Research Article

Assessment of Microbiological Quality, Phytochemicals, Trace Elements and Proximate Analysis of Bio-Clean II Herbal Remedy

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The aim of this study was to determine the microbiological quality, phytochemicals, trace elements and proximate analysis of Bio-Clean II. Samples of the herbal remedy were procured from the manufacturer and were analyzed using standard procedure of analysis including sterility test, Spectrophotometry, Atomic Absorption spectroscopy, and gravimetric method. All the batches of Bio-Clean II examined pass sterility test, as no single microbial colony was recovered from them. Physicochemical characterization of the herbal remedy was as follows: pH (6.86), temperature (25^0C), relative density (1.08), conductivity (272uS/cm) and total dissolved solid (163mg/L). Phytochemical screening of the herbal remedy revealed the presence of the following bioactive secondary metabolites in varied quantity: resin (+), Flavonoids (+++), Saponins (+), Anthraquinones (+++), Tannin (++), Alkaloids (++) and Cardiac Glycoside (++); except for Steroids which was absent (-). Trace elements analysis revealed the presence of the following in varied quantity: Iron (2.526mg/L), Zinc (0.033mg/L) and Iodine (0.025mg/L), except for Copper and Cobalt which were absent. The results of the proximate analysis were as follows: % Moisture (54.25), % Carbohydrate (11.23), % Crude Protein (12.41), % Crude Fat (9.25), % Crude Fibre (10.65) and % Ash Content (2.21). The outcome of the study shows that all the samples of Bio-Clean II examined were of high microbiological quality and therefore poses no microbial threat to the safety of potential consumers; while the presence of appreciable quantities of some important phytochemical compounds and trace elements in the herbal remedy further underscores its medicinal benefits as earlier reported.

Key Words: ***Herbal remedy, Safety, Active Compounds, Mineral Elements, Medicinal values***

The practice of herbal medicine has continued to gain worldwide recognition (Falodun, 2010). Globally, over 80% of the general populace depends on some form of herbal medicine due to their availability, affordability, cultural acceptability, efficacy and safety claims (Nontokozo et al., 2018; Krishna et al., 2022). Owing to its popularity, the issue of efficacy and safety, as well as quality control of complementary, alternative and traditional medicine has become important concerns for many countries (Enitan et al., 2014; Asomugha et al., 2015; Chei et al., 2017; Ansley, 2020).

Currently, many herbal remedies are available in the markets, which the manufacturers and vendors claim to be more effective than the conventional antibiotics in the treatment of several illnesses (Ekor, 2013; Enitan et al., 2022a). However, due to the differences in the quality of different batches of herbal remedies there is therefore a strong need to validate and re-validate the assertions made by the manufacturers of these herbal remedies concerning their microbiological quality amongst other considerations. While the making and sales of standardized herbal remedies is being encouraged by the World Health Organization (WHO, 2005, 2007), the need to assured their microbiological quality cannot be overemphasized. As far as herbal drug's standardization is concerned, WHO has also emphasized on the need and importance of determining proximate and micronutrients analysis. Such herbal formulations must pass through standardization processes (Niranjan and Kanaki, 2008; Ojokoh, 2008; Hussain et al., 2009).

A few studies have shown that Bio-Clean II herbal remedy boosts immunity and fights viral infection like HIV. It has been reported to perform well on HIV patients and had shown evidence of total restoration of damaged tissues. Specifically, it has been shown to induce an increase in CD4 Cell Count and body weight, whilst decreasing viral loads in a cohort of HIV positive women (Ibeh et al., 2013; Ibeh et al., 2016a, 2016b). It has also been previously shown to boost immunity by modulating the levels of T-Helper 4 and Cytotoxic T-Lymphocytes, as well as serum level of some inflammatory cytokines in rats exposed to purified bacterial lipopolysaccharide (Enitan et al., 2022b, 2022c; 2022d). Although, many of the protective and healing effects of Bio-Clean II are thought to be directly or indirectly related to its immunomodulatory properties (Ibeh et al., 2016b). To the best of our knowledge, no work has been done to assess the microbiological quality, phytochemicals, trace elements and proximate analyses of the herbal remedy. Since Bio-Clean II like other commercially sold herbal remedies is used orally, it is therefore crucial to determine the microbiological quality, phytochemicals, trace elements and proximate analyses of the herbal remedy in order to determine its nutritional significance and health effects on consumers.

METHODOLOGY

Study Design

This is an analytical study.

Duration of Study

The study lasted for a period of two (2) months (April and May, 2021).

