc (mg/kg)	3.080±0.28	3.580±0.21	1.942±0.12	213.38±15.41
3	Selenium (mg/kg)	0.001±0.00	0.003±0.00	0.001±0.00	ND
4	Calcium (mg/kg)	0.12±0.001	0.15±0.005	0.18±0.003	ND
5	Manganese (mg/kg)	0.00	0.00	0.00	ND

ND = Not Detected.

Table 4. Proximate analysis of the suspensions.

S/N	Parameter	Cov-Pla1	Cov-Pla2	Cov-Pla3
1	Moisture Content (%)	97.12±5.50	97.09±5.47	96.88±5.40
2	Protein (%)	1.68±0.12	1.52±0.11	1.66±0.12
3	Fat (%)	0.18±0.01	0.15±0.01	0.16±0.01
4	Crude Fibre (%)	0.15±0.01	0.17±0.02	0.17±0.02
5	Ash (%)	0.08±0.004	0.05±0.002	0.05±0.02
6	Carbohydrate (%)	0.79±0.11	1.02±0.24	1.08±0.24
7	Energy (kcal/kg)	11.3025±2.54	11.255±2.52	12.13±2.61

Table 5. Proximate analysis of the herbal tea.

S/N	Parameter	PlaBoost herbal tea
1	Moisture content (%)	9.12±1.68
2	Total ash content (%)	7.19±1.04
3	Acid insoluble ash (%)	0.30±0.02
4	Water soluble Ash (%)	3.12±0.56
5	Crude fibre (%)	5.17±1.88
6	Alkalinity of soluble ash (as K_2O) (dry weight) (%)	1.25±0.05
7	Water extracts (dry weight) (%)	38.00±2.77
8	Ether extracts (%)	0.00±0.00
9	Caffeine (dry weight) (%)	0.00±0.00
10	Tannins (dry weight) (%)	8.12±1.35

Table 6. Antimicrobial evaluation (% Potency) of the formulations.

S/N	Organism	Cov-Pla1 (%)	Cov-Pla2 (%)	Cov-Pla3 (%)	PlaBoost Herbal Tea (%)
1	*Staphylococcus aureus*	60.00	58.00	50.00	28.00
2	*Pseudomonas aeruginosa*	50.00	45.00	50.00	15.00
3	*Proteus vulgaris*	50.00	45.00	40.00	35.00
4	*Candida albicans*	58.00	40.00	48.00	20.00

Table 7. Evaluation of the bacterial contaminant in the formulations.

S/N	Bacterial Contaminant	Cov-Pla1	Cov-Pla2	Cov-Pla3	PlaBoost Herbal Tea
1	Total coliform count (cfu/ml)	0.00	0.00	0.00	0.00
2	Total viable count (cfu/ml)	0.00	0.00	0.00	0.00
3	Yeast/Mould (cfu/ml)	0.00	0.00	0.00	0.00

Table 8. Phytochemical screening of the formulations.

S/N	Cov-Pla1	Cov-Pla2	Cov-Pla3
1	Flavonoids, Saponins, Steroids present	Flavonoids, Saponins, Steroids present	Flavonoids, Saponins, Steroids present

Table 9. Stability studies of the formulations.

S/N	Cov-Pla1	Cov-Pla2	Cov-Pla3
1	No discolouration No foul smell within 30 days	No discolouration No foul smell within 30 days	No discolouration No foul smell within 30 days

Table 10. Antioxidant activity of the formulations.

S/N	Sample	IC_{50} (µg/mL)
1	Cov-Pla1	125.87 ± 0.02
2	Cov-Pla2	63.09 ± 0.01
3	Cov-Pla3	27.29 ± 2.17
4	Pla-Boost Herbal Tea	251 ± 0.01
5	Ascorbic Acid (Standard)	2.53 ± 1.25

composition which will be useful in their standardization.

The products were found to have good organoleptic properties (Table 1) and it is envisaged that they would be acceptable to the sensory faculties of the consumers when they are eventually put to human use. The suspensions were also all found to have slightly acidic pH (about 5) and this may be linked to the type and nature of phytochemicals present in them. Phytochemical screening revealed the presence of steroids, saponins and flavonoids (Table 8) in the three suspensions and the

presence of polyphenolic metabolites may account for the slightly acidic pH obtained. This will however not be a problem during therapy with the suspension as the dose to be administered is very unlikely to cause any significant change in physiological pH *in vivo*.