Study Area

The research was carried out at the Medical Microbiology Laboratory Unit of the Department of Medical Laboratory Science, School of Public and Allied Health, Babcock University, Ilishan-Remo, Ogun State; a Seventh-day Adventist Institution of higher learning

Source of Test Herbal Products

Samples of Bio-Clean II herbal remedy which comes in liquid form was procured from the manufacturer/maker on demand.

Sterility test

Method described by Okunlola et al. (2007) was used to detect the presence of potential microbial contaminants in the herbal remedy solution. Briefly, the mouth of each sealed bottle of the herbal preparation was sterilized by rapidly passing it through the flame of the Bunsen burner thrice before opening to prevent self-contamination. Afterwards, about 0.1g of each sample of herbal remedy solution was streaked directly on the plates containing Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) medium already prepared according to manufacturer's instruction. The NA plates were incubated at 37°C for 24-48 hours and observed for any presence of bacterial growth; while the SDA plates were incubated at 25°C for 5-7 days and observed for any presence of fungal growth.

Physiochemical characterization

The physiochemical characteristics of the herbal remedy carried out on the Bio-Clean II includes: Turbidity, Conductivity, pH, Temperature, Relative density and Total dissolved solid.

Turbidity

A turbidity meter was used to measure the turbidity of the herbal remedy. Sample of the Bio-Clean II was poured in the sample holder and kept inside for a few minutes. After achieving the reading stability, the value was recorded (Swanson and Baldwin, 1965).

Conductivity

A conductivity meter was used to measure the conductivity of the herbal remedy. The probe of the meter was submerged in the Bio-Clean II solution and the reading was recorded after the disappearance of the stability indicator. After the measurement of the conductivity of the sample, the probe was rinsed with deionized water to avoid cross contamination among different samples (Rahmanian et al., 2015).

pH

The pH test strip was dipped into the Bio-Clean II solution for a few seconds and after a while, the indicator bars on the paper changed color. The tip of the test strip was compared with the standard color chart that came with it to establish the pH level of the herbal remedy solution (Claire, 2018).

Temperature

A thermometer was used to measure the temperature of the herbal remedy. Without touching the walls or bottom of the container, the tip of the thermometer was carefully suspended below the surface of the herbal solution to measure the temperature of the herbal solution and not that of the glass container. To provide a better representation of the entire solution, the herbal solution was properly stirred according to the method described by Stanley et al (2018).

Relative density

The relative density of the herbal remedy was determined using a hydrometer, which consists of a bulb attached to a stalk of constant cross-sectional area. The hydrometer was placed in the jar and was given a quick twirl to dislodge any air bubbles. When the hydrometer settled, the reading was taken from the right scale (Jeff, 2015).

Total dissolved solid

The total dissolved solids were determined by filtering a measured volume of the Bio-Clean II through a standard glass fiber filter. The filtrate (*i.e.,* filtered liquid) was then added to a preweighed ceramic dish that was placed in a drying oven at a temperature of 103^{0}C (Brain, 2018).

Phytochemical Screening

Phytochemical screening of the Bio-Clean II herbal remedy was done using methods described by Sofowora (1982), as well as Trease and Evans (1996).

Test for Alkaloids

A 1mL of the herbal solution was added to 3 mL of 1% aqueous hydrochloric acid, stirred on a steam bath and filtered. Then, 1 mL of the filtrate was treated with few drops of the following reagents: Wagner's reagent, Mayer's reagent and Draggendorf's reagent. Precipitation (Cream, Reddish-brown or Orange or reddish-brown) with either of these reagents was taken as preliminary evidence for the presence of alkaloids.

Test for Resins

A 2mL of the herbal solution was added to 5 mL of boiling ethanol. This was filtered through Whatman No. 1 filter paper and the filtrate was diluted with 4 mL of 1% aqueous HCl. Formation of resinous precipitate indicates the presence of resins.

Test for Tannins

A 1 mL of the herbal solution was added to 1 mL of distilled water, stirred and filtered. Ferric chloride solution was then added to the filtrate. A blue-black, blue-green or green precipitate was taken as the evidence for the presence of tannins.

Test for Steroids

About 1 mL of the herbal solution was added to 2 mL of chloroform. Then sulphuric acid was carefully added to form a lower layer. A reddish-brown color at the inter-phase indicates the presence of steroidal ring.

Test for Saponins

About 1 mL of the herbal solution was thoroughly mixed with water in a test tube. Frothing which persists on warming was taken as a preliminary evidence for presence of saponins.

Test for Anthraquinones

A 1 mL of herbal solution was taken into a dry test tube and 5 mL of chloroform was added and shaken for 5 minutes. It was filtered and the filtrate was thoroughly mixed with equal volume of 100% ammonia solution. Red or pink violet colour in the ammoniacal layer (lower layer) indicates the presence of free anthraquinones.

Trace element analysis

Trace elements present in the herbal remedy solution were detected using atomic absorption spectroscopy as described by Robert and Gustav (2018). Briefly, the sample was digested using Nitric acid and was then later subjected to Atomic Absorption Spectrophotometer (Perkin Elmer AA Analyst

700) using varying cathode lamp to detect different metals. Absorption for each element is specific and no other elements absorb this wavelength.

Proximate analysis

For proximate analysis, standard techniques of AOAC (1990) and AOCS (2000) were followed. The proximate analyses (moisture fiber, ash, fats, proteins and carbohydrates) of freeze dried sample of the herbal remedy were determined. Briefly, the moisture and ash were determined using weight difference method. Fiber content was estimated from the loss in weight of the crucible and its content on ignition. Carbohydrate was determined by difference method. The sum of the percentages of moisture, ash, crude protein, either extract and crude fiber were subtracted from 100. The nitrogen value, which is the precursor for protein of a substance, was determined by micro Kjeldahl method, involving digestions, distillation and finally titration of the sample (Pearson, 1976). The nitrogen value was converted to protein by multiplying a factor of 6.25. All the proximate values were presented in percentage (AOAC, 1990; AOCS, 2000).

RESULTS

In order to understand the scientific reason underpinning the medicinal benefits of Bio-Clean II, the herbal remedy was assessed for its microbiological quality, physicochemical properties, phytochemical constituents, trace elements and proximate analysis.

Microbiological Quality

The four batches of Bio-Clean II (100%) examined for the presence of microbial contaminants were found to be sterile. All the samples of Bio-Clean II examined were of high microbiological quality as no single microbial contaminant was found in them (Table 1).

Table 1: Microbiological quality of Bio-Clean II

Batch Number	Growth	No. of microbial colonies counted	Isolate recovered	Remark
1	Absent	0	None	Sterile
2	Absent	0	None	Sterile
3	Absent	0	None	Sterile
4	Absent	0	None	Sterile
5	Absent	0	None	Sterile

Physicochemical Characterization

The physicochemical characterization of Bio-Clean II is presented in Table 2. Bio-clean II is a brown colored turbid herbal solution with a pH of 6.86, temperature of 25^{o}C, relative density of 1.08, conductivity of 272uS/cm and a total dissolved solid of 163mg/L.

Table 2: Physicochemical Characterization of Bio-Clean II

Physicochemical Parameters	Result
Color	Brown
Transparency	Turbid
pH	6.86
Temperature	25^{o}C
Relative Density	1.08
Conductivity	272uS/cm
Total Dissolved Solid	163mg/L

Phytochemicals Screening

The outcome of the semi-quantitative phytochemical screening of Bio-Clean II is presented in Table 3. The following phytochemicals were found to be present in various quantity: resin (+), Alkaloids (++), Saponins (+), Anthraquinones (+++), Tannin (++), and Cardiac Glycoside (++); except for Steroids (-) and Flavonoids (+++).

Table 3: Semi-quantitative Phytochemical Screening of Bio-Clean II

Phytochemicals	Result
Resin	+
Flavonoids	+++
Saponins	+
Anthraquinones	+++
Tannin	++
Alkaloids	++
Cardiac Glycoside	++
Steroids	-

Trace Elements Analysis

The result for the qualitative and quantitative analysis of trace elements found in Bio-Clean is presented in Table 4. Iron, Zinc and Iodine were present at different levels: 2.526mg/L, 0.033mg/L and 0.025mg/L, respectively, except for Copper and Cobalt which were absent.

Table 4: Qualitative and Quantitative Analysis of Trace Element in Bio-Clean II

Trace Element	Qualitative	Quantitative (mg/L)
Iron (Fe)	+	2.526
Zinc (Zn)	+	0.033
Iodine (I)	+	0.025
Cobalt (Co)	-	0.000
Copper (Cu)	-	0.000

Key: *+ = Present; - Absent*

Table 5: Proximate Analysis

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Parameters	Mean±SEM
% Moisture	54.25±0.05
% Carbohydrate	11.23±0.01
% Crude Protein	12.41±0.02
% Crude Fat	9.25±0.03
% Crude Fibre	10.65±0.06
% Ash Content	2.21±0.02

DISCUSSION

With herbal medicine making significant contribution to modern medical practice Almas et al. (2001), the use of herbal remedy against conventional standard drugs for the purpose of treatment of various ailments is fast becoming a very popular practice among some folks, and deliberate efforts must be made to assess and reassess the acclaimed health benefits of these products. This present study assessed the microbiological quality, phytochemicals, trace elements and proximate analysis of the herbal remedy Bio-clean II.