Heavy metals such as lead, mercury and arsenic are not essential in the human body and can have harmful effects even at very low concentrations in the body. High levels may be carcinogenic and can cause central nervous dysfunction, kidney and liver damage (Friberg et

al., 1986). The formulations were found to have low levels of lead (Table 2) which were below the WHO recommended limit of 10 mg/kg while arsenic and mercury were completely absent in all the formulations (WHO, 2007). Trace elements such as chromium, iron, cobalt, zinc and copper are essential in very little quantities for enzyme function, synthesis of vitamins and haemoglobin formation (Sadhu et al., 2015). The formulations were found to contain reasonable amounts of iron and zinc, moderate amounts of selenium and calcium while manganese was absent (Table 3). The presence of zinc and selenium in the formulations may particularly prove to be beneficial in the management of Covid-19 as they are known to have immunomodulatory properties (de Almeida Brasiel, 2020; Gombart et al., 2020; Maggini et al., 2008) and are usually part of the regimen used in the orthodox management of Covid-19 (Gasmi et al., 2020; Shakoor et al., 2021).

Proximate analysis involves quantitative analysis of a sample to determine the percentage content of macronutrients found in it. The components usually quantified include: Moisture content, crude fibre, ash, protein, carbohydrate and fats (Basu, 2013). The results of the proximate analysis of the suspensions are shown in Table 4 while that of the herbal tea can be found in Table 5. It can be seen that the Cov-Pla suspensions have high moisture content ranging between 96.88 and 97.12% and this is due to the fact that they are liquid preparations formulated in a hydroethanolic vehicle while the tea is packaged in dry form and hence has a low moisture content of 9.12%. The ash values which are indicative of the presence of natural impurities like oxalate, silicate and carbonate was found to range from 0.05 to 0.08% for the suspensions while the total ash value for PlaBoost herbal tea was determined to be 7.19%. Determination of these parameters plays an important role in standardization of herbal medicines as they can serve as indices of the quality, purity and authenticity of the preparations (Fazal et al., 2011).

Evaluation of the antimicrobial activities of the formulations in comparison to standard antibiotics against Gram positive (*Staphylococcus aureus*), Gram negative (*Pseudomonas aeruginosa, Proteus vulgaris*) and fungal (*Candida albicans*) organisms showed that they all possessed activity against these organisms. Furthermore, Cov-Pla1 showed the greatest percentage potency ranging from 50% against *Pseudomonas aeruginosa* and *Proteus vulgaris* to 60% against *Saphylococcus aureus*, implying that it had greater activity against Gram positive than Gram negative organisms. PlaBoost consistently had the lowest activity against all the organisms tested while Cov-Pla2 and Cov-Pla3 demonstrated intermediate potency against the organisms. The formulations are primarily not intended to be used as antibacterial or antifungals but against Covid-19 which is caused by a virus (SARS-CoV-2). However,

their antimicrobial activity may be useful in preventing the growth of spoilage causing micro-organisms thereby enhancing their stability and shelf-life.

The formulations were found to be free of microbial contamination as the total coliform, total viable and yeast/mould counts were all found to be zero (Table 7). Freedom from microbial contamination is critical for herbal medicines as their presence can accelerate deterioration and spoilage of the herbal medicines. This result was further strengthened by the results of the stability studies (Table 9) which showed that the products remained stable for one month when stored under appropriate storage conditions which is between 2 and 8°C (refridgerator) for the Cov-Pla suspensions and a cool, dry location for the herbal tea.

The Cov-Pla formulations were all found to possess antioxidant activity. Cov-Pla3 had the highest antioxidant activity with IC_{50} of 27.29 μg/mL while PlaBoost had the least with IC_{50} of 251 μg/mL. Cov-Pla 1 and Cov-Pla2 had intermediate values of 125.87 and 63.09 μg/mL, respectively (Table 10). A review of the pathogenesis of Covid-19 shows that one of the downstream end products of the cytokine storm is the increased production of free radicals including reactive oxygen species (ROS) that results in oxidative stress and this has been identified as one of the main causes of local or systemic tissue damage that occurs in severe Covid-19 (Schönrich et al., 2020). The antioxidant property of the Cov-Pla formulations will therefore be very useful in scavenging these free radicals and attenuating the oxidative stress linked to the disease.

Conclusion

The study indicates that the Cov-Pla formulations have low heavy metal content which are within acceptable limits while some essential trace elements with immunomodulatory properties such as zinc and selenium were found to be present in reasonable amounts in the formulations. Proximate analysis has also established the nutritional characteristics of the formulations which will be useful in their authentication and quality assessment when commercialized. They were similarly found to have good antioxidant property which may be beneficial in ameliorating oxidative stress linked to Covid-19.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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