All the samples of Bio-Clean II examined in this study were found to be sterile (No growth). This suggests that the herbal remedy met the drug standard which excludes the presence of indicator, index or pathogenic organism in products to be consumed by end users. This observation is in agreement with those of Okpalugo *et al.* (2009), Oranusi and Akhigbe (2013), as well as that of Mamatha and Kumar, 2017), who reported that all the brands of herbal products examined in their studies were sterile and complied with specified standards. The reason for this may be accrued to the high quality preparation and handling ensured by manufacturers of these herbal products. On the other hand, the outcome of this study differ from that of Enitan *et al.* (2022) who reported that half (50%) of the herbal remedy samples examined were contaminated with a microbial count of <10CFU/mL.

To this end, it is important for manufacturers of herbal products to be aware of possible sources of microbial contamination of their products. These include the maker/handler's own normal flora, clothes, bags, shoes; as well as activity of makers/handlers like coughing, sneezing, yawning and

talking. Poor hygiene amongst the makers/handlers of these products either along the line of production, packaging, storage or transportation is another important factor contributing to the occurrence of bacterial contaminants in commercially sold herbal products. Most people lack good hand and toilet hygiene after making use of the toilets or after eating and because the human hand harbors a lot of pathogenic bacteria, it becomes a good medium for transfer of bacterial contaminants to whatever that is been handled when not sanitized or washed properly with soap and water (Enitan et al., 2022a).

In addition, the manufacturer's equipment and materials such as grinder, mixer, dryer, dispenser, sealer, containers, utensils, water, herbs and other ingredients use for preparing the herbal products may also serve as viable sources of bacterial contaminants. Still, house-keeping activity such as using dry dust mops or sweeping in the production or dispensing room can aerosolize particles containing microorganisms (Pasquarella et al., 2000; ASHRAE, 2004). Other sources of contamination of herbal materials includes environmental pollution (that is, contaminated emissions from factories and leaded petrol and contaminated water including runoff water which finds its way into rivers, lakes and the sea and some pesticides), soil composition and fertilizers (WHO, 2007; Hussain et al., 2009). The absent of bacterial contaminants in the test herbal product (Bio-Clean II) is a clear indication that all the human and environmental factors mentioned above were properly taken off by the maker.

Phytochemical, proximate and trace elemental analyses of herbs and herbal products are ways of profiling them so as to determine the class of chemicals and trace elements in them which are all associated with their use and authenticity. The nutritional and pharmacological activities of herbs and herbal products are a function of these phytochemical compounds and trace elements. The goal is to explore and improve their medicinal benefits, enhance safety when consumed and provide evidence-based insight as to how the herbs should be collected, processed, handled and stored. These constituents vary with the environmental and climatic conditions present in the areas where the plants are grown (Marshall, 1988; Quadril et al., 2021) and also dependent on the kind and amount of constituents present in them (Almeida et al., 2011).

Bio-Clean II has been proven to fights viral infections like HIV by increasing CD4 Cell Count, while decreasing viral load in a group of HIV positive women. It has also been shown to boost immunity by modulating the levels of T-Helper 4 and Cytotoxic T-Lymphocytes, as well as inflammatory cytokines in rats exposed to purified bacterial lipopolysaccharide (Ibeh et a., 2013; 2016a; 2016b; Enitan et al., 2022a; 2022b). These reported effects of the herbal remedy may be due to the presence of some

important phytochemicals (Resins, Flavonoids, Saponins, Anthraquinones, Tannin, Alkaloids and Cardiac Glycoside) detected in this study. Generally, phytochemicals are known to exhibit various biological and physiological effects including antidiarrhoeal, free radical scavenging abilities, anti-inflammatory, anticarcinogenic and antimicrobial (Liu, 2004; Okwu and Okwu, 2004; Evans, 2005). Various mechanisms of action of these phytochemicals have been suggested. The antimicrobial activities of these phytochemicals are thought to be related to inhibition of virulence factors, modulation of microbial pathogenesis, interference with some metabolic processes, intercalation into microbial cell wall and DNA, inactivation or destruction of microbial DNA, regulation of gene expression and signal transduction pathways, interference with the phospholipids bilayer of the cell membrane, disruption of the electron flow and active transport leading to increased cellular permeability and loss of cellular constituents, coagulation of cell contents amongst others. Synergistic interactions between the active constituents of these herbs are considered to be a vital part of their efficacy (Fatima et al., 2000; Bou-Chacra et al., 2005; Madhumitha and Fowsiya, 2017).

Proximate analysis refers to the identification of the main components of medicinal plant extracts for the purpose of determining whether a sample is within the parameters of its typical composition or has been contaminated. With the help of this technique, the nutrients in the extracts were divided into five categories: moisture, ash, crude protein, and fat (AOAC, 2010; Quadri et al., 2021).

The amount of moisture in a plant depends on its storage condition, as well as environmental factors such humidity, temperature, harvest season, and climate. Depending on the type of plant and the plant portion under examination, different plants have different moisture contents (James et al., 2010). A desirable stability feature of herbal products is low moisture content (below 15%), which lowers the possibility of microbial growth and contamination in medicinal plants during storage (Adegbe et al., 2016). Although, the moisture content (54.25%) reported in this study was higher than the one reported by Quadri et al. (2021) for *Ocimum gratissimum* (12.65±0.20), there was no incidence of microbial contamination in the herbal remedy; suggesting that high quality control measures were observed in the preparation of the herbal remedy which comes in liquid form.

The crude fat (9.25%) observed in this study was lower than the 43.51±0.32 reported by Quadri et al. (2021) for *Ocimum gratissimum*; suggesting that Bio-clean II may not be a very good source of nutritional fat relative to *Ocimum gratissimum* plant.

Still, the amount of minerals (inorganic components) contained in plant portions is measured by ash content. While some of these minerals may enhance nutritional advantages, others may have an

adverse effect on the physical integrity of the plant product or be able to prevent the growth of pathogens (Julian, 2016). The ash content recorded in this study was extremely lower (2.21%) than that reported by Quadri *et al.* (2021) (28.00±0.32). This may partly explain the lack of microbial contamination observed in this study.

Furthermore, protein is essential for many bodily processes, including growth, fluid-balance, hormone and enzyme production, and the maintenance of a robust immune system (Mau et al., 1999). The crude protein (12.41) reported in this study was higher than the one reported by Quadri et al. (2021) for *Ocimum gratissimum* (0.23±0.02). This may partly explain why the herbal remedy was effective in boosting the immune function of HIV infected persons with significant increase in body weight (Ibeh et al., 2013; 2016a; 2016b).

Dietary fibers have a variety of biological roles in the treatment of colon cancer, gastrointestinal diseases, high blood pressure, high cholesterol, high blood sugar, and waste removal (Hussain et al., 2009). Although, the crude fibre (%10.65) observed in this study was lower than that observed by Quadril et al. (2021) in *Ocimum gratissimum* (43.51±0.32); nevertheless, the outcome of this study suggest that Bio-Clean II may aid in the management of some disorders as earlier reported.

In addition, certain amounts of mineral elements are physiologically necessary for the appropriate growth, development, and operation of the system (Igwenyi et al., 2014). According to Kruczek (2005), mineral element content of plants is influenced by the properties of soils. Mineral elements in plants and plant products are also responsible for their anti-disease effects because there is a link between the mineral composition of the human body and various disease conditions. The iron and zinc content (though very low) found in Bio-Clean II suggest that the herbal remedy may have some little hematopoietic and immune stimulating properties. Although the iron and zinc level in Bio-Clean II falls below the WHO (2007) recommended dose, an inclusion of other plant or plant parts rich in iron and zinc in the formulation of the herbal remedy is very critical to optimizing the hematopoietic and immune stimulating potentials of the herbal remedy.

Conclusion

Bio-Clean II is of high microbiological quality and contains some important phytochemicals, as well as some trace elements; though in low quantities. The outcome of this current study gives further credence to the medicinal benefits of the herbal remedy as earlier reported.

Ethical Approval

Ethical approval (Registration number: *BUHREC369/21*) was sought for and obtained from the Babcock University Health Research Ethics Committee (BUHREC) before the commencement of the study.

Disclosure statement

The authors report no conflict of interest.

Data Availability

The data that support the findings of this study are available from the corresponding author, [Enitan S.

S.], upon reasonable request.

